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Mercury distribution in the mouse brain after mercury vapour exposure

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Summary. Female SJL/N mice were exposed to mercury vapour 5 days/week for 10 weeks, at a mercury concentration of approximately 0.5 mg/m^3, 19 h/day; 1 mg/m^3, 3 h/day; 0.3 mg/m^3, 6 h/day or 1 mg/m^3, 1.5 h/day. The total mercury concentrations in the brain were 6.4, 6.3, 1.6 and 0.64 μg/g tissue, respectively. The mercury distribution in the brains was examined. Mercury was found in almost the whole brain in the two groups with the highest exposure. In the third group, mercury was primarily found in the neocortical layer V, the white matter, thalamus, and the brain-stem. In the fourth group, the white matter and the brain-stem were the targets for mercury accumulation. Similarities and differences between rats and mice in the distribution pattern are discussed.

Keywords: mercury vapour, SJL/N mouse, mercury distribution, brain

The distribution of mercury in the rat brain after administration of different mercury compounds has been extensively studied by Möller-Madsen (1990, 1991) and by Möller-Madsen and Danscher (1986, 1991). Recently, two papers dealing with mercury distribution in the rat brain after mercury vapour exposure have been published (Möller-Madsen, 1992; Warfvinge *et al.* 1992). The investigation by Möller-Madsen concerned Wistar rats and 7 different exposure levels (range 50 μg/m^3, 4 h–550 μg/m^3, 24 h). We used BN rats, which are immunologically hyperreactive to mercury and prone to develop an autoimmune disease upon mercury exposure (Hua *et al.* 1993). The rats were long-term exposed at 1 mg/m^3 air (Warfvinge *et al.* 1992). The distribution patterns in the two reports corresponded well, although they comprised different strains of animals and exposure levels. However, an interesting discrepancy between the two studies was that after short-term exposure mercury was detected in neocortical layer III and after long-term exposure mainly in layer V. Does the slightly different distribution reflect strain specificity, which might give us a clue to whether mercury induced autoimmune defect animals have different mercury induced toxic injury of the CNS? Or does it simply reflect the choice of method for the visualization of mercury, which might give us more information concerning the pool(s) of mercury that is (are) visualized? The methods used claim to visualize only inorganic mercury, but it is uncertain whether all inorganic mercury is visualized. In the latter case, the fact that the two studies differed in exposure duration might be important for what mercury molecule is present for visualization.

The present study is part of a project at our laboratory to investigate the effects of mercury vapour exposure on the developing and mature central nervous system and the immune system of squirrel monkeys and mercury sensitive rats and mice. The present study demonstrates the mercury distribution in the brain of a mercury sensitive mouse strain after mercury vapour exposure.

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Material and methods

Inbred female SJL/N mice, 7–8 weeks of age at the

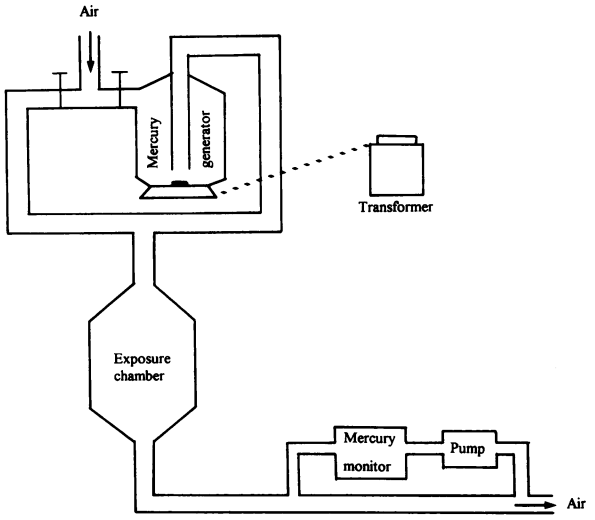

Figure 1. The schematic drawing demonstrates the exposure chamber and the mercury vapour generation.
→, Air direction; ⊣, valve.

beginning of the experiments, were used throughout the study. They were obtained from BomMice Breeding and Research Centre (Ry, Denmark).

Experimental procedure

Two plastic cages with mice were enclosed beside each other in the middle of a chamber with stainless steel and glass walls of Rochester type. The mice were kept in the chamber throughout the 10 weeks of exposure. The volume of the chamber was approximately 1 m^3 and it was ventilated with an air flow of 2 m^3/h with controlled temperature (22°C) and humidity (about 50%). Part of the air passed through a mercury vapour generator before entering the chamber. Air was also continuously drawn through a mercury meter (type Milton Roy P-0408, sensitivity 7 $\mu g/m^3$) from the middle of the chamber, just above the cages. Thus, mercury vapour concentration in the chamber was continuously recorded. The calibration was carried out by sampling 10 l of chamber air into mercury absorbing acid solution (0.4 M $KMnO_4$ and 2% H_2SO_4). Mercury contents were assayed with the aid of cold vapour atomic absorption spectrophotometry. The animals were kept in groups of five to seven in ordinary plastic cages with steel wire tops. They had free access to food (placed on the wire top) and water during the 10 weeks of exposure.

Mercury vapour was generated by stirring metallic mercury in a 10-l glass flask with a magnetic stirrer (Figure 1).

Mercury absorption was calculated as follows: mercury concentration in the chamber × time of exposure × alveolar ventilation of mice × 80%. The mercury concentration was measured by the mercury meter as described above, the alveolar ventilation of mice is 1.5 l/h (Green, 1975) and 80% absorption is assumed, as has been shown for man (Hursh *et al.*, 1976).

Groups of mice tested

Four groups (A, B, C, and D) of ten mice were exposed to mercury vapour 5 days a week (Table 1). A control group (group E) of ten mice received clean air without mercury from the same source. All mice were killed after 10 weeks of exposure.

Mercury analyses

The caudal half of the right hemisphere was taken for mercury analysis. The total mercury content was determined by a method described by Einarsson *et al.* (1984). The procedure was modified for higher sensitivity by enrichment (amalgamation) of the mercury vapour on a gold wire filter. Briefly, homogenized brain samples were digested with a mixture of $HClO_4$ and HNO_3 overnight at 68°C. Chemical blanks and reference samples (Seronorm Trace Elements, batch 904, Nycombed AS, Oslo) were included in the sample series.

Group ($n = 10$)	Hg conc. (mg/m^3)	Hg exposure (h/day)	(h)	Total absorption (μg Hg)	Brain concentration (μg/g tissue)	(range)	(±s.d.)
A	0.5	19	695	473	6.4	(4.3–9.9)	(1.6)
B	1	3	126	138	6.3	(5.3–7.5)	(0.8)
C	0.3	6	232	96	1.6	(1.2–2.3)	(0.3)
D	1	1.5	65	72	0.64	(0.50–0.72)	(0.07)
E	–	–	–	–	0.01	(0–0.01)	(0.005)

Table 1. Groups of mice tested and mercury exposure. When calculating total mercury absorption, 80% absorption is assumed, as has been shown for man (Hursh *et al.* 1976)

Histology

After sacrifice, the brains were removed and placed in a solution of 4% formaldehyde in phosphate buffer. Two to 3-mm thick slices of the brain were then cut. The tissues were dehydrated and embedded in paraffin. Five-μm thick sections were cut and placed on glass slides and further processed according to a method described by Danscher and Möller-Madsen (1985). The method is based on the ability of metal sulphides to catalyse the reduction of silver ions to metallic silver (Danscher & Möller-Madsen 1985). Briefly, the slides were deparaffinized, coated with gelatine, developed at 26°C for 1 h in a dark box (the developer contained gum arabic, sodium citrate buffer, hydroquinone and silver lactate). The gelatine was washed out in 40°C tap water followed by distilled water, exposed to 5% sodium thiosulphate and counterstained with haematoxylin-eosin. The method has been shown, in several reports by Danscher and Möller-Madsen (Danscher & Möller-Madsen 1985; Möller-Madsen & Danscher 1986; 1991; Möller-Madsen 1990; 1991; 1992), to be specific for metallic gold and inorganic mercury. As the experimental animals have not been exposed to gold, mercury is the only possible catalyst for the reduction.

At the microscopical examination, the amount of visualized mercury in each cell was estimated according to a three-grade scale: + a few grains in the cytoplasm, ++ a moderate amount of grains in the cytoplasm, +++ the cytoplasm was loaded with grains. In Figure 2 this scale is represented by three different sizes of dots: small dots represent cells with a few grains in the cytoplasm, medium-sized dots a moderate amount of grains in the cytoplasm and large dots indicate cells which were loaded with grains. The relative densities of stained cell-bodies are illustrated by the distance between the dots. A quantitative assessment of grain density and its variability was not performed.

The Latin nomenclature is based on the *Nomina anatomica* (Excerpta Medica 1968) and the mouse brain atlas of Sidman *et al.* (1971).

Results

Mercury exposure

Data concerning mercury exposure, assumed mercury absorption and mercury concentration in the brains are presented in Table 1.

Clinical observations

No clinical signs of toxic effect were observed in any of the groups during the 10 weeks of exposure. However, no specific neurological examination was performed in this study.

Mercury distribution

Mercury was not detected in the brains of group E. The results from the examination of the brains of groups A, B, C, and D are presented in Figure 2. The drawings illustrate representative sections of the brains, although minor variations in staining intensity of the brains were present within each group. The variation was restricted to quantitative differences in visualized mercury, not only to the localization of mercury.

In all mice from groups A, B and C, mercury was found in the capillary walls. In groups A and B, the ependyma lining the ventricles and plexus choroideus contained mercury.

In group A, mercury was found throughout the whole brain, although the amount of mercury visualized within the cells varied. The pyramidal cells of the neocortical layer V were heavily loaded, as well as some of the neurons in nucleus caudatus/putamen, nuclei of the brain-stem, and the Purkinje cell layer and nuclei of the cerebellum.

In group B, the distribution pattern was the same as for group A, although the density of mercury containing cells and the amount of visualized mercury in each cell were in some areas lower. Claustrum, cortex piriformis and the hippocampal formation CA1 contained no visualized mercury.

In group C, lamina cellularum of the olfactory bulb, the neocortical layer V (Figure 3a), the white matter, nucleus caudatus/putamen, nuclei of thalamus and the brainstem (Figure 3c), Purkinje cells (Figure 3b) and nuclei of the cerebellum contained mercury.

In group D, the white matter and nuclei of the brainstem contained mercury.

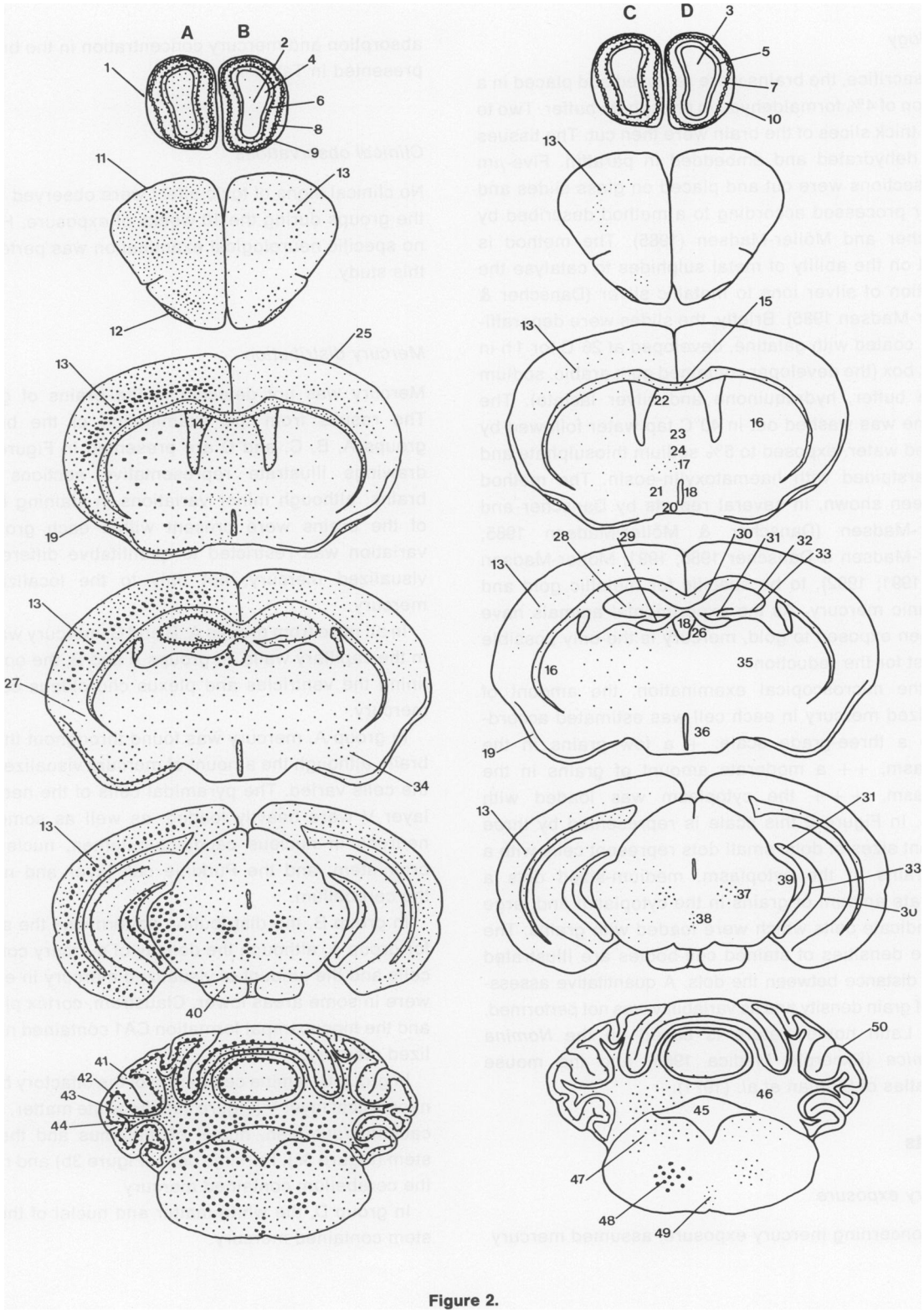
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Figure 2.

Figure 2. Brain sections from groups A, B, C and D. Mercury containing cell-bodies are shown as black dots. Large dots indicate cell-bodies which stained especially deeply, while the relative densities of stained cell-bodies are illustrated by the distance between the dots.

1 Bulbus olfactorius, 2 stratum subependymale ventriculi olfactorii, 3 lamina medullaris interna bulbi olfactorii, 4 lamina granularis interna bulbi olfactorii, 5 lamina plexiformis interna bulbi olfactorii, 6 lamina cellularum mitralium bulbi olfactorii, 7 lamina plexiformis externa bulbi olfactorii, 8 lamina granularis externa bulbi olfactorii, 9 lamina glomerulosa bulbi olfactorii, 10 lamina fibrorum n. olfactorii, 11 cortex cerebri, area frontalis, 12 tuberculum olfactorium, 13 neocortical layer V, 14 ventriuculus lateralis, 15 genu corporis callosi, 16 nuc. caudatus/putamen, 17 commissura anterior, pars anterior, 18 ventriculus tertius, 19 cortex cerebri, area pyriformis, 20 chiasma opticum, 21 nuc. preopticus, 22 nuc. lateralis septi, 23 nuc. fimbrialis septi, 24 nuc. triangularis septi, 25 cortex cerebri, area parietalis, 26 nuc. medialis habenulae, 27 claustrum 28 fissura hippocampi, 29 hippocampus CA4, 30 hippocampus CA1, 31 gyrus dentatus, 32 hippocampus CA2, 33 hippocampus CA3, 34 nuc. amygdaloideus lateralis, 35 nuclei thalami, 36 nuclei hypothalami, 37 nuc. ruber, 38 nuc. accessorius n. oculomotorii, 39 nuc. corporis geniculati medialis, 40 nuclei pontis, 41 stratum moleculare, 42 Purkinje cells, 43 stratum granulosum, 44 corpus medullare, 45 ventriculus quartus, 46 nuclei cerebelli, 47 nuclei vestibularis, 48 nuc. gigantocellularis, 49 nuc. raphe magnus, 50 cerebellum.

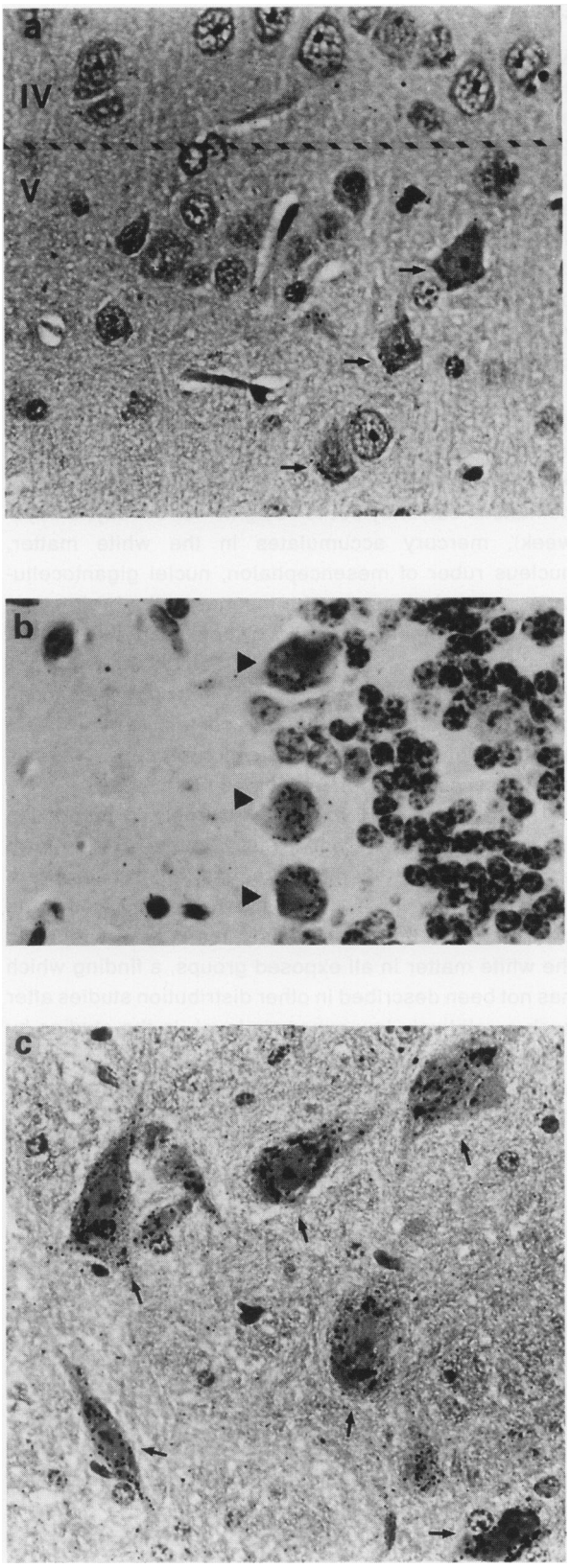

Figure 3. a, The neocortical layer V (13 in Figure 2) in a brain from group C. The picture shows the deeper part of layer IV and three mercury containing pyramidal cells in layer V (arrows). × 450. b, Cerebellum from the mouse described in a. The picture shows from left to right the molecular layer (41), Purkinje cell layer (42), and a part of the granular layer (43). The Purkinje cells (arrowheads) contain mercury. × 660. c, A picture taken from the brain-stem from the same mouse as in a and b. The mercury containing neurons (arrows) belong to nuc. gigantocellularis (48). ×450.

Discussion

Mercury treated, genetically susceptible mice of the H-2s haplotype exhibit a general activation of the immune system with splenic cell hyperplasia, a strong B-cell activation, an increased number of immunoglobulin-secreting cells (Hultman & Enestrom 1989) and hyperimmunoglobulinaemia (Hultman & Enestrom 1987). How the immune disease is linked to the mercury distribution in the brain is not known. However, the present study demonstrates the distribution of mercury within the brain of a mercury sensitive mouse strain (the SJL/N strain).

The present study on mercury sensitive mice reveals that after 10 weeks of mercury vapour exposure at a level (group D) of 1 mg Hg/m^3 air for 1.5 h/day, 5 days/week (the threshold limit for occupational exposure of humans is currently set to 50 $\mu g/m^3$ for 8 h/day, 5 days/week), mercury accumulates in the white matter, nucleus ruber of mesencephalon, nuclei gigantocellularis, vestibularis and raphe magnus of the brain-stem. Mercury accumulates in the cytoplasm (Fowler *et al.* 1974; Danscher & Schröder 1979; Schiönning & Möller-Madsen 1991), a localization that might suggest that cell groups accumulating mercury contain certain lysosomes involved in the detoxification process. This has been discussed earlier (Warfvinge *et al.* 1992).

The results of the present study largely correspond to the results of Möller-Madsen (1992) following short-term exposure of Wistar rats, although differences were found between the two investigations concerning the distribution. In the present study, mercury was found in the white matter in all exposed groups, a finding which has not been described in other distribution studies after such a relatively low exposure level. In the studies by Möller-Madsen (1992) and our laboratory (Warfvinge *et al.* 1992) on mercury-sensitive BN rats, it was found that the white matter was not a target for mercury accumulation.

In the present study, neocortical layer V is the target for mercury accumulation. This is in accordance with results obtained after long-term exposure of mercury sensitive rats (Warfvinge *et al.* 1992), but not after short-term exposure of Wistar rats (which are not mercury sensitive) in which layer III is the target (Möller-Madsen 1992). The laminar specificity would rather suggest a different uptake mechanism(s) and/or oxidative capacity than transport mechanism of mercury in different populations of pyramidal cells, as the topographical differences in mercury accumulation could not be correlated to the capillary density.

A striking resemblance between rats and mice is the early and heavy accumulation in nucleus ruber. In the series of investigations on mercury distribution in rat brain after administration of different mercury compounds (Möller-Madsen & Danscher 1986; 1991; Möller-Madsen 1990; 1991; 1992), it was shown that nucleus ruber accumulates mercury independently of the type of mercury molecule administered. The nucleus ruber belongs to the motor systems, and the accumulation of mercury might explain the neurological symptoms obtained after mercury intoxication.

In conclusion, white matter (among other structures) in the young mouse brain is a target for mercury accumulation after long-term exposure to mercury vapour of a mercury sensitive mouse strain. This is in contrast to other distribution studies. In addition, neocortical layer V is the main cortical target for mercury accumulation in the present study.

Acknowledgements

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