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The Relationship between Progression of PBC Assessed by Biochemical Analysis Results and Level of Met-Enkephalin in the Liver and Blood Plasma

Progresja pierwotnej żółciowej marskości wątroby
oceniana badaniami biochemicznymi
a stężenie Met-enkefalin w osoczu krwi
i jej zawartość w tkance wątrobowej

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Abstract

Background. Met-enkephalin is a pentapeptide which belongs to opioid system. In the liver disorders, the concentration rates of opioid peptides in blood plasma and liver tissue are changed. On the grounds of biochemical analysis results, the following episodes are confirmed to occur in patients with PBC: some symptoms of cholestasis appear, the activity of aspartate and alanine transaminase is either within the standard ranges or insignificantly higher. When inflammatory processes are intensified, as well as during new attacks of necrosis, the serum aminotransferases activity essentially grows. During the end-stage of the PBC, clinical and biochemical symptoms of decompensation of the liver cirrhosis occur.

Objectives. Is there a relationship between progression of PBC assessed by biochemical analysis results and level of Met-enkephalin in the liver and blood plasma?

Material and Methods. Forty female patients with PBC were examined; their disease was determined with the use of biochemical and serological investigations as well as of the histopathological biopsies estimation. In each case, the activity of the following parameters was determined: aspartate and alanine transaminase, alkaline phosphatase, γ glutamyl transpeptidase, cholinesterase, bilirubin concentration level, immunoglobulins and acute phase proteins. In each case, the percutaneous thick-needle liver biopsy was performed and a histopathological estimation was prepared. In each case, the Met-enkephalin concentration rates in plasma and its contents in liver tissue were determined.

Results. A statistically significant correlation exists between the plasma Met-enkephalin concentration and the activity levels of alanine transaminase ($p = 0.0052$), aspartate transaminase ($p = 0.0001$), alkaline phosphatase ($p = 0.0001$) and the level of bilirubin ($p = 0.0129$) in blood plasma; but there is no statistically significant correlation between the activity levels of γ glutamyl transpeptidase, cholinesterase, level of haptoglobin, ceruloplasmin, antytrypsin, glycoprotein acid and immunoglobulins (IgA, IgG, IgM). Statistically significant difference has been demonstrated in the Met-enkephalin contents in the liver tissue and activity of the serum aspartate transaminase ($p = 0.0427$), the serum haptoglobin ($p = 0.0029$) as well as the ceruloplasmin level ($p = 0.0418$) in group II (patients with histopathological changes of liver tissue – grades III and IV according to the Ludwig scale). In group I included patients with histopathological changes of liver tissue grades I and II according to the Ludwig scale no statistically significant correlation has been demonstrated between Met-enkephalin content in liver tissue and activity of the serum aspartate transaminase, the serum haptoglobin and the ceruloplasmin level. No statistically significant correlation has been demonstrated between Met-enkephalin contents in liver tissue and other measured biochemical parameters in both groups.

Conclusions. In PBC patients, when Met-enkephalin concentration increases in plasma, the activity of the serum aspartate transaminase, alkaline phosphatase and the level of bilirubin in blood are although increased. If the liver tissue damages are advanced (the stage III and IV according to the Ludwig scale), both the Met-enkephalin con-

tent in the liver tissue, and the acute phase protein level in the blood decrease. On the basis of the performed investigations, it can be stated that the plasma Met-enkephalin concentration rate is an important and useful parameter that should be measured because it allows for the determination of the PBC disease progress/advancement in the organism of a patient with PBC (*Adv Clin Exp Med* 2005, 14, 1, 23–29).

Key words: Met-enkephalin, primary biliary cirrhosis, biochemical analysis, liver tissue.

Streszczenie

Wprowadzenie. Met-enkefalina jest pentapeptydem należącym do układu opioidowego. W schorzeniach wątroby zmienia się stężenie peptydów opioidowych w osoczu krwi oraz w mięszu wątroby. W początkowym okresie pierwotnej żółciowej marskości wątroby w badaniach biochemicznych stwierdza się cechy cholestazy; aktywność aminotransferazy alaninowej i asparaginianowej natomiast może być w granicach normy lub nieznacznie podwyższona. W okresie nasilenia procesów zapalnych oraz w rzutach martwiczych aktywność aminotransferaz znacznie wzrasta. W schyłkowym okresie choroby występują objawy kliniczne i biochemiczne niewyrównanej marskości wątroby.

Cel pracy. Czy progresja pierwotnej żółciowej marskości wątroby, oceniana badaniami biochemicznymi, przebiega równolegle ze zmianą stężenia Met-enkefaliny w osoczu krwi i jej zawartością w wątrobie?

Materiał i metody. Badana grupa obejmowała 40 chorych kobiet, u których rozpoznanie pierwotnej żółciowej marskości wątroby zostało potwierdzone w badaniach biochemicznych, serologicznych i w ocenie histopatologicznej biopatów wątroby. U każdej chorej pobierano krew w celu oznaczenia: stężenia bilirubiny, aktywności aminotransferaz, cholinesterazy, fosfatazy alkalicznej, γ glutamylotranspeptydazy, stężenia immunoglobulin i białek ostrej fazy oraz oznaczono stężenie Met-enkefaliny w osoczu krwi. Wykonano również biopsję gruboigłową wątroby i dokonano oceny histopatologicznej oraz oznaczono zawartość Met-enkefaliny w biopsacie wątroby.

Wyniki. W badanej grupie chorych na pierwotną żółciową marskość wątroby stwierdzono statystycznie istotną korelację między stężeniem Met-enkefaliny w osoczu krwi a aktywnością AlAT ($p = 0,0052$), AspAT ($p = 0,0001$), fosfatazy alkalicznej ($p = 0,0001$) i stężeniem bilirubiny ($p = 0,0129$) w surowicy krwi, a dla γ glutamylotranspeptydazy, cholinesterazy, haptoglobiny, ceruloplazminy, antytrypsyny, kwaśnej glikoproteiny i immunoglobulin (IgA, IgG, IgM) nie wykazano istotnej statystycznie korelacji. Stwierdzono istotną statystycznie korelację między zawartością Met-enkefaliny w tkance wątrobowej a stężeniem AspAT ($p = 0,0427$), haptoglobiny ($p = 0,0029$) i ceruloplazminy ($p = 0,0418$) w surowicy krwi w II grupie (chore ze zmianami histopatologicznymi w wątrobie III i IV stopnia według klasyfikacji Ludwiga). W grupie I obejmującej chore ze zmianami w stopniu I i II według klasyfikacji Ludwiga nie wykazano współzależności zawartości Met-enkefaliny w tkance wątrobowej ze stężeniem AspAT, haptoglobiny i ceruloplazminy w surowicy krwi. W przypadku pozostałych oznaczanych parametrów biochemicznych nie wykazano istotnej korelacji między zawartością Met-enkefaliny w tkance wątrobowej zarówno w grupie I, jak i w grupie II.

Wnioski. Wraz ze wzrostem stężenia Met-enkefaliny w osoczu krwi, zwiększa się aktywność aminotransferaz, fosfatazy alkalicznej i stężenie bilirubiny. W zaawansowanym uszkodzeniu mięszu wątroby (III i IV stopień według Ludwiga) maleje zawartość Met-enkefaliny w tkance wątrobowej oraz stężenie białek ostrej fazy w surowicy krwi. Dotychczasowe badania mogą wskazywać na przydatność pomiaru stężenia Met-enkefaliny w osoczu krwi jako wskaźnika pozwalającego oceniać progresję pierwotnej żółciowej marskości wątroby (*Adv Clin Exp Med* 2005, 14, 1, 23–29).

Słowa kluczowe: Met-enkefalina, pierwotna żółciowa marskość wątroby, badania biochemiczne, tkanka wątrobowa.

The system of opioid peptides is widely represented in many organs and tissues. The endogenous opioid peptides were discovered in the late 1970s [1].

Their presence was identified both in the central nervous system, where as neurotransmitters or neuromodulators they take part in converting the nervous signals into suitable physiological responses, as well as in the peripheral structures that regulate basic vital functions. Presence of opioid peptides in the above-mentioned regions reflects their participation in the phenomena related to modulation of pain perception, regulation of emotional conditions and memory, intake of foods and water, and regulation of the immune system [2–4].

Development of new biochemical and pharmacological methods allowed to establish the presence

of over 20 endogenous opioid peptides. Opioid peptides, similarly to the majority of other peptide hormones or neuromodulators, derive from high molecular weight precursors in result of cleavage of active fragments. Met-enkephalin precursor is proenkephalin composed of 263 amino acids. Enzymatic hydrolysis results in separation of smaller fragments, including Met- and Leu-enkephalin. These two compounds possess identical sequence of initial four amino acids: tyrosine – glycine – glycine – phenylalanine, and differ with the fifth amino acid – methionine in case of Met-enkephalin (methionine enkephalin) and leucine in case of Leu-enkephalin (leucine enkephalin). Met-enkephalin participates in regulation of the gastrointestinal motility, in gastric, pancreatic and intestinal secretion, and influences the carbohydrate metabolism [5].

Changes in the opioid system were identified in alcoholism, epilepsy, schizophrenia, manic-depressive syndrome, anxiety syndrome, neoplastic diseases and hepatic disorders [6]. Liver diseases are associated with changed opioid peptide concentration in the blood plasma and hepatic parenchyma [7, 8]. In acute liver disorders, blood plasma levels of Met- and Leu-enkephalin rise to values several times higher than levels of these enkephalins in plasma of healthy persons. Elevation of blood plasma enkephalin level is proportional to the degree of liver damage. The highest values are reached in hepatic cirrhosis with ascites; in such patients Met-enkephalin level is significantly higher than in patients with hepatic cirrhosis not complicated by ascites [9].

Many studies have concluded that opioid peptides play a role in aetiopathogenesis of ascites, and have vasodilating properties. Chronic vasodilation leads to activation of the sympathetic system with subsequent elevation of catecholamines level and stimulation of non-osmotic release of vasopressin [10]. It has been revealed that opioid peptides release histamine, which in turn interferes with hepatic flow of the lymph, thus increasing lymph penetration. Higher Met-enkephalin level in blood plasma is also detected in liver diseases associated with cholestasis [11]. Origin of cholestasis-associated pruritus has not been clarified in detail, however it possibly occurs due to interaction of various substances present in the blood plasma. The studies published previously indicate the significance of opioid peptides in pathogenesis of cholestasis-associated pruritus. Attempts to treat pruritus with naltrexone carried out in recent years yield positive results, thus confirming that opioid peptides and their receptors are engaged in the aetiology of pruritus [12, 13].

Administration of opioid peptides produced a change of blood serum liver enzymes activity. An animal model has demonstrated that administration of a single dose of Leu-enkephalin leads to increased activity of acid and alkaline phosphatase and of lactate dehydrogenase, and this result is dose-independent. During this period, the levels of acid phosphatase in the liver and of sialic acid in the spleen are reduced, and these changes are related to the dose of Leu-enkephalin [14]. Comparison of the activity of Met-enkephalin revealed only slight and transitory effect on activity of these enzymes. Administration of Dolargan, a synthetic Leu-enkephalin analogue, leads to reduction of xanthine oxidase activity in the liver tissue and blood serum, and increased level of histidine and urokinase in liver parenchyma, and decreased – in blood serum [15].

A direct relationship has been identified between Met-enkephalin blood serum concentration and total bilirubin concentration.

Material and Methods

This study included 40 female patients with primary biliary liver cirrhosis, confirmed by biochemical and serologic tests and by histopathological evaluation of liver biopsates. All patients were under surveillance of the Clinical Department of the Clinic of Gastroenterology of the University Hospital in Cracow. Excluded from the study were carriers of hepatitis B and C viruses, alcoholics and patients with coexistent diseases that could injure the liver parenchyma. Mean age of the patients was 54.1 years (30–68 years of age). Carrier state of hepatitis B and C viruses was excluded by means of enzymatic immune tests and polymerase chain reaction performed in every patient.

Blood was sampled from each patient for measurement of: bilirubin level, activity of aminotransferases, cholinesterase, alkaline phosphatase, γ glutamyl transpeptidase, level of immunoglobulins (IgA, IgG and IgM) and acute phase proteins (haptoglobins, ceruloplasmin, antitrypsin and acid glycoprotein), and concentration of Met-enkephalin concentration in blood plasma. A thick-needle liver biopsy was also performed with histopathologic evaluation and Met-enkephalin content determination in the liver biopsate. Measurement of Met-enkephalin blood plasma concentration and hepatic tissue content was carried out according to a method by Pierzchała-Koziec [16]. This analysis was performed in the Chair of Animal Physiology of Agricultural University of Cracow. Results of these analyses are expressed in pg per ml of plasma and pg per milligram of hepatic tissue.

Liver biopsy was done with Menghini method, with a Hepafix needle manufactured by Braun Company, 1.6 mm in cross section. The obtained biopsate was divided into 2 parts. One part was weighed and placed in a test tube containing 500 μ l of 0.05 N hydrochloric acid, and then frozen at -80°C until measurement of Met-enkephalin content in the liver tissue. The other was fixed in 80% buffered formalin and transferred for histopathologic testing in the Chair of Pathomorphology of Jagiellonian University Medical College. Stage of progression of histopathologic changes was assessed according to Ludwig classification.

The results were subjected to statistical analysis with application of the Mann-Whitney U test. Test results, for which the significance level was below 0.05 ($p < 0.05$), were assumed as statistically significant.

Results

In the tested group of PBC patients, the concentration of Met-enkephalin in the blood plasma was compared with that of bilirubin, activity of aminotransferases, cholinesterase, alkaline phosphatase, γ glutamyl transpeptidase, level of immunoglobulins and acute phase proteins. Table 1 presents the activity of aminotransferases, cholinesterase, alkaline phosphatase, γ glutamyl transpeptidase expressed in U/l, their mean levels with standard deviations in the tested group of patients. Table 2 presents the concentration of bilirubin expressed in $\mu\text{mol/l}$, its mean value and standard deviation in the observed group.

A statistically significant correlation was identified between the blood plasma concentration of Met-enkephalin and the blood serum level of AlAT ($p = 0.0052$), AspAT ($p = 0.0001$), alkaline phosphatase ($p = 0.0001$) and bilirubin ($p = 0.0129$). Conversely, no statistically significant correlation was demonstrated for γ glutamyl transpeptidase, cholinesterase, haptoglobin, ceruloplasmin, anti-trypsin, acid glycoprotein and immunoglobulins (IgA, IgG, IgM).

The histopathologic examination of liver biopsies in the tested groups of patients with PBC revealed the following progression of liver histological changes according to Ludwig classification: stage I in 17.5% of patients, stage II in 37.5% of patients, stage III in 35% of patients and stage IV

in 10% of patients. With regard to the Ludwig classification, the patients with PBC were divided into 2 groups. To group I the authors qualified 22 patients with stage I and II according to Ludwig classification, and to group II, 18 patients with stage III and IV, in whom fibrosis and cirrhosis of the liver were discovered.

Statistically significant correlation was identified between the content of Met-enkephalin in hepatic tissue and the blood serum level of AspAT ($p = 0.0427$), haptoglobin ($p = 0.0029$) and ceruloplasmin ($p = 0.0418$) in group II. In group I, comprising the patients with stage I and II according to Ludwig classification, no relationship was demonstrated between the content of Met-enkephalin in hepatic tissue and the blood serum level of AspAT, haptoglobin and ceruloplasmin. For levels of the remaining measured biochemical parameters, no significant correlation was revealed between the content of Met-enkephalin in hepatic tissue in group I and group II.

Table 3 presents the Spearman r_s coefficients, standardised Z-score and significance level p of the test for blood serum aminotransferase activity in group I and group II of the tested female patients. Table 4 presents the Spearman r_s coefficients, standardised Z-score and significance level p of the test for the blood serum levels of haptoglobin and ceruloplasmin in group I and group II of the tested female patients.

Table 1. Activity of aspartate and alanine transaminase, cholinesterase, alkaline phosphatase, γ glutamyl transpeptidase (U/l) in serum, average levels and standard deviation in group of PBC patients

Tabela 1. Aktywność aminotransferaz, cholinesterazy, fosfatazy alkalicznej, GGTP wyrażona w U/l, średnie stężenia wraz z odchyleniami standardowymi w badanej grupie chorych na pierwotną żółciową marskość wątroby

Enzyme (Enzym)	Average level (Wartość średnia)	Standard deviation (Odchylenie standardowe)	r_s	Z	p	Pearson correlation coefficient (Współczynnik korelacji liniowej)
AlAT	138.73	128.42	0.4096	2.5578	0.0052	0.2815
AspAT	88.85	48.96	0.5902	3.6858	0.0001	0.4291
ChE	1969.7	548.85	-0.0574	-0.3588	0.3594	-0.036
AP	930.65	450.18	0.8458	5.2819	0.0000	0.8616
GGTP	295.15	200.97	0.2266	1.4154	0.0778	0.1973

Table 2. A bilirubin concentration level in serum ($\mu\text{mol/l}$), average level and standard deviation in group of PBC patients

Tabela 2. Stężenie bilirubiny w $\mu\text{mol/l}$, wartość średnia i odchylenie standardowe obserwowane w badanej grupie chorych na pierwotną żółciową marskość wątroby

	Average level (Wartość średnia)	Standard deviation (Odchylenie standardowe)	r_s	Z	p	Pearson correlation coefficient (Współczynnik korelacji liniowej)
Bilirubin (Bilirubina)	48.96	30.03	0.3572	2.2306	0.0129	0.4313

Table 3. Spearman rank coefficient (r_s), standardised Z-score and significance level p in groups I and II for blood serum activity of ASPAT and ALAT

Tabela 3. Współczynnik rang Spearmana, wartość standaryzowana Z oraz poziom istotności p w grupie I i II dla aktywności AspAT i AlAT w surowicy krwi

Group (Grupa)	AspAT		AlAT	
	I	II	I	II
r_s	-0.0808	0.4180	0.1621	0.3230
Z	-0.370	1.7233	0.7426	1.3318
p	0.3557	0.0427	0.2296	0.0917

Discussion

Biochemical tests performed in the initial stage of PBC may reveal the features of cholestasis, with elevated activity of alkaline phosphatase, γ glutamyl transpeptidase and bilirubin level. Serum activity of alkaline phosphatase exceeds the reference ranges several fold, and bilirubin level increases with progression of liver injury. Besides, increased concentration of immunoglobulin M and presence of antimitochondrial antibodies is identified in the blood serum. In initial stage of PBC, the activity of alanine and aspartate aminotransferase may be within the reference range or only slightly elevated. In the period of intensification of inflammatory processes and in acute necrotic phases, activity of aminotransferases increases very markedly.

In the tested group of patients with PBC, a statistically significant correlation was established between the values of blood plasma Met-enkephalin concentration and aminotransferase activity, both for AlAT at significance level of $p = 0.0052$, and AspAT ($r_s = 0.5902$) at significance level of $p = 0.0001$. It points to a relationship between the blood plasma Met-enkephalin concentration and activity of enzymes that indicate the degree of hepatocyte injury.

Studies of Thornton et al. [17] similarly demonstrated a statistically significant correlation between the blood plasma concentration of enkephalins and activity of alanine aminotransferase.

When testing the relationship between the Met-enkephalin concentration and activity of cholestatic enzymes, the authors established a statistically significant correlation between Met-enkephalin blood plasma values and alkaline phosphatase level at significance level of $p = 0.0000$, whereas for GGTP, the studies failed to demonstrate any statistical correlation ($p = 0.0778$). Linear correlation between alkaline phosphatase and Met-enkephalin is 0.8616 and approximates $r_s = 0.8458$.

Table 4. Blood serum haptoglobin and ceruloplasmin level, expressed in mg/ml, Spearman r_s coefficient, standardised Z-score and significance level p in group I and group II PBC patients

Tabela 4. Stężenie haptoglobiny i ceruloplazminy w surowicy krwi wyrażone w mg/ml, współczynnik r_s Spearmana, wartość standaryzowana Z oraz poziom istotności p w grupie I i grupie II chorych na pierwotną żółciową marskość wątroby

Group (Grupa)	Haptoglobin (Haptoglobina)		Ceruloplasmin (Ceruloplazmina)	
	I	II	I	II
r_s	-0.1793	0.6682	0.0243	0.4195
Z	-0.8216	2.7551	0.1113	1.7297
p	0.2061	0.0029	0.4562	0.0418

Previous studies on the relationship between the concentration of opioid peptides and the activity of cholestatic enzymes were carried out in animal models [18]. They confirmed that administration of Leu-enkephalin to mice at a dose of 10 mg/kg resulted in significant elevation of serum activity of alkaline phosphatase, but administration of Met-enkephalin at identical dose was followed by less significant elevation of alkaline phosphatase activity. Conduct of these studies also employed the control of activity of acid phosphatase and lactate dehydrogenase, and serum concentration of both enzymes rose after administration of opioid peptides. These studies are consistent with results obtained in our work, which indicate the relationship between Met-enkephalin in blood plasma and alkaline phosphatase activity.

In the primary biliary liver cirrhosis, bilirubin constitutes one of the major parameters that qualify a patient for liver transplantation. Bilirubin level above 150 $\mu\text{mol/l}$ is an indication for liver transplantation in a PBC patient. In the tested PBC patients group, a statistically significant correlation was identified between blood plasma concentration of Met-enkephalin and bilirubin concentration at significance level of $p = 0.0129$. Additionally, a linear correlation coefficient approximates the r_s value of the Spearman rank order correlation test. It permits a conclusion that the observed correlation between Met-enkephalin and bilirubin has linear characteristics. The authors additionally observed that the linear correlation coefficient was two-fold higher if the Met-enkephalin level exceeded 400 pg/ml. These results are consistent with reports from other authors, who demonstrated the correlation between Met-enkephalin concentration and bilirubin blood serum level [19].

This work involved the evaluation of cholinesterase activity in PBC patients. A statistical

analysis of the relationship between Met-enkephalin concentration and ChE activity in blood plasma, carried out with Spearman rank order correlation test, failed to demonstrate any statistically significant correlation ($p = 0.3594$). Probably, the lack of such correlation results from the fact that among the 40 PBC patients in the tested group, only 5 patients demonstrated the symptoms of decompensated liver failure. In the tested group of patients, an analysis was performed comparing the content of Met-enkephalin in liver tissue with the activity of indicator, cholestatic and secretory enzymes. The results of executed analysis indicate a statistically significant correlation between Met-enkephalin content in hepatic parenchyma and blood serum activity of AspAT, at significance level of $p = 0.0427$ in group II. Qualification for group II comprised patients with histological liver changes stage III and IV according to Ludwig classification. These data allow a conclusion that reduction of Met-enkephalin in hepatic tissue is followed by reduction of blood serum activity of AspAT, this being related to the presence of extensive fibrosis and necrosis of hepatic parenchyma. The analysis carried out with the Spearman rank test failed to demonstrate any statistically significant correlation between the content of Met-enkephalin in hepatic parenchyma, and blood serum levels of alkaline phosphatase, GGTP, ChE, and bilirubin.

The authors did not identify any statistically significant correlation between blood plasma Met-enkephalin concentration and blood serum levels of haptoglobin, acid glycoprotein, ceruloplasmin and antitrypsin.

However, a statistically significant correlation was demonstrated between Met-enkephalin content in hepatic parenchyma, and blood serum lev-

els of haptoglobin and ceruloplasmin in patients of group II; in these patients, histopathological liver changes were qualified as stages III and IV according to Ludwig classification. It allows a conclusion that reduction of Met-enkephalin content in the liver parenchyma is analogous to the reduction of hepatic synthesis of acute phase proteins in the stage of extensive hepatic tissue necrosis. In group I, comprising patients with histological changes stage I and II according to Ludwig classification, no correlation was demonstrated between Met-enkephalin level in the liver tissue and blood serum levels of haptoglobin and ceruloplasmin. Similarly, no statistical correlation was revealed between Met-enkephalin level in the liver parenchyma, and levels of antitrypsin and acid glycoprotein both in group I and group II of PBC patients. In the discussed group of patients, no statistically significant correlation was found between Met-enkephalin blood plasma concentration and level of immunoglobulins class IgA, IgG and IgM. Likewise, no relationship was demonstrated between Met-enkephalin liver content, and levels of the above-mentioned immunoglobulins.

The presented work describes an attempt to evaluate the usefulness of measurement of blood plasma concentration of Met-enkephalin and its content in the liver parenchyma in patients with primary biliary liver cirrhosis. Although this study included only a small number of participants, it yielded encouraging results. The results obtained in this research, as well as experience of other authors who investigate this subject, rationalise acceptance of a hypothesis that measurement of blood plasma Met-enkephalin concentration may be useful in monitoring the course of primary biliary liver cirrhosis.

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