Pharmacological studies of dopamine transporter imaging agent $^{125/131}$I-β-CIT

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Abstract To prepare $^{125/131}$I-β-CIT (2β-carbomethoxy-3β-(4-iodophenyl)tropane) as an imaging agent for dopamine transporter (DAT), the labeling method from tributylstannyl precursor with peracetic acid has been reported in this article. The radiochemical purity (RCP) of the labeled compound was over 95% determined by HPLC and TLC. The stability, partition coefficients were also determined. The pharmacological studies of the imaging agent were performed in rats, mice, rabbits and normal monkey. The ligand showed preferable uptake in brain (1.9%ID/organ in rats and 4.5%ID/organ in mice at 5 min). The ratios of striatum/cerebellum, hippocampus/cerebellum and cortex/cerebellum were 28.9, 3.97 and 4.75 at 6 h in rats, and 8.52, 2.99 and 3.06 at 6 h in mice, respectively. In monkey brain imaging the ratios of striatum/frontal cortex (ST/FC) and striatum/occipital cortex (ST/OC) were 5.14 and 5.97 at 4 h, respectively. All of above showed the high affinity of the ligand to DAT. The compound was primarily metabolized in liver because the hepatic uptake was much higher than other organs (75.4%ID/organ at 18 h). The half-life of blood elimination was 5 min. The dose received by mice was 2500 times as high as that received by human in the test of undue toxicity, which evaluated the safety of the agent. All the results suggest that β-CIT can be used as a potential DAT imaging agent.

Keywords $^{125/131}$I-β-CIT, Labeling, Dopamine Transporter, Biodistribution, Parkinson's disease

CLC numbers R742.5, R817, R962, O628.5^{+}1 A

1 INTRODUCTION

Parkinson's diseases are frequently observed in old people. The postmortem studies of the patients demonstrated the degeneration of dopaminergic neurons in the substantia nigra, which leads to a reduction of neuronal projections to the striatum and consequently a significant reduction of the dopamine transporters (DAT) which was presynaptically located in striatum.[1] In the patients with Alzheimer's diseases, the similar

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phenomenon can also be observed.[1] Thus, the decrease of the DAT may indicate the pathological changes of the dopamine neurons, and positron emission tomography (PET) imaging and single photon emission computed tomography(SPECT) imaging using tracers of DAT can be valuable tools for early stage diagnosis of the diseases mentioned above. 2β-carbomethoxy-3β-(4-iodophenyl)tropane(β-CIT) bound with high affinity to DAT, therefore, is a marker of the neurons that degenerate in Parkinson's disease and shows promise as a clinical useful agent to diagnose and evaluate patients with idiopathic Parkinson's disease.[2~4] β-CIT can be labeled with ^{123}I for SPECT[2,3] and ^{11}C for PET[5] in clinical application. The purpose of this article was to evaluate the pharmacological character of our self-made β-CIT[6] labeled with $^{125/131}$I through the animal studies.

2 MATERIALS AND METHODS

2.1 Instruments

Varian SY 5000 HPLC (USA), PACKARD COBRA automatic gamma counter (USA), Bio-Rad GS-250 Molecular Imager (USA), AO America Optical Hislo Stat cryostat microtome (USA), Picker AXIS DHTC SPECT (USA)

2.2 Reagents

The tributylstannyl precursor of β-CIT was synthesized by our group.[6] Na^{131}I was purchased from China Institute of Atomic Energy, and Na^{125}I was the product from DuPont NEN.

2.3 Animals

Rats (Sprague-Dawley) and mice (NIH) were provided by the Center of Experimental Animals of our laboratory. Rabbits (New Zealand) were from the Center of Experimental Animals of Suzhou University.

2.4 Preparation of $^{125/131}$I-β-CIT

To the ethanol solution of β-CIT precursor (20 μg/20μL), 100 μL ammonium acetate buffer(pH4.0, 0.1 mol/L), a sufficient quantity of Na*I and 100μL freshly prepared 3.2% peracetic acid solution were added. After being shaken up, the mixture was allowed to stand for 15~30 min. Then the reaction was ceased by 100μL aqueous solution of $Na_2S_2O_5$(0.3 g/mL), and 0.7 mL saturated $NaHCO_3$ solution was added to adjust the pH of the solution to 5~7. In the purification, the labeled compound was extracted from aqueous reaction medium with ethyl acetate (1 mL×2) and passed through a small column of Na_2SO_4, then the solvent was removed under nitrogen flow, and the residue

was dissolved in water.

2.5 Determination of labeling yield and radiochemical purity (RCP)

The labeling yield and RCP were determined by HPLC and TLC.

HPLC : C18 reverse phase column(150×4 mm) with MeOH/H_2O/Et_3N(70/10/0.1) as mobile phase and at a flow rate of 1.0 mL/min.

TLC: Silica gel sheets eluted with EtOH/$CHCl_3$/NH_4OH (1/9/0.2).

2.6 Determination of partition coefficient

The labeled compound (10μL)was added to the two-phase system of 3.0 mL n-octanol and 3.0 mL 0.1mol/L phosphate buffer(PB). Two kinds of PB (pH 7.4 and pH 7.0) were applied respectively. After the mixture was vortexed for 3×1 min and centrifuged for 5 min at 4,000r/min, 1.0 mL n-octanol and 1.0 mL PB were taken out from each pH and counted for the radioactivity respectively. Then the 1.0 mL n-octanol was transferred to another tube containing 3.0 mL PB and 2.0 mL n-octanol, and the procedure above was repeated for 5 times. The partition coefficient can be calculated as the ratio of the cpm/mL in organic phase to that in PB.

2.7 Determination of stability

The labeled compound (111 MBq/mL for ^{131}I-β-CIT, 1.85 MBq/mL for ^{125}I-β-CIT) was allowed to stand at 4–8°C, and the RCP was determined with HPLC every day.

2.8 Biodistribution in rats and the inhibition of β-CIT

Seven groups of rats(180~220 g), four per group, were injected ^{131}I-β-CIT (0.2 mL, 3.7 MBq) each via the tail veins, and were sacrificed by cervical dislocation at different time intervals postinjection. The organs of interest were removed, weighed and counted for the radioactivity, and then by comparing the sample counts with the counts of the diluted initial injected dose, we calculated the percent of injected dose (%ID) per gram and per organ. For regional distribution in brain, samples from different brain regions (cortex [CX], striatum [ST], hippocampus [HP] and cerebellum [CB]) were separated, weighed and counted, and the %ID per gram was obtained as described above. Another four rats were injected intravenously unlabeled β-CIT (5 mg/kg) 5 minutes prior to the injection of ^{131}I-β-CIT and sacrificed 2hr later, then the similar brain regional distribution as above was determined to evaluate the in vivo competitive binding of unlabeled β-CIT.

2.9 Biodistribution in mice

Five groups of mice (18-20g), five per group, and ^{131}I-β-CIT (0.2 mL, 1.85 MBq) were injected into the tail vein of each. The mice were sacrificed at different time intervals postinjection, with the organs of interest removed, weighed and counted for radioactivity

to calculate the %ID/gram.

2.10 Kinetics of blood-drug clearance in rabbits

Four rabbits were injected ^{131}I-β-CIT (0.4 mL, 37 MBq) each via the ear-edge vein. Then a series of blood samples (20μL) were obtained from the other ear at different time intervals postinjection, and were counted for radioactivity, expressed as percent of the injected radioactivity per milliliter of blood. Then a blood time-radioactivity curve was plotted.

2.11 In vitro autoradiography in rat brains

Two normal rats, each was injected intravenously ^{125}I-β-CIT (0.2 mL, 7 MBq), and was sacrificed under anesthesia (diethyl ether) by cervical dislocation 2 hr later. The whole brains were rapidly removed, placed in OCT embedding medium, and frozen at -15°C. After reaching equilibrium at that temperature, cut the coronal sections (20 μm) consecutively with a cryostat microtome. Then the sections were mounted on gelatin-coated microscope slides, and air-dried at room temperature. The slides containing the brain sections were exposed to GS-250 Phosphor Imaging Screen-BI for 2 h and imaged in GS-250 molecular imager, and the OD (optical densities) ratios of striatum/parietal cortex (ST/PC) were calculated.

2.12 Imaging in monkey

Prior to imaging, the rhesus monkey (10 kg) was anesthetized with 0.1 g ketamine and 5 mg diazepam (administered intramuscularly), and then additional 0.05 g ketamine was given every 0.5 h. $KClO_4$ 100 mg was poured into the stomach for blocking the choroid pluxus and thyroid, and 60 min later, 148 MBq ^{131}I-β-CIT was injected into the elbow vein of the monkey. At 90 min a nd 240 min postinjection, brain SPECT imaging was performed and the ratios of ST/FC and ST/OC (occipital cortex) were calculated.

2.13 Undue toxicity test

According to the regulations of Pharmacopoeia of China(1995), the undue toxicity was determined by observing the death and survival of five mice(18-20 g) within a period of time after receiving an injection of (0.2 mL, 10μg) unlabeled β-CIT.

3 RESULTS AND DISCUSSIONS

3.1 Labeling yield and radiochemical purity

The HPLC retention time of $(t_R)^{125/131}$I-β-CIT and $^{125/131}$I$^-$ were 4.6 min and 1.4 min, respectively. The TLC Rf values of both were 0.8~0.9 and 0.0~0.1, respectively. The t_R and R_f value of unlabeled β-CIT were the same as the labeled compound. RCP of $^{125/131}$I-β-CIT was greater than 95%, and Labeling yield was greater than 90%.

The standing time in the process of labeling β-CIT with peracetic acid was concerned with the volume of NaI' solution added, the larger the volume, the longer the time

3.2 Stability

The radiochemical purity of ^{131}I-β-CIT in aqueous solution was greater than 90% after standing at 4~8°C for three days, and that of ^{125}I-β-CIT was over 90% after standing at 4~8°C for 30 days.

3.3 Partition coefficient

The partition coefficients were 8.10 and 21.0 at pH7.0 and pH7.4, respectively. The drug was proved to be more lipophilic at pH7.4, which contributes to entering into the brain.

3.4 Biodistribution in rats and mice

As is shown in Table 1, the uptake and retention of ^{131}I-β-CIT in rats was satisfying in brain. The %ID/g was 1.9, 1.4 and 1.3 at 5 min, 1 h and 2 h postinjection, respectively, and striatum uptake was high within 2~6 h. In lung, the uptake was high in the beginning but the clearance was also rapid. The radioactivity was constantly accumulated in liver, to 75.4%ID/organ at 18 hr postinjection, which suggests that the major radioactivity was excreted by the hepatobiliary system.

Table 1 Biodistribution of ^{131}I-β-CIT in rats %ID/organ (n=4)

Organ	5 min	1 h	2 h	6 h	18 h	24 h
Heart	0.73±0.06	0.24±0.03	0.12±0.02	0.05±0.01	0.03±0.00	0.03±0.00
Liver	10.8±1.2	36.9±3.7	46.7±3.3	71.9±11.3	75.4±7.7	74.0±8.4
Spleen	1.10±0.17	0.68±0.03	0.42±0.04	0.13±0.01	0.04±0.01	0.06±0.01
Lung	11.9±3.4	3.8±1.3	2.3±0.6	0.8±0.2	0.2±0.1	0.3±0.1
Kidneys	8.6±1.4	9.5±2.4	9.8±2.9	6.6±2.5	3.9±2.2	3.8±2.3
Stomach	0.44±0.18	0.42±0.12	0.3±0.07	0.19±0.01	0.08±0.01	0.07±0.02
Thyroid	0.10±0.02	0.20±0.01	0.35±0.04	0.81±0.44	1.14±0.98	1.10±0.88
Blood	4.4±0.5	2.0±0.2	1.3±0.1	0.8±0.1	0.5±0.0	0.5±0.0
Brain	1.9±0.3	1.4±0.2	1.3±0.1	0.5±0.0	0.1±0.0	0.1±0.0
Muscle	25.6±2.1	14.7±1.1	12.0±2.2	4.4±1.1	2.3±0.3	1.9±0.9
Bone	4.4±1.0	2.0±0.4	1.3±0.2	0.8±0.2	0.5±0.2	0.3±0.1

The result of regional brain distribution in rats is shown in Fig.1. The ratio of striatum and cerebella (ST/CB) reached peak (28.9) at 6 h (Fig.2). In comparison with the distribution in rats, the result of distribution in mice can be seen in Fig.3, which shows the highest uptake in striatum (32.7±2.99%ID/g) at 4 h. The ratio of ST/CB was 9.82 at 4 h and 8.52 at 6 h (Fig.4), and the uptake in whole brain was 4.90±0.55%ID/organ at 1 h (Table 2). The liver uptake in mice was 26.8 at 2 h, which was apparently lower than in rats. The difference may be explained by the absence of gallbladder in rats, which resulted in the concentration of radioactivity in liver.

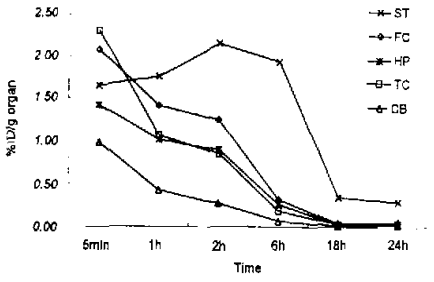

Fig.1 Biodistribution of ^{131}I-β-CIT in Rats' brains

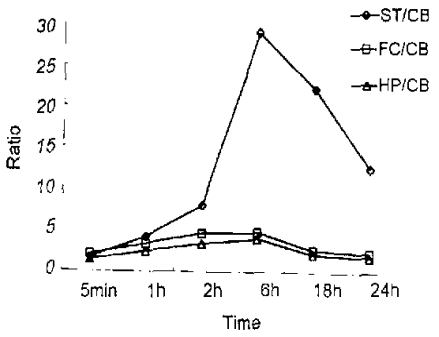

Fig.2 Radioactivity ratios of brain tissues in different regions to cerebellum at different time in mice

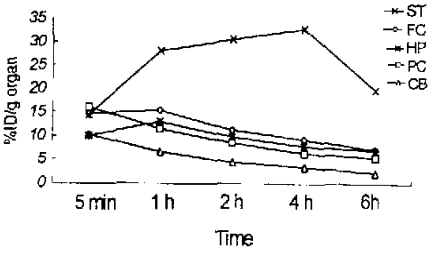

Fig.3 Biodistribution of ^{131}I-β-CIT in Rats' brains

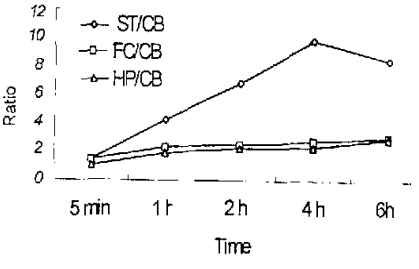

Fig.4 Radioactivity ratios of brain tissues in different regions to cerebellum at different time in mice

Table 2 Biodistribution of ^{131}I-β-CIT in mice %ID/organ (n=5)

Organ	5 min	1 h	2 h	4 h
Heart	1.14±0.087	0.54±0.05	0.40±0.02	0.34±0.03
Liver	17.9±3.55	29.0±3.24	26.8±2.06	25.0±2.72
Spleen	1.21±0.43	0.92±0.096	0.73±0.16	0.69±0.14
Lung	6.96±1.21	2.04±0.14	1.65±0.33	1.44±0.15
Kidneys	5.48±0.85	2.56±0.33	2.3±0.30	1.88±0.11
Thyroid	0.171±0.038	0.13±0.011	0.15±0.02	0.16±0.02
Blood	7.836±1.148	4.88±0.69	3.89±0.50	3.99±0.35
Brain	4.50±0.651	4.90±0.55	4.13±0.26	3.36±0.29
Muscle	38.81±8.85	18.4±1.24	15.0±2.41	14.4±1.21
Bone	7.12±0.95	5.44±0.48	4.46±0.88	4.40±1.71

The ratio of ST/CB in rats which were pretreated with β-CIT was apparently lower than the ratio of those were not, and so was the ratio of FC/CB, which indicate the inhibition of β-CIT to striatum and frontal cortex. But the difference in hippocampus wasn't distinct (Fig.5). The phenomenon may result from three facts: the high content of

5-hydroxytryptamin transporter(5-HTT) in FC, the high affinity of β-CIT to 5-HTT, and the high dose of the β-CIT applied. We have repeated the distribution experiments in rats several times, and obtained the data with good recurrence. The difference between our results (in rats) and the results reported in other literature[7~9] may be explained by the different animals and different specific radioactivity of the tracer applied in experiments.

3.5 Kinetics of blood clearance

The blood time-activity curve is shown in Fig.6, in which the half-life of blood elimination of radioactivity was about 5 min. The result shows that the blood clearance of ^{131}I-β-CIT was rapid, and the ascent of the radioactivity during 15-30 min may be concerned with the metabolism of ^{131}I-β-CIT in body.

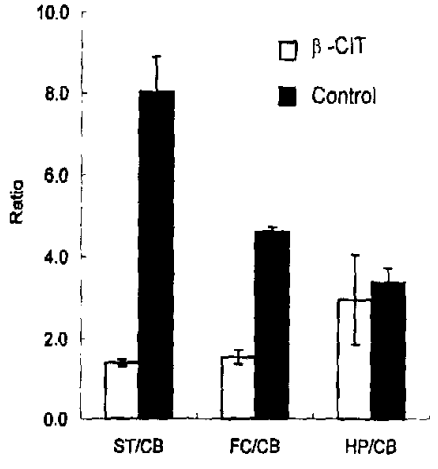

Fig.5 The comparison of the ratios of ST/CB, FC/CB and HP/CB in control and β-CIT pretreated rats

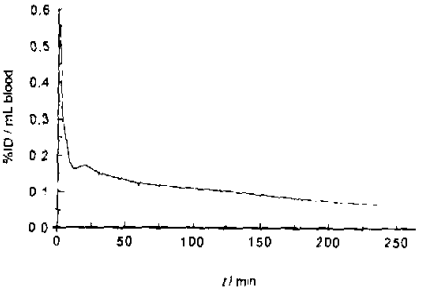

Fig.6 The blood time-radioactivity curve of ^{131}I-β-CIT in rabbits

3.6 In vitro autoradiography in rat brains

As can be seen in Fig.7, the radioactivity of striatum was apparently higher than that of cortex in normal rats. The ratios of ST/PC (parietal cortex) in both left and right side were 5.1.

3.7 Imaging in monkey

Fig.8 is the ^{131}I-β-CIT SPECT image of monkey brain, in which shows the uptake and retention in striatum, and also indicates the concentration of DAT in striatum. Although the uptake of the frontal cortex was quite high at 90 min, but the clearance was rapid as well, for its uptake approached the level of occipital cortex (OC) at 240 min. The ratios of ST/FC and ST/OC were 5.14 and 5.97 at 240 min, respectively, which

shows the apparently higher uptake of striatum than that of cortex and cerebellum at this time.

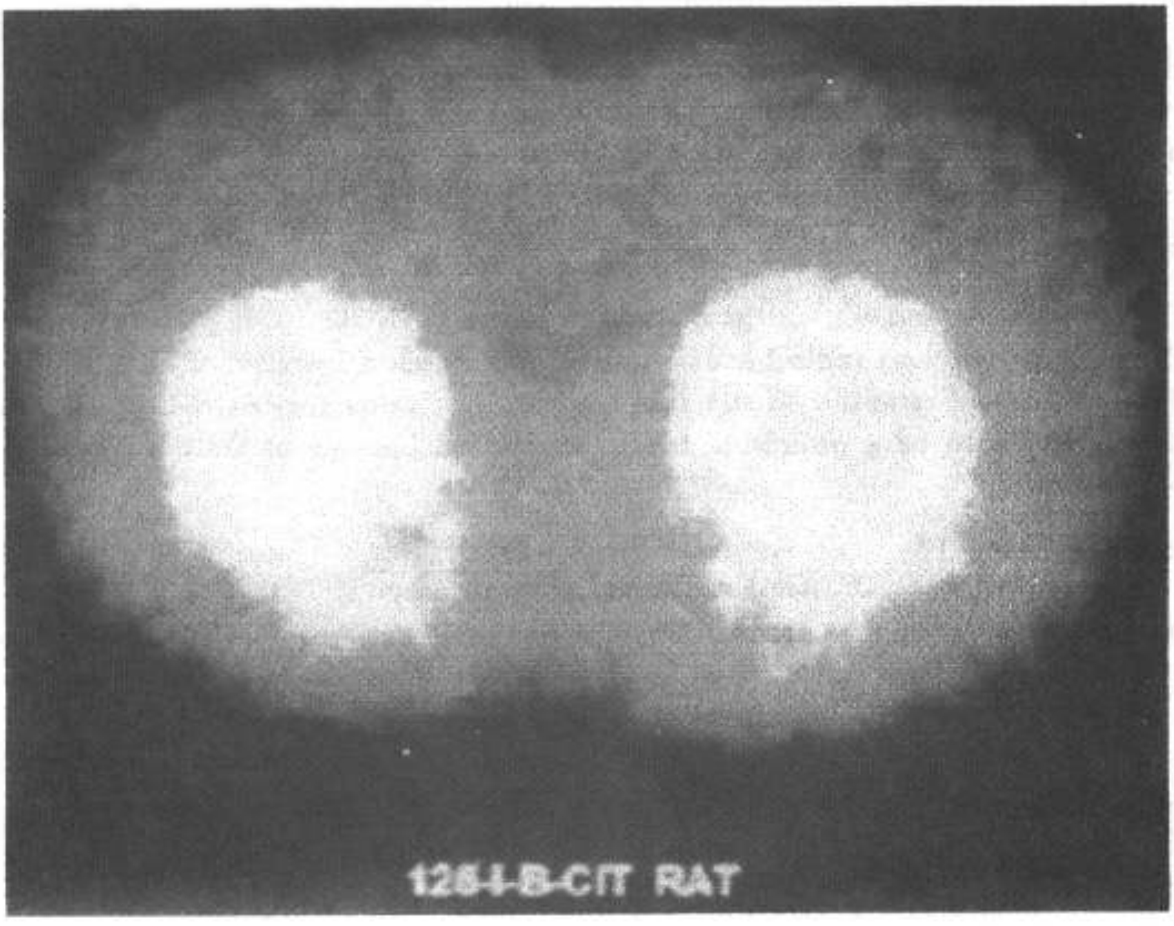

Fig.7 Autoradiography of ^{125}I-β-CIT in rat's brain

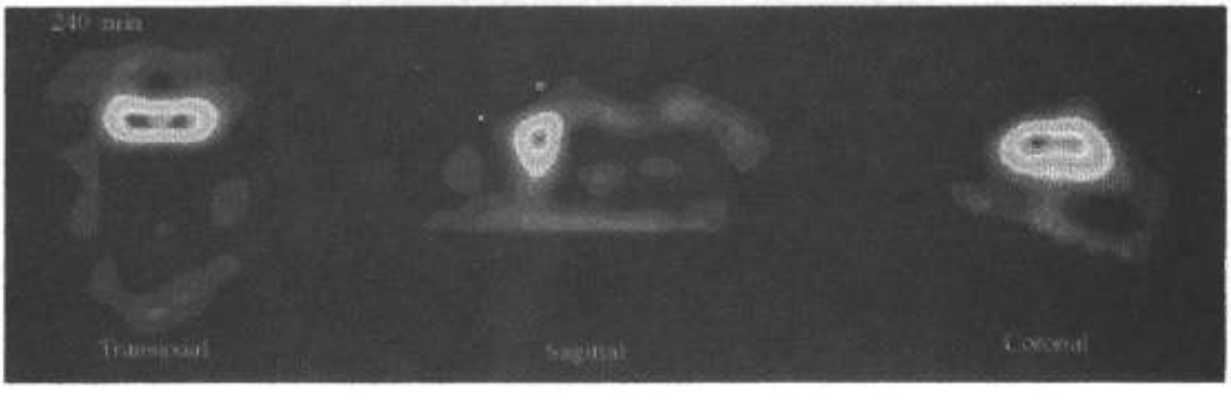

Fig.8 SPECT imaging of ^{131}I-β-CIT in monkey brain

3.8 Test of undue toxicity

After being injected with the regular dose on human (if assuming a weight of 50 kg), and raised regularly for 48 h, none of the mice died, and no abnormality was observed in all central organs after dissection. The dose per kilogram administered to the mice was 2500 times greater than that received by human.

4 CONCLUSION

It is a simple and convenient method to obtain ^{125}I-β-CIT using peracetic acid, with high labeling yield and radiochemical purity, and stability in vitro. β-CIT displays excellent uptake and retention in striatum comparing to other regions of brain in animals, which proves it to be a promising tracer for clinical imaging of DAT with safety and validity.

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