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# EFFECTS OF ALTERED BRAIN 5-HYDROXYTRYPTAMINERGIC ACTIVITY ON BRAIN TRYPTOPHAN, 5-HYDROXYTRYPTAMINE AND 5-HYDROXYINDOLEACETIC ACID

C. A. MARSDEN and G. CURZON
Department of Neurochemistry, Institute of Neurology, Queen Square, London WC1 3BG

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**Summary**—The effects of procedures known to alter 5-hydroxytryptaminergic activity (raphe stimulation, raphe lesions and exposure to elevated temperature) on brain tryptophan, 5-hydroxytryptamine and 5-hydroxyindoleacetic acid concentrations were investigated.

Electrical stimulation of the dorsal raphe nucleus of unanaesthetised unrestrained rats for 1 hr, using implanted electrodes, significantly increased 5-hydroxyindoleacetic acid concentrations in the hypothalamus, striatum, midbrain and cortex but not in the hippocampus. The level of 5-hydroxytryptamine fell proportionately with the rise of 5-hydroxyindoleacetic acid in the midbrain but did not alter significantly in any other region. Tryptophan concentration was not significantly altered except in the hypothalamus where it increased moderately. Rats kept at 40°C for 60 min had significantly increased plasma and brain tryptophan and brain 5-hydroxyindoleacetic acid. Lysergic acid diethylamide prevented only the increase in 5-hydroxyindoleacetic acid. Electrolytic lesions in the dorsal and median raphe nuclei markedly lowered brain 5-hydroxytryptamine and 5-hydroxyindoleacetic acid but had no effect on brain or plasma tryptophan.

Therefore, evidence is against a major role for brain tryptophan changes in the regulation of 5-hydroxytryptamine synthesis following alteration of 5-hydroxytryptaminergic activity. Other possible factors involved in the relationship of 5-hydroxytryptamine metabolism to neuronal activity are discussed.

Rat brain 5-hydroxytryptamine (5-HT) turnover is influenced by tryptophan changes because tryptophan hydroxylase is unsaturated at normal brain tryptophan concentrations (FRIEDMAN, KAPPELMAN and KAUFMAN, 1972). Thus, L-tryptophan and drugs or alterations of the milieu that increase its concentration in the brain increase brain 5-HT turnover (ECCLESTON, ASHCROFT and CRAWFORD, 1965; TAGLIAMONTE, TAGLIAMONTE, PEREZ-CRUET, STERN and GESSA, 1971; KNOTT and CURZON, 1972). However, it is not clear whether changes in 5-HT turnover following altered activity of 5-HT neurones are related to changes in brain and plasma tryptophan.

We studied this problem using two procedures known to cause increased 5-HT neuronal activity (stimulation of 5-HT-containing cell bodies and exposure to increased temperature) and one procedure (lesioning of the 5-HT cell bodies) causing decreased activity. Electrical stimulation of the dorsal or median raphe nuclei, which contain the 5-HT cell bodies (DAHLSTRÖM and FUXE, 1964), increases brain concentrations of the 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) (SHEARD and AGHAJANIAN, 1968a), presumably due to increased release of 5-HT from nerve endings. It also increases 5-HT synthesis (SHIELDS and ECCLESTON, 1972). The 5-HT neuronal firing is also increased at elevated environmental temperature (WEISS and AGHAJANIAN, 1971). In this situation rat brain tryptophan (TAGLIAMONTE *et al.*, 1971) and 5-HIAA are increased while 5-HT shows little change, suggesting that synthesis is increased (AGHAJANIAN and WEISS, 1968; WEISS and AGHAJANIAN, 1971). The increases in firing and 5-HT metabolism appear to be related as both are blocked by D-lysergic acid diethylamide (LSD) (AGHAJANIAN and WEISS, 1968): their relation to the increase in tryptophan is not clear. Electrolytic lesions in the raphe nuclei destroy 5-HT cell bodies and thus must decrease 5-HT neuronal activity. The concentrations of 5-HT and 5-HIAA in brain also decrease (KOSTOWSKI, GIACOLONE, GARATTINI and VALZELLI, 1968; LORENS and GULDBERG, 1974; JACOBS, WISE and TAYLOR, 1974).

The effects of these three procedures on brain tryptophan, 5-HT and 5-HIAA were measured in order to elucidate the relationship between neuronal firing and brain tryptophan concentration. Part of this work has already been briefly reported (CURZON and MARSDEN, 1976).

## METHODS

Male Sprague–Dawley rats (Anglia Laboratory Animals, Alconbury, Huntington, U.K.) kept at 25° ± 3°C under a 12 hr light–dark cycle and given food (Oxoid 41B) and water *ad lib.*, were used in all experiments.

### *Raphe stimulation*

Bipolar steel electrodes (Plastic Products, Roanoke, VA) insulated with bakelite, except for the tip, were

*Key words*: 5-hydroxytryptamine, 5-hydroxyindoleacetic acid, tryptophan, raphe lesions, raphe stimulation, LSD.

chronically implanted into the dorsal raphe nucleus of rats (220–240 g) under sodium pentobarbital (40 mg/kg, i.p.) anaesthesia, using a stereotaxic frame (Stoelting, Chicago, IL) and the following co-ordinates (PELLEGRINO and CUSHMAN, 1967): sagittal, 0.0 mm; frontal, 0.4 mm; horizontal, 0.6 mm. The electrode was fixed to the skull surface with dental cement and three small screws. Rats were housed individually for 7–12 days after placement of electrodes. Stimulation of the dorsal raphe was performed without anaesthesia as follows. Rats were transferred into individual perspex cages (35 × 35 × 45 cm) and the electrode leads attached to the stimulator via a swivel system (Lehigh Valley Co., Foglesville, PA), thus permitting unrestrained movement. Stimulation parameters were: 10 pulse/sec, 2 msec duration at 6 V for 60 min. One rat was stimulated and one rat sham stimulated concurrently without passage of current. Each rat was stimulated on one occasion only between 13.00–15.30 hr (during the light period of the light–dark cycle) in experiments spread over 6 days. Following stimulation the animals were immediately killed, the brains removed and either used as whole brain or divided into the following regions: hypothalamus, hippocampus, striatum, midbrain, cortex, brainstem (including raphe) plus cerebellum. All except the latter were frozen over dry ice and stored at −25°C. To determine the sites of the electrodes, the brainstem was cut into 30 μm sections using a cryostat.

*Raised environmental temperature*

Rats (180–200 g) were caged in eight groups of three in a chamber at 25° ± 3°C. Four groups were transferred to a chamber at 40° ± 3°C for 60 min. All groups were then killed. The brains were removed, frozen over dry ice and stored at −25°C. Plasma was also collected and stored at −25°C. Two test and two control groups were administered with LSD (Sandoz, 500 μg/kg i.p.) immediately before transfer of test groups to the 40°C chamber. In another experiment, probenecid (200 mg/kg, i.p.) was given 15 min before transfer.

*Raphe lesions*

Electrolytic lesions were placed in both dorsal and median raphe nuclei of the same animal (220–240 g) under ether anaesthesia using the stereotaxic frame. A 2.0 mA current was passed for 20 sec at each lesion site through a steel electrode insulated with teflon except for 0.5 mm at its tip. The following co-ordinates (PELLEGRINO and CUSHMAN, 1967) were used: sagittal, 0.0 mm; frontal 0.4 mm; horizontal (dorsal), 0.6 mm, (median) 2.5 mm. In sham operated animals electrodes were placed but no current passed. The rats were then housed individually. As raphe lesions cause a transient fall in food intake and body weight (KOSTOWSKI *et al.*, 1968) which might influence brain tryptophan concentration (CURZON, JOSEPH and KNOTT, 1972), the rats were killed 48 hr after the weight gain per day of both sham operated and lesioned groups were similar, i.e. 8 days after making the lesions (Fig. 1). The brains were removed and the brainstem (including raphe) plus cerebellum separated from the rest of the brain. The latter was frozen over dry ice, stored at −25°C and used for the determination of tryptophan, 5-HT and 5-HIAA. Blood was collected into heparinized tubes and plasma separated and stored at −25°C for the determination of free and total tryptophan in plasma. The brainstem was cut into 30 μm sections on a cryostat in order to determine the sites of the lesions.

*Biochemical determinations*

Plasma and brain tyrosine, tryptophan, 5-HT and 5-HIAA were determined as described previously (CURZON *et al.*, 1972); the method being modified slightly when brain regions were used (KNOTT and CURZON, 1974). Plasma total and free tryptophan

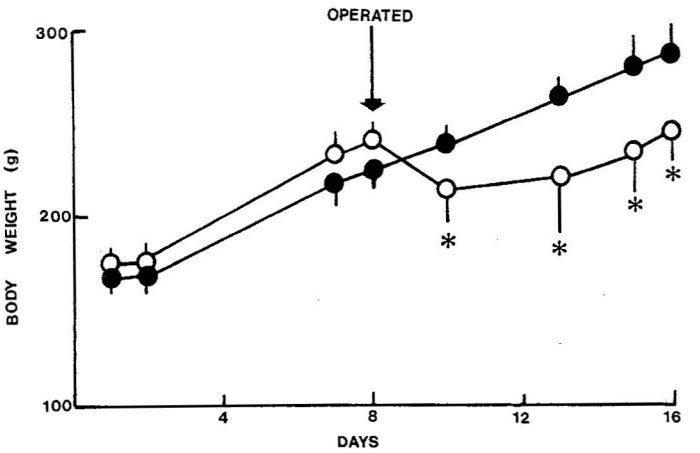


Fig. 1. Effect of lesions in the dorsal and median raphe nuclei on rat body weight. The lesions were made on day 8 when the controls were sham operated. All rats were killed on day 16 (i.e. 8 days after operation.)

Sham operated, $n = 6$ (●—●); lesioned, $n = 8$ (○—○). * $P < 0.01$.

Table 1. Effect of electrical stimulation of the dorsal raphe nucleus on rat brain* tryptophan, 5-HT and 5-HIAA

| | Tryptophan (μg/g) | 5-HT (μg/g) | 5-HIAA (μg/g) |
|---|---|---|---|
| Sham stimulated (4) | 1.76 ± 0.20 | 0.42 ± 0.03 | 0.36 ± 0.05 |
| Stimulated (4) | 1.88 ± 0.17 | 0.38 ± 0.06 | 0.57 ± 0.08 $P < 0.01$ |

The dorsal raphe was stimulated for 1 hr in unrestrained unanaesthetised rats. Results given as mean ± S.D. Figures in brackets refer to number of animals.
* Whole brain less brainstem and cerebellum.

were determined as described by KNOTT and CURZON (1972).

## RESULTS

### *Effect of dorsal raphe stimulation*

Stimulation of the dorsal raphe produced no obvious behavioural effects apart from some tremor of the facial muscles and whiskers as previously described (SHEARD and AGHAJANIAN, 1968b). One rat, in which the electrode was found to be placed lateral to the dorsal raphe, exhibited circling on stimulation. Stimulation of the dorsal raphe significantly increased whole brain 5-HIAA but not 5-HT or tryptophan (Table 1). When brain regions were studied (Fig. 2), 5-HIAA was found to be significantly increased in the hypothalamus, striatum, midbrain and cortex with essentially no change in the hippocampus. Percentage increases were greater in the hypothalamus and striatum than in the midbrain and cortex. There was a moderate but significant percentage fall of 5-HT in the midbrain which was essentially identical with the percentage rise of 5-HIAA, while in the cortex there was a smaller 5-HT fall which approached significance. In all other regions 5-HT changes were negligible. Tryptophan showed a slight tendency to increase in all regions except the midbrain but only in the hypothalamus was the percentage increase significant and even here it was rather less than that of 5-HIAA.

If it is assumed that raphe stimulation does not appreciably affect the rate of egress of 5-HIAA from brain, then net increase of 5-HIAA + 5-HT should give an index of increase of 5-HT synthesis. These values were significantly increased in the hypothalamus and striatum only ($P < 0.01$).

### *Effect of raised environmental temperature*

Exposure to 40°C for 60 min markedly and significantly increased brain tryptophan (+199%), moderately but significantly increased 5-HIAA (+29%) and did not significantly alter 5-HT (Table 2). Plasma total and free tryptophan were both increased to the same relative extent (+110%) (Table 2). The increases of plasma and brain tryptophan were not specific to this amino acid as corresponding tyrosine values also increased (75 and 95% respectively). When LSD was given immediately before placing the rats in the 40°C

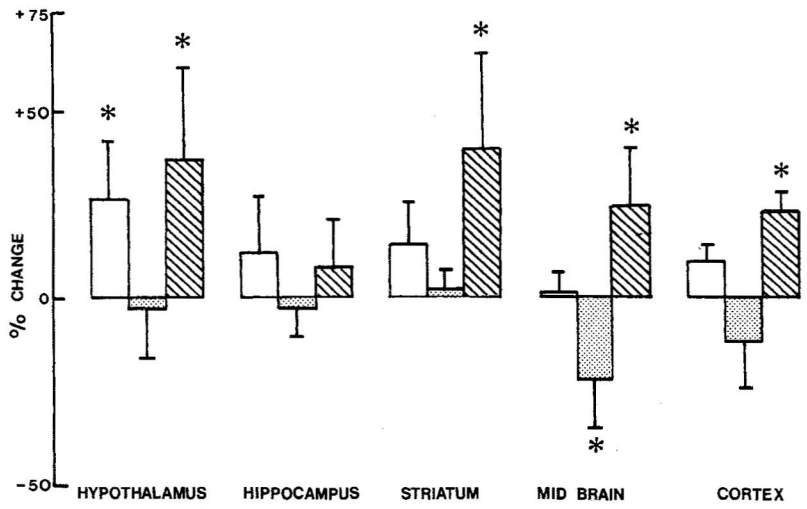


Fig. 2. Effect of electrical stimulation of the dorsal raphe on rat brain regional tryptophan (open bar), 5-HT (stippled bar) and 5-HIAA (hatched bar). The dorsal raphe was stimulated for 1 hr in unrestrained unanaesthetised rats. Results shown as % change from control (sham stimulated) ± S.D. * $P < 0.01$, $n = 6$. Four additional rats were stimulated but biochemical findings on these are not included because histological examination of brain stem sections revealed that the electrode had been placed outside the dorsal raphe nucleus.

Table 2. Effects of LSD and raised environmental temperature on rat plasma free and total tryptophan and brain tryptophan, 5-HT and 5-HIAA

| | Plasma | | Brain | | |
|---|---|---|---|---|---|
| | Total tryptophan (μg/ml) | Free tryptophan (μg/ml) | Tryptophan (μg/g) | 5-HT (μg/g) | 5-HIAA (μg/g) |
| 0.9% Sodium chloride 25°C 6 | 13.6 ± 4.0 | 2.01 ± 0.31 | 3.02 ± 0.52 | 0.76 ± 0.04 | 0.56 ± 0.04 |
| LSD (500 μg/kg) 25°C (6) | 14.8 ± 2.5 | 2.43 ± 0.39 | 3.23 ± 0.36 | 0.76 ± 0.06 | 0.48 ± 0.05* |
| 0.9% Sodium chloride 40°C (6) | 28.9 ± 3.6** | 4.39 ± 0.60** | 9.04 ± 2.43** | 0.88 ± 0.09 | 0.72 ± 0.07** |
| LSD (500 μg/kg) 40°C (6) | 25.3 ± 0.4** | 3.60 ± 0.73** | 7.85 ± 1.16** | 0.83 ± 0.12 | 0.49 ± 0.04*** |

Rats placed in 40°C environment for 60 min and LSD or 0.9% sodium chloride administered at the start of this period. Results given as mean ± SD. Figures in brackets refer to number of animals.

Significantly different from 0.9% sodium chloride injected rats (25°C): $P < 0.02$, *; $P < 0.01$, **.

Significantly different from 0.9% sodium chloride injected rats (40°C): $P < 0.001$, ***. Although mean brain tryptophan was slightly lower in LSD- than in 0.9% sodium chloride-injected rats at 40°C this was not confirmed in subsequent experiments.

chamber in order to block neuronal firing and the associated rise of brain 5-HIAA (WEISS and AGHAJANIAN, 1971), it did not significantly diminish the rises of plasma and brain tryptophan. The latter gross increase was not associated with a significant increase of 5-HT or 5-HIAA above their values in LSD-treated rats kept at 25°C.

To investigate whether egress of 5-HIAA was affected by exposure to 40°C, probenecid was given to a group of rats before exposure in order to inhibit egress. Results in Table 3 show that the percentage increase of 5-HIAA due to exposure to the higher temperature was unaffected by probenecid and the drug caused similar percentage increases of 5-HIAA at both temperatures. The increase of brain tryptophan upon exposure to 40°C was much less marked than in the previous experiment. However the percentage increase of 5-HIAA was almost identical in both experiments.

*Effect of raphe lesions*

The lesioned rats lost weight for the first two days after operation. However, when the animals were killed (8 days after operation), although the lesioned group were significantly lighter than the sham operated group, both were gaining weight at a similar rate (Fig. 1) suggesting similar food intake in both groups.

Raphe lesions markedly and significantly reduced brain 5-HT (−66%) and 5-HIAA (−78%) but had essentially no effect on brain tryptophan or on either plasma total or free tryptophan (Table 4).

## DISCUSSION

In two situations, dorsal raphe stimulation and exposure to elevated temperature, increased firing of 5-HT neurones occurs and is associated with increased 5-HT synthesis (SHEARD and AGHAJANIAN, 1968a; WEISS and AGHAJANIAN, 1971). The latter change was also indicated in the present study by whole brain determinations.

Stimulation caused negligible changes of whole brain tryptophan concentration, suggesting that the increase of 5-HT synthesis did not occur through a closed feedback loop in which depletion of neuronal

Table 3. Effect of probenecid on rat brain tryptophan, 5-HT and 5-HIAA at 25°C and 40°C

| Treatment | Tryptophan (μg/g) | 5-HT (μg/g) | 5-HIAA (μg/g) |
|---|---|---|---|
| 25°C + Saline (6) | 2.83 ± 0.25 | 0.61 ± 0.08 | 0.47 ± 0.02 |
| 40°C + Saline (6) | 4.17 ± 0.69<br>+ 47%<br>$P < 0.01$ | 0.56 ± 0.04<br>− 8% | 0.59 ± 0.06<br>+ 26%<br>$P < 0.01$ |
| 25°C + probenecid (6) | 2.93 ± 0.29 | 0.62 ± 0.05 | 0.68 ± 0.07 |
| 40°C + probenecid (6) | 4.67 ± 0.68<br>+ 59%<br>$P < 0.01$ | 0.64 ± 0.06<br>+ 3% | 0.86 ± 0.07<br>+ 26%<br>$P < 0.01$ |

Probenecid (200 mg/kg, i.p.) was given 15 min before placing the rats in the 40°C chamber. They were killed after 60 min at 40°C. Figures in brackets refer to number of animals. Mean increase of 5-HIAA due to probenecid was 45% at 25°C and 46% at 40°C.

Table 4. Effect of combined lesions in the dorsal and median raphe nuclei on rat plasma free and total tryptophan and brain* tryptophan. 5-HT and 5-HIAA

| Treatment | Plasma | | Brain | | |
|---|---|---|---|---|---|
| | Total tryptophan | Free tryptophan | Tryptophan | 5-HT | 5-HIAA |
| | ($\mu$g/ml) | | ($\mu$g/g) | ($\mu$g/g) | ($\mu$g/g) |
| Sham operated | 19.4 ± 3.1 (5) | 1.52 ± 0.24 (5) | 1.80 ± 0.09 (6) | 0.46 ± 0.06 (6) | 0.42 ± 0.07 (6) |
| Lesioned | 21.3 ± 4.1 (6) | 1.46 ± 0.20 (6) | 1.95 ± 0.26 (8) | 0.16 ± 0.05 (8) $P < 0.001$ | 0.09 ± 0.02 (8) $P < 0.001$ |

* Whole brain less brainstem and cerebellum. Raphe lesions made 15 days before killing. Results given as mean ± S.D. Figures in brackets refer to number of experiments.

5-HT stores on firing led to increased uptake by brain of the 5-HT precursor tryptophan. This finding agrees with that of SHIELDS and ECCLESTON (1972) who stimulated the median raphe of anaesthetized rats previously given the monoamine oxidase inhibitor pargyline.

Results obtained in the present study on regional changes after dorsal raphe stimulation are more complex. Thus, although significant percentage increases of 5-HIAA were found in all regions, except the hippocampus where 5-HT terminals derive mainly from the median raphe (LORENS and GULDBERG, 1974; JACOBS *et al.*, 1974), synthesis kept pace with increased catabolism only in the hypothalamus and striatum. In the midbrain, 5-HT fell in proportion with the rise of 5-HIAA, suggesting that increased synthesis was not occurring here.

In the striatum, the apparent increase of 5-HT synthesis is not explicable by the insignificant tryptophan changes. However, results are less clearly interpretable in the hypothalamus, where the significant rise of tryptophan could well be responsible for at least part of the increase of 5-HT synthesis. Hypothalamic tryptophan concentration may be under some special influence, as in agreement with previous findings (KNOTT and CURZON, 1974; CURZON and MARSDEN, 1975; COLMENARES, WURTMAN and FERNSTROM, 1975), control levels were found to be considerably higher than in other regions.

In agreement with TAGLIAMONTE *et al.* (1971), exposure of rats to a 40°C environment caused considerable increases of plasma tryptophan and tyrosine and proportionately rather larger increases of these amino acids in brain. Increases of brain 5-HIAA were small in comparison with those of tryptophan. If exposure to 40°C increases egress of 5-HIAA from brain, then comparison of its concentrations in rats exposed to 40°C and 25°C would give a falsely low estimate of the increase of 5-HT synthesis due to exposure to the higher temperature. However, egress does not appear to be affected by the increased temperature since 5-HIAA values rose upon probenecid treatment in the same proportion in rats at both temperatures. When LSD was given to prevent neuronal firing and the increase of 5-HT turnover, at 40°C the increases of plasma and brain tryptophan were unaffected. Therefore the latter seem to be unrelated to altered 5-HT neuronal activity.

The above findings indicate that increased brain 5-HT synthesis following firing of 5-HT neurones does not require increased brain tryptophan. Conversely, when 5-HT nerve cells were destroyed by electrolytic lesions, the resultant decrease of 5-HT synthesis was not associated with a decrease of brain tryptophan concentration nor with an alteration in its uptake by the brain as indicated by the unaltered ratio of brain tryptophan to plasma total or free tryptophan. Similarly, LYTLE, JACOBY, NELSON and BAUMGARTEN (1975) found that irreversible depletion of brain 5-HT after intraventricular injection of 5,7-dihydroxytryptamine did not lead to decreased brain tryptophan. Findings with 5,6-dihydroxytryptamine appear to be in conflict because falls of both 5-HT and tryptophan are reported. However, the latter change was suggested to be merely a result of prolonged decreased food intake (HYYPPA, CARDINALI, BAUMGARTEN and WURTMAN, 1973). These findings do not strengthen the recent suggestion by NISKANEN, HUTTUNEN, TAMMINEN and TAASKELAINEN (1976) that a postulated 5-HT defect in depressive illness may lead to compensatory plasma tryptophan changes.

The present findings answer in the affirmative the question (GESSA and TAGLIAMONTE, 1974) whether changes of 5-HT synthesis can occur without parallel changes of brain tryptophan concentration. It is also of interest that the considerable increase of brain tryptophan on exposure of LSD-treated rats to 40°C occurs without apparent effect on 5-HT synthesis. The findings in general support the suggestion of SHIELDS and ECCLESTON (1972) that increased activity of existing tryptophan hydroxylase molecules is responsible for the increased 5-HT synthesis following firing. This might occur through allosteric changes or through increased availability of oxygen (DAVIS, CARLSSON, MACMILLAN and SIESJÖ, 1973) or of the cofactor of the enzyme. A possibility which is not excluded is that the increased 5-HT synthesis occurs through an increase of tryptophan uptake specifically by 5-HT neurones and without striking whole brain tryptophan changes.

While altered 5-HT function on the whole did not

lead to brain tryptophan changes (though the increase in the hypothalamus following raphe stimulation is of interest) it may be that when brain tryptophan does rise, 5-HT function can be enhanced. Increasing brain tryptophan does increase 5-HT turnover (FERNSTROM and WURTMAN, 1972; KNOTT and CURZON, 1972; TAGLIAMONTE, BIGGIO, VARGIU and GESSA, 1973) and while all the additional 5-HT synthesized could be metabolized intraneuronally without release to receptors (GRAHAME-SMITH, 1971), such a release may occur especially if the pre-existing level of brain 5-HT stores is low (MARSDEN and CURZON, 1976).

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