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# Research on the Mechanism of Dahuang Mudan Decoction in Treating IBD Based on Metabolomics

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**Abstract:** *Objective*: This study aims to systematically explore the mechanism of Dahuang Mudan Decoction in treating inflammatory bowel disease through metabolomic analysis, revealing its therapeutic effects and potential pathways in mice, and providing a scientific basis for clinical treatment. *Methods*: An acute colitis model in mice was established by administering dextran sodium sulfate (DSS) in drinking water continuously for 7 days. After successful modeling, the mice were randomly divided into an ulcerative colitis model group, a mesalazine group, and low-, medium-, and high-dose Dahuang Mudan Decoction groups. The intervention was administered via continuous gavage for 7 days. Body weight changes and colon length were recorded, and the disease activity index (DAI) and fecal occult blood status were monitored. Colon length was measured, and colon tissue was subjected to HE staining and pathological scoring to assess inflammatory damage. Intestinal tissue samples were collected for metabolomic analysis to screen for differential metabolites and perform pathway enrichment analysis. *Results*: The experimental results indicated that, compared with the model group, intervention with Dahuang Mudan Decoction significantly improved the colitis phenotype induced by DSS, as evidenced by alleviated weight loss, reduced DAI scores, decreased colon shortening, and mitigated histopathological damage, along with improved inflammatory cell infiltration and crypt structure destruction. Metabolomic analysis revealed a clear separation in metabolic profiles between the model and normal groups. *Conclusion*: Dahuang Mudan Decoction induced a holistic shift in the metabolic phenotype of the model mice, partially reverting/remodeling it toward the normal state.

**Keywords:** Dahuang Mudan decoction; Inflammatory bowel disease; Metabolomics; Metabolic pathway

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## 1. Introduction

Inflammatory bowel disease (IBD), primarily comprising ulcerative colitis and Crohn's disease, is a chronic, relapsing inflammatory disorder of the gastrointestinal tract with an unclear pathogenesis. Current pharmacological interventions, such as aminosalicylates and immunosuppressants, often have limited efficacy and are associated with significant adverse effects, highlighting the urgent need for safer and more effective therapeutic strategies. Dahuang Mudan Decoction (DMD), a classic traditional Chinese medicine formula, has been historically used to treat intestinal ailments and shows promise in managing inflammation. However, the specific mechanisms by which it exerts its therapeutic effects on IBD remain to be fully elucidated. This study aims to investigate the therapeutic efficacy of DMD in a dextran sodium sulfate (DSS)-induced acute colitis mouse model and to explore its underlying mechanisms through a comprehensive metabolomic approach. By analyzing the alterations in the metabolic profile and identifying key differential metabolites and pathways, we seek to provide a scientific foundation for the clinical application of DMD in IBD treatment.

## 2. Research background

Inflammatory Bowel Disease (IBD) is a prevalent chronic intestinal disorder. The pathogenesis of IBD is complex, involving multiple aspects such as the immune system, gut microbiota, and genetic factors. Currently, the treatment of IBD primarily relies on medication; however, medications often come with significant side effects and suboptimal therapeutic outcomes.

Traditional Chinese medicine (TCM) offers unique advantages in treating IBD. Dahuang Mudan Decoction, a traditional TCM formula composed of rhubarb, moutan cortex, mirabilite, peach kernel, and waxgourd seed, possesses functions such as clearing heat and detoxifying, cooling blood and relieving pain, and promoting blood circulation to remove blood stasis. In recent years, an increasing number of studies have demonstrated the favorable therapeutic effects of Dahuang Mudan Decoction in treating IBD.

## 3. Research significance

### 3.1. Exploring the mechanism of Dahuang Mudan decoction in treating IBD

Currently, the mechanism by which Dahuang Mudan Decoction treats IBD remains unclear. Through metabolomic research, the mechanism of action of Dahuang Mudan Decoction in treating IBD can be elucidated, providing a scientific basis for its clinical application.

### 3.2. Identifying new therapeutic targets

Metabolomics can reveal the active components and targets of action within Dahuang Mudan Decoction. These new targets can be utilized to develop novel therapeutic drugs, enhancing treatment efficacy and reducing medication side effects.

### 3.3. Promoting the integration of traditional Chinese and Western medicine

TCM offers unique advantages in treating IBD; however, its complex mechanism of action makes it challenging to integrate with Western medicine. Through metabolomic research, a deeper understanding of the mechanism of action of Dahuang Mudan Decoction can be achieved, facilitating the integration of traditional Chinese and Western medicine and improving treatment outcomes.

## 4. Overview of domestic and international research

Studies both domestically and internationally have demonstrated that Dahuang Mudan Decoction possesses various pharmacological effects, including anti-inflammatory, antioxidant, and immune-regulatory properties, exerting its therapeutic effects through multiple pathways. However, the precise mechanism of action of Dahuang Mudan Decoction remains incompletely understood, necessitating further in-depth research.

Inflammatory bowel disease (IBD) is a chronic inflammatory condition caused by factors such as genetics, environment, dysregulation of mucosal immune responses, and damage to the intestinal mucosal barrier. It encompasses Crohn's disease (CD) and ulcerative colitis (UC). Clinically, CD can affect any part of the intestine, whereas UC is confined to the mucosal layer of the colon or rectum [1]. Although the pathogenesis of IBD is not fully elucidated, epidemiological and experimental data indicate a close association between gut microbiota and the onset and progression of IBD. For instance, under sterile conditions, IBD does not develop or its incidence significantly decreases. Furthermore, compared to healthy individuals, the diversity, composition, or abundance of gut microbiota in IBD patients is altered [2,3]. Trillions of microorganisms in the gut maintain a symbiotic or mutualistic relationship with the host. In a healthy state, gut microbiota can sustain an anti-infective and anti-inflammatory state and play a crucial role in maintaining immune and intestinal homeostasis through specific host-microbe interactions [4,5]. Multiple studies have shown that the balance between pro-inflammatory and anti-inflammatory cytokines in mucosal immune cells is critical for the development of IBD, as they not only regulate the epithelial barrier but also control the interaction between the immune system and the microbiota [6].

Ulcerative colitis (UC) is characterized by clinical symptoms such as abdominal pain, diarrhea, and mucopurulent bloody stools [7]. Currently, the incidence of IBD is on the rise globally, with a prevalence rate of 10–20% in Western countries and even higher in developing countries or regions [8,9]. Western medicine primarily treats IBD with drugs such as 5-aminosalicylic acid (sulfasalazine) and immunosuppressants, but these medications are associated with side effects such as drug resistance and intestinal dysbiosis, severely affecting patients' quality of life [10]. Therefore, exploring the pathological mechanisms of IBD and identifying new therapeutic targets and drugs are of great significance in addressing the current challenges in IBD prevention and treatment [11].

UC is currently believed to be an intestinal immune abnormality influenced by environmental factors and gut microbiota on the basis of genetic susceptibility. Commonly used clinical drugs include aminosalicylic acid, hormones, and immunosuppressants, but issues such as drug resistance, drug dependence, and adverse reactions limit their clinical use [12]. UC lesions can extend from the rectum proximally to the entire colon, with clinical pathological features such as colonic mucosal erosion or ulcers. Patients may experience symptoms such as abdominal pain, diarrhea, and mucopurulent bloody stools, with a disease course characterized by alternating episodes of exacerbation, remission, and recurrence, making treatment challenging and potentially leading to complications such as colorectal cancer [13,14]. The specific etiology of UC remains unclear, and its mechanism is still not fully understood. Clinical treatment presents challenges due to the alternating episodes of exacerbation, remission, and recurrence, making UC one of the difficult-to-treat conditions [15]. Currently, Western medicine primarily uses mesalazine to treat UC patients, which can effectively suppress intestinal mucosal inflammation and alleviate related clinical symptoms. However, long-term use can lead to numerous adverse reactions and even induce hepatitis [16]. Traditional Chinese medicine has demonstrated certain advantages in controlling UC progression and alleviating symptoms. Its multi-target and multi-pathway pharmacological effects provide a beneficial approach for the clinical treatment of UC [17]. Dahuang Mudan Decoction, originating from "Jin Gui Yao Lue", has the effects of clearing heat, removing dampness, and dispersing masses and reducing swelling. Animal experimental studies have confirmed that Dahuang Mudan Decoction can effectively suppress the inflammatory state in UC model animals [18,19].

## 4.1. Body weight changes

The results of body weight changes in each group of mice are shown in **Figure 1**. The body weight of the normal group increased steadily, while the body weight gain in each model group was slower compared to the normal group. After modeling, the body weight in the model group decreased, whereas the body weight in the high-, medium-, and low-dose groups of Dahuang Mudan Decoction, as well as the positive control group, increased more gradually compared to the model group.

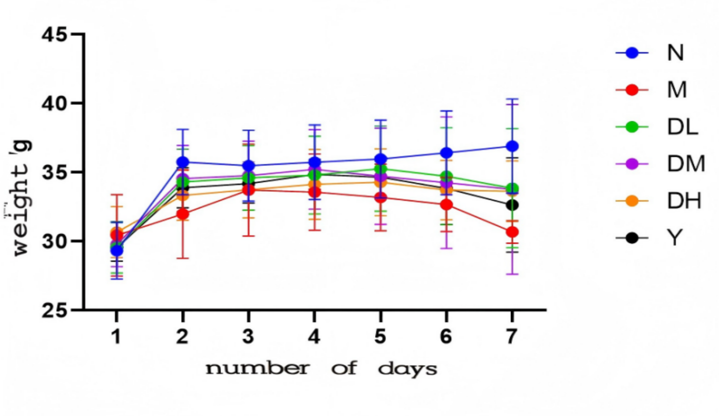


**Figure 1.** Changes in body weight of mice.

## 4.2. Pathological changes in colonic mucosa

HE staining revealed that after modeling, the crypts and mucosal layer of the colon were damaged, accompanied by significant infiltration of inflammatory cells (**Figure 2**). Following treatment with different doses of Dahuang Mudan Decoction, the damage to the crypts and mucosal layer was ameliorated, and the infiltration of inflammatory cells in the colonic tissue was reduced. AB-PAS staining results showed a decreased content of goblet cells in the colonic tissue of Group M, which was significantly restored after treatment with different doses of Dahuang Mudan Decoction, indicating an improvement in colonic injury. Based on the aforementioned results, it is evident that Dahuang Mudan Decoction exhibits a favorable therapeutic effect on ulcerative colitis, prompting further analysis of its underlying mechanism of action.

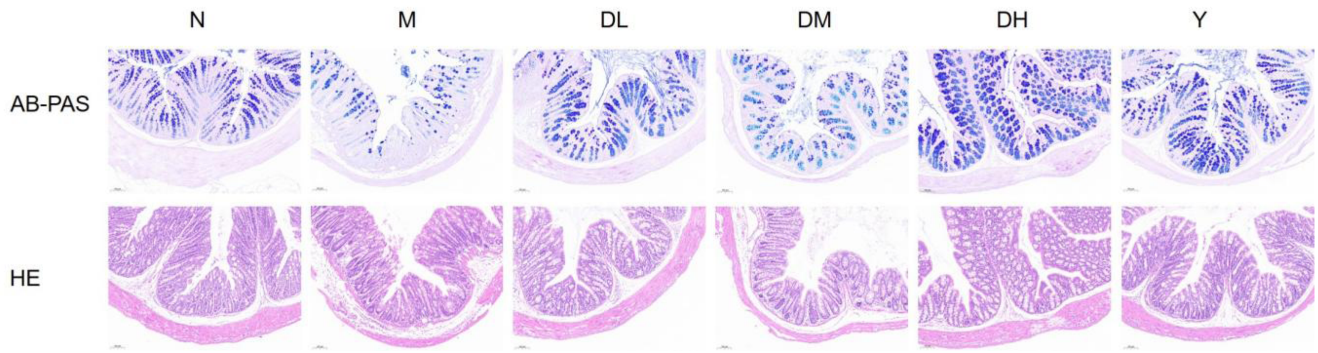


**Figure 2.** HE and AB-PAS staining of goblet cells at 100x magnification.

## 5. Metabolomics analysis

### 5.1. PCA analysis (Group N, Group M, Group DM)

PCA analysis, or principal component analysis, is employed to assess the similarity of sample species composition, with closer distances on the PCA plot indicating greater similarity. **Figure 3** illustrates the distribution and dispersion of samples along the PC1 axis. The model group is positioned far from the blank group, indicating that the intestinal metabolite composition of mice with ulcerative colitis differs from that of normal mice. In comparison to the model group, the Dahuang Mudan Decoction group is closer to the blank control group, suggesting that Dahuang Mudan Decoction can modulate the changes in intestinal metabolites in mice with ulcerative colitis.

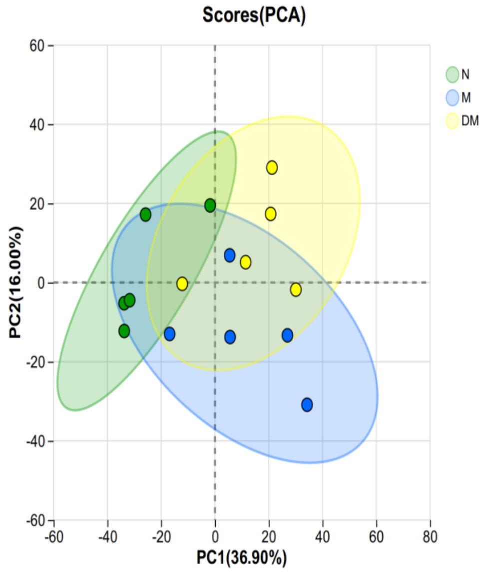


**Figure 3.** PCA analysis.

### 5.2. Venn analysis

The Venn diagram for the metabolomic analysis of mouse intestinal tissue is shown in **Figure 4**. Group N has 1,125 metabolites, Group M has 1,174 metabolites, and Group DM has 1,144 metabolites. There are 715 metabolites shared among all three groups, while Group N, Group M, and Group DM have 16, 21, and 6 unique metabolites, respectively.

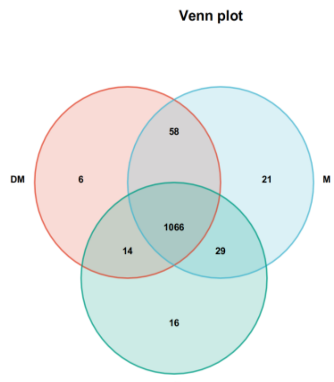


**Figure 4.** Venn diagram.

### 5.3. PLS-DA analysis

The PLS-DA results are depicted in the figure: there is a significant separation between Group N, Group M, and Group DM, indicating substantial differences among the groups. The aforementioned results demonstrate that the metabolite profiles of the three sample groups exhibit distinct differences (**Figure 5**).

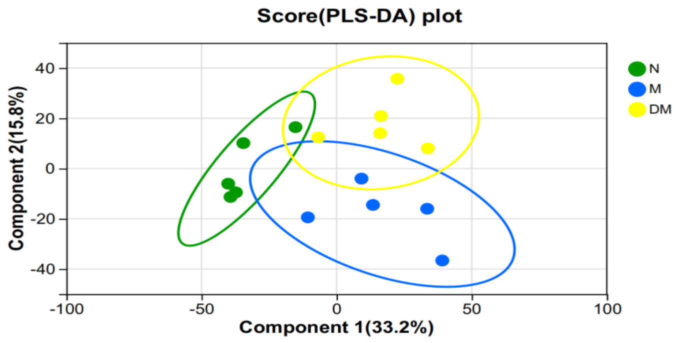


**Figure 5.** PLS-DA analysis.

## 6. Comparative analysis of differential metabolites

### 6.1. Comparative analysis between two groups

The differential metabolites between each group are shown in the figure: Compared to Group N, Group M has 240 differential metabolites, with 165 upregulated and 75 downregulated; compared to Group M, Group DM has a total of 67 differential metabolites, with 8 upregulated and 59 downregulated (**Figure 6**,**7**,**8**).

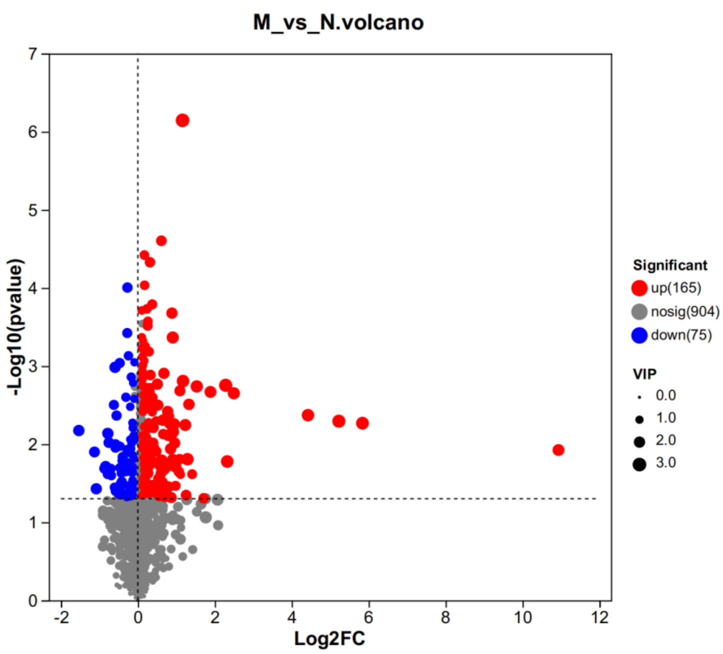


**Figure 6.** Volcano plot of differentially expressed genes between Group M and Group N.

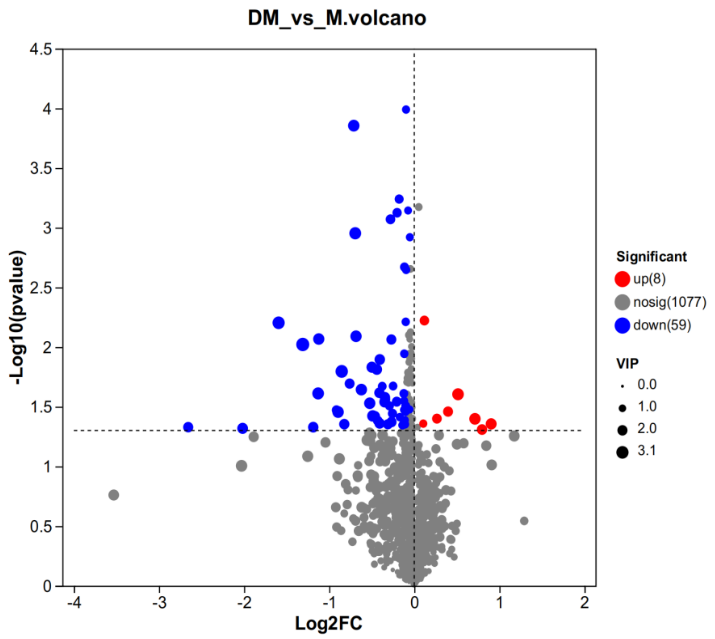


**Figure 7.** Volcano plot of differentially expressed genes between Group DM and Group M.

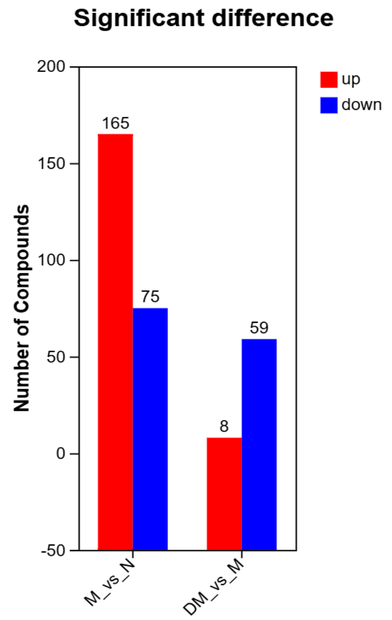


**Figure 8.** Graph of changes in the number of differential metabolites.

## 6.2. KEGG enrichment pathways

Enrichment analysis of differential metabolites between groups was conducted using the KEGG Pathway database. The main pathways are illustrated in **Figures 9** and **10**: Compared to Group N, the enrichment pathways of differential metabolites in Group M exhibit significant differences. The top 20 pathways, ranked from smallest to largest *p*-value, primarily include nucleotide metabolism, lysine degradation, autophagy in animals, etc. Compared to Group M, Group DM shows significant differences in the enrichment pathways of differential metabolites, with a total of 20 pathways, mainly including neural signaling pathways, PD-L1 expression and PD-L1 checkpoint pathway in cancer, glioma, T-cell signaling pathways, etc.

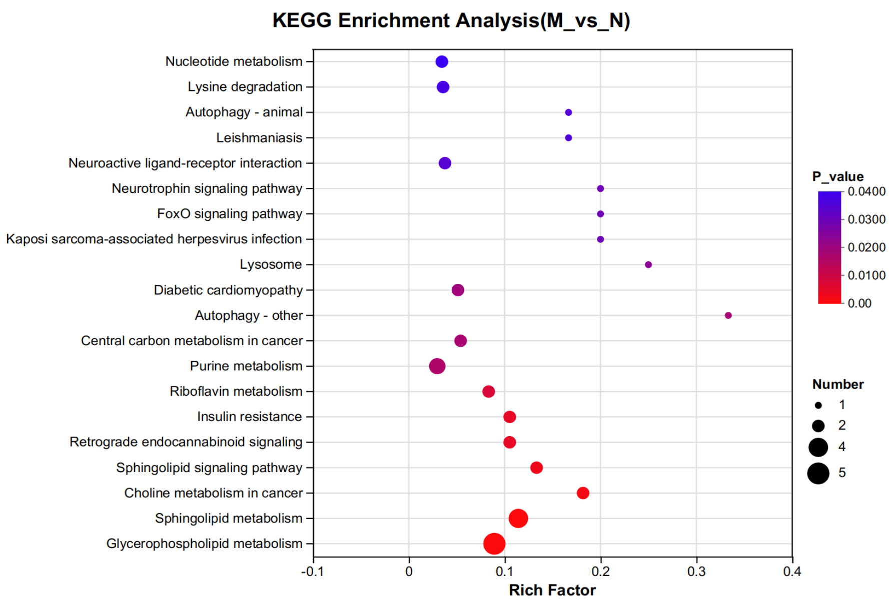


**Figure 9.** KEGG enrichment pathways of differential metabolites between Group M and Group N.

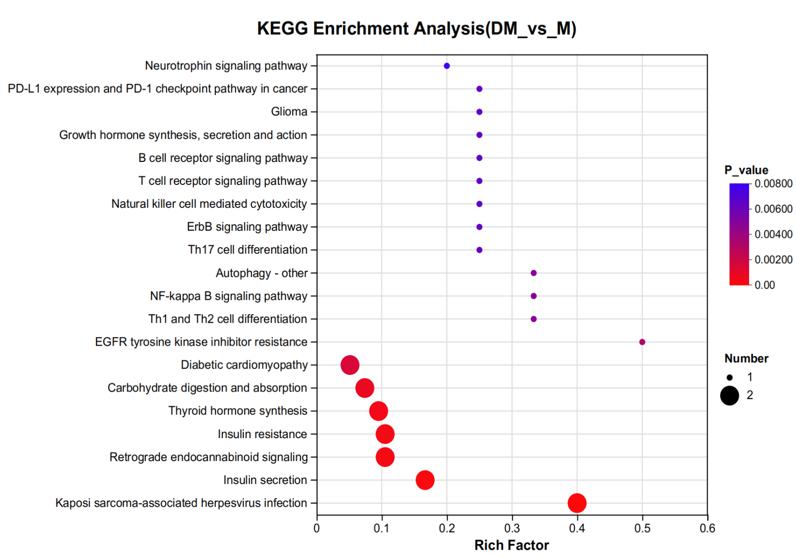


**Figure 10.** KEGG enrichment pathways of differential metabolites between group DM and Group M.

## 7. Conclusion

Dahuang Mudan Decoction can alleviate clinical symptoms such as weight loss, diarrhea, and bloody stool in IBD mice, and improve damage to goblet cells in the intestinal mucosa. It can also alleviate intestinal inflammation by regulating gut microbiota dysbiosis. This study provides experimental and theoretical evidence for the application of Dahuang Mudan Decoction in the treatment of IBD, promoting the in-depth application of traditional Chinese medicine in modern medicine.

Metabolomics methods can offer new perspectives and approaches for studying the mechanisms of Dahuang Mudan Decoction. In the future, further in-depth research is needed to explore the mechanisms of action of Dahuang Mudan Decoction, with the aim of providing more effective drugs and treatment plans for IBD.

## Disclosure statement

The authors declare no conflict of interest.

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