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MECHANISM OF INFLUENCE OF MOLECULAR HYDROGEN ON THE FUNCTION OF THE PROXIMAL TUBULE OF THE NEPHRON IN IRRITABLE BOWEL SYNDROME

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Abstract

Aim. To find out a pathophysiological protective role of influence of molecular hydrogen on a functional condition of a proximal tubule of kidneys at an irritable bowel syndrome.

Materials and methods. 60 patients with irritable bowel syndrome were examined. Men - 18, women - 42 aged from 28 to 62 years. Of which: with irritable bowel syndrome with constipation were - 28 patients, irritable bowel syndrome with diarrhea - 32. The control

group consisted of 25 almost healthy patients. General clinical (Likert scale), functional (proximal reabsorption of sodium ions in the kidneys), enzyme-linked immunosorbent assay (concentration of cytokines in blood plasma: tumor necrosis factor- α , interleukin-1 β , interleukin-6), microbiological (population levels of anaerobic B. Bifidum, B. lactis in lg CFO/g in the lumen of the colon), biochemical (the total, enzymatic fibrinolytic activity of urine) research methods were used.

All patients received molecular hydrogen in the form of an aqueous solution in the amount of 1 liter per day in four doses of 250 ml for 7 days with a concentration of H₂ 1.0 - 1.2 ppm and redox potential from -250 to -350 mV, which was obtained using a generator of a new generation of antioxidant solution saturated with hydrogen Blue Water 900 (Korea), mode 1. Statistical data processing was performed using computer programs "Statgrafics" and "Exel 7.0".

Results and discussion. The protective effect of molecular hydrogen improves the functional state of the proximal renal tubule in irritable bowel syndrome due to its high permeability and selective antioxidant activity against hydroxyl radical and peroxy nitrite, which contributes to the rupture of large and small vicious circles in the combined pathological process.

Key words: proximal tubule; water diuresis; renal function; molecular hydrogen; irritable bowel syndrome.

INTRODUCTION. Irritable bowel syndrome according to the IV Roman Consensus is known to be a functional bowel disease with recurrent abdominal pain associated with bowel movements or changes in stool type, such as diarrhea or constipation, and flatulence. These symptoms appear at least 6 months before diagnosis and occur periodically during the last three months, with the frequency of abdominal pain should be at least once a week [1, 2]. In the pathogenesis of irritable bowel syndrome can play a significant role proinflammatory cytokines with the formation of inflammasome [3], changes in which can lead to increased inflammatory process with activation of lipid peroxidation and renal dysfunction, because the proximal nephron is very sensitive to active forms of oxygen [4].

On the other hand, it is known that molecular hydrogen has selective antioxidant, anti-inflammatory and anti-apoptotic properties, inhibits oxidative stress, atherosclerosis, prevents cognitive impairment, has a hepatoprotective effect, protects against the harmful effects of ischemia-reperfusion. H₂ can be used as an effective antioxidant therapy; due to its ability to diffuse rapidly through membranes, it can penetrate the mitochondria and react with the most dangerous cytotoxic reactive oxygen species, such as hydroxyl radical, peroxy nitrite, and thus

protect against oxidative damage phospholipids of membranes, proteins, manifestations of inflammation and apoptosis [6, 7].

Aim. To find out a pathophysiological protective role of influence of molecular hydrogen on a functional condition of a proximal tubule of kidneys at an irritable bowel syndrome.

METHODS. 60 patients with irritable bowel syndrome were examined. Men - 18, women - 42 aged from 28 to 62 years. Of which: with irritable bowel syndrome with constipation were - 28 patients, irritable bowel syndrome with diarrhea - 32. Control group - 25 almost healthy patients. General symptoms (summary assessment of symptoms: pain, bloating, frequency of defecation) in patients with irritable bowel syndrome were evaluated on a seven-point Likert scale [1, 2]. The functional state of the kidneys was studied under water load. Patients consumed tap water heated to a temperature of 37°C in the amount of 2% of body weight. The amount of diuresis (V) was estimated in 1/2 h · 1.72 m² of body surface area. After water loading to obtain plasma, blood from a vein was collected in tubes with heparin. In plasma of blood and urine concentration of creatinine on reaction with picric acid, sodium ions by a method of a photometry of a flame on FPL-1 was defined. Calculated the proximal reabsorption of sodium ions (T^PNa⁺) by the formula: $T^PNa^+ = (C_{cr} - V) \cdot PNa^+$; where C_{cr} is the glomerular filtration rate, V is diuresis, PNa⁺ is the concentration of sodium ions in blood plasma [5, 8]. Population levels of anaerobic (B. Bifidum, B. lactis.) in Ig CFO/g in the lumen of the colon were determined [9]. Studies of the concentration of cytokines in blood plasma: tumor necrosis factor-α, interleukin-1β, interleukin-6 were performed by enzyme-linked immunosorbent assay using kits from Diaclone (France) [10]. Fibrinolysis of urine was assessed by determining the lysis of azofibrin with the assessment of total (TFA), non-enzymatic (NFA) (incubation of samples in the presence of a blocker of enzymatic fibrinolysis ε-aminocaproic acid) and enzymatic fibrinolytic activity (EFA), which was calculated by the formula: EFA = TFA-NFA [5, 8].

All patients received molecular hydrogen in the form of an aqueous solution in the amount of 1 l per day in four doses of 250 ml for 7 days with a concentration of H₂ 1.0 - 1.2 ppm and redox potential from -250 to -350 mV, which was obtained using a generator of a new generation of antioxidant solution saturated with hydrogen Blue Water 900 (Korea), mode 1.

All studies were performed in compliance with the "Rules of ethical principles of scientific medical research with human participation", approved by the Declaration of Helsinki (1964-2013), ICH GCP (1996), EEC Directive №609 (dated 24.11.1986), orders of

the Ministry of Health of Ukraine № 690 dated 23.09.2009. № 944 dated 14.12.2009, № 616 dated 03.08.2012. Statistical data processing was performed using computer programs "Statgraphics" and "Exel 7.0".

RESULTS. The development of irritable bowel syndrome with constipation and diarrhea led to an increase in the level of pro-inflammatory cytokines in the blood plasma: interleukin-1-beta, interleukin -6, tumor necrosis factor -alpha. The use of molecular hydrogen led to the correction of the detected changes (Fig. 1).

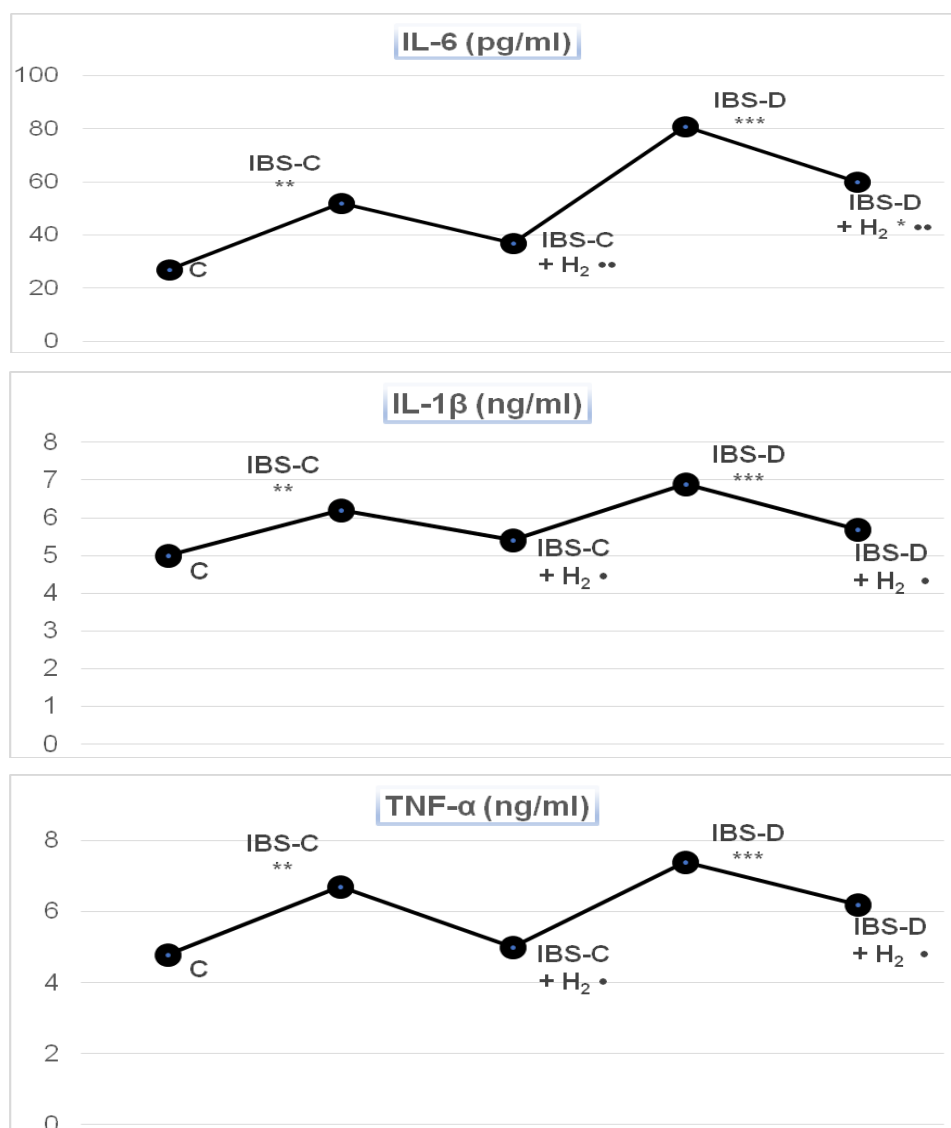


Fig. 1. Influence of molecular hydrogen (H₂) on the concentration of cytokines in blood plasma in patients with the development of irritable bowel syndrome with constipation (IBS-C) and diarrhea (IBS-D). The probability of differences was noted in comparison: before control (C): * - p <0,05; ** - p <0.02; *** - p <0.01; to irritable bowel syndrome with constipation and diarrhea • - p <0,05.

The use of molecular hydrogen led to the correction of the detected violations. The study of the indicators of total and enzymatic fibrinolytic activities of urine showed inhibition of these parameters in the urine in irritable bowel syndrome with constipation and diarrhea. These disorders were combined with inhibition of proximal reabsorption of sodium ions in the kidneys in irritable bowel syndrome with constipation and diarrhea. The use of molecular hydrogen led to the correction of the detected changes (Fig. 2).

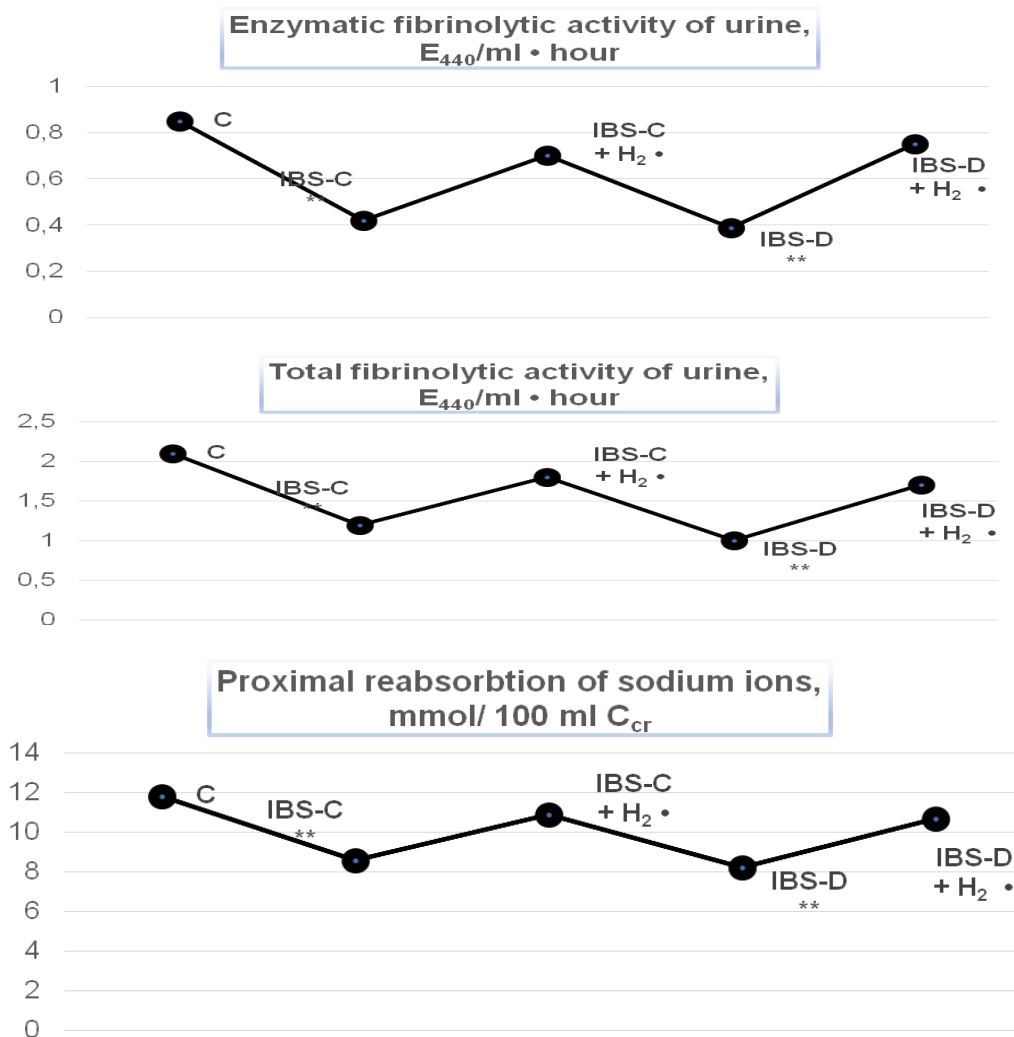


Fig. 2. Influence of molecular hydrogen (H₂) on the total, enzymatic fibrinolytic activity of urine and proximal reabsorption of sodium ions of kidneys in patients with the development of irritable bowel syndrome with constipation (IBS-C) and diarrhea (IBS-D). The probability of differences was noted in comparison: before control (C): ** - p < 0.02; to irritable bowel syndrome with constipation and diarrhea • - p < 0.05.

Assessment of the general condition of the patient on the Likert scale showed a deterioration in patients with irritable bowel syndrome with constipation and diarrhea, which was accompanied by a quantitative increase in this indicator. These changes were combined with a decrease in the number of protective microflora of *B. bifidum*, *B. lactis* in the lumen of the colon. Molecular hydrogen under these conditions also had a protective effect (Fig. 3).

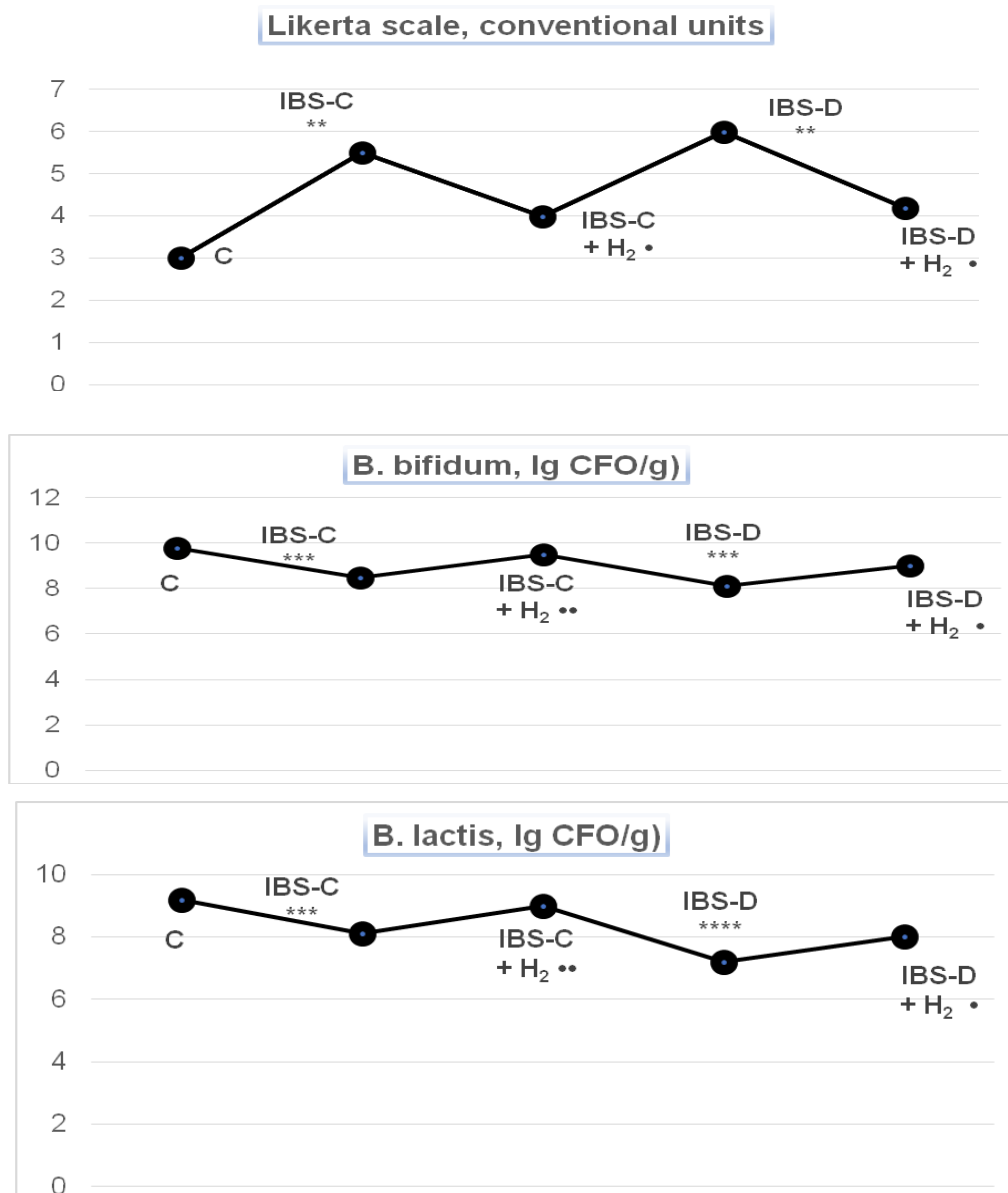


Fig. 3. Influence of molecular hydrogen (H₂) on the Likert scale, population levels of anaerobic microflora of the large intestine in patients with the development of irritable bowel syndrome with constipation (IBS-C) and diarrhea (IBS-D). The probability of differences was noted in comparison: before control (C): ** - p < 0.02; *** - p < 0.01; **** - p < 0.001; ***** - p < 0.0001; to irritable bowel syndrome with constipation and diarrhea • - p < 0,05; •• - p < 0,02.

DISCUSSION. The protective effect of molecular hydrogen is realized due to its high permeability and ability to neutralize hydroxyl radicals and peroxyxynitrite by reducing the manifestations of chronic stress, significant psychogenic maladaptation, anxiety response, which inhibited hyperplasia of APUD cells (EC-enterochromaffin), active substances: serotonin, motilin, substance P. Accordingly, there was no hyperfunction [11, 12] and the development of local inflammatory process with disorders of the barrier function of the intestine.

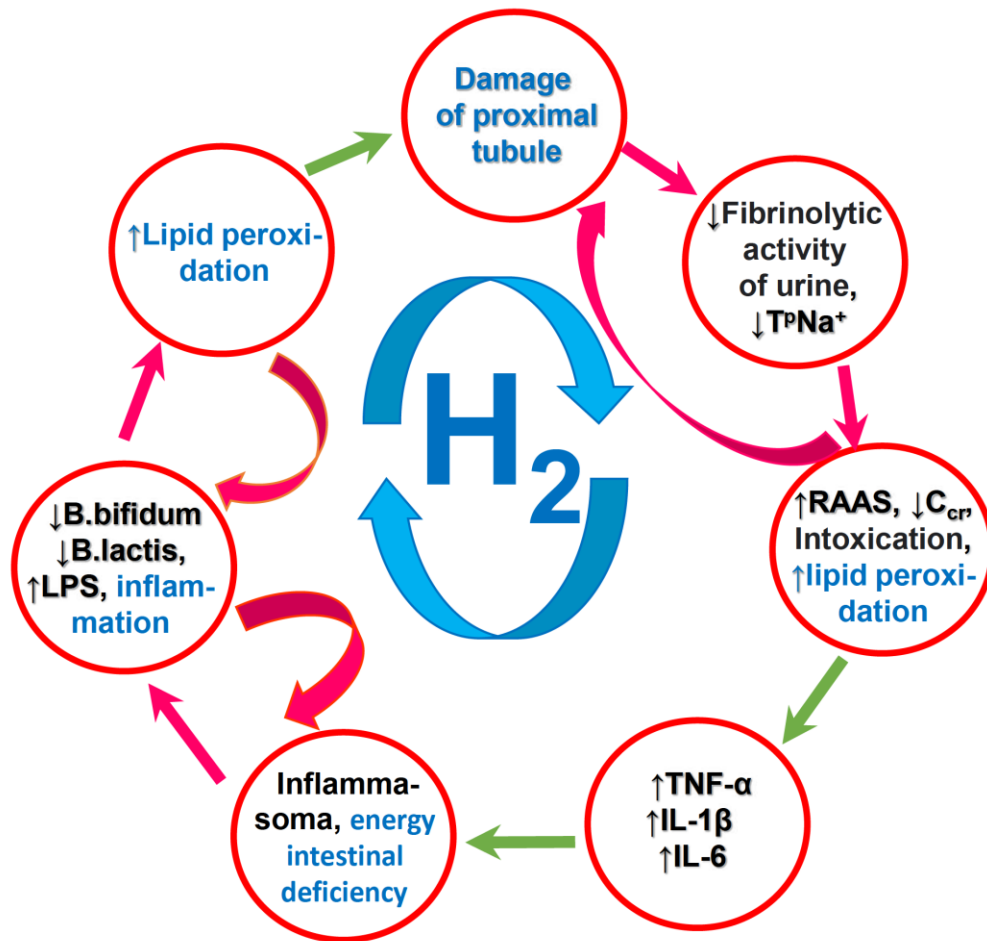


Fig. 4. Break of large and small vicious circles in damage to the proximal nephron and intestines in irritable bowel syndrome with constipation and diarrhea in patients using the antioxidant properties of molecular hydrogen (H_2). $\downarrow T^PNa^+$ - a decrease in the proximal reabsorption of sodium ions, $\downarrow C_{cr}$ - a decrease in the glomerular filtration rate, $\uparrow RAAS$ - an increase in the activity of the renin-angiotensin-aldosterone system, $\uparrow LPS$ increase in the lipopolysaccharide content in the intestine, $\uparrow TNF-\alpha$ increased blood tumor necrosis factor alpha, $\uparrow IL-1\beta$ increased blood interleukin 1 beta, $\uparrow IL-6$ increased blood interleukin 6.

Which did not lead to an increase in proinflammatory cytokines of tumor necrosis factor α , interleukin- 1β , interleukin -6. Accordingly, there was no hyperfunction of the large intestine, its energy deficiency, increased use of B. Bifidum, B. Lactis to ensure the regeneration of the intestinal epithelium.

Due to the lack of decrease in the level of B. Bifidum, B. Lactis did not develop dysbacteriosis and there was no increase in lipopolysaccharide concentrations, activation of lipid peroxidation. As a result, the lipopolysaccharide did not interact with the CD14 receptor and the Toll-like receptor type 4 (TLR4) on monocytes (macrophages), which did not infiltrate the intestinal wall, which probably did not lead to the formation of an inflammasome (inflammation). - multiprotein complex [4], which is responsible for activating the inflammatory response. Inflammasoma promotes the maturation and secretion of proinflammatory cytokines interleukin- 1β and interleukin 18.

As a result of these processes, pyroptosis does not develop - a special type of programmed cell death. Inhibition of these processes did not lead to damage to the proximal tubule and inhibition of reabsorption of sodium ions [13, 14, 15], and, accordingly, did not create conditions for the formation of large and small vicious circles, when cause (etiological factors) causes the pathologic reactions (process) and then these reactions return to the first agent (etiological factor) and intensify of the combined pathological process (Fig. 4).

CONCLUSION

The protective effect of molecular hydrogen improves the functional state of the proximal renal tubule in irritable bowel syndrome due to its high permeability and selective antioxidant activity against hydroxyl radical and peroxynitrite, which contributes to the rupture of large and small vicious circles in the combined pathological process.

The prospect of scientific research is to conduct further research on the protective effect of molecular hydrogen in other diseases that are accompanied by the development of inflammation and activation of lipid peroxidation.

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