THE UPTAKE AND DISTRIBUTION OF ^{14}C-MESCALINE IN DIFFERENT ORGANS OF DEVELOPING RAT

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ABSTRACT

Rats of 1, 4, 8, 12, 20, and 60 days postnatal age were injected ip with ^{14}C-mescaline (50 nCi/g). The levels of mescaline and its deaminated metabolite, 3,4,5-trimethoxyphenylacetic acid, were examined in the brain, liver, heart, spleen, lung, and kidney at 30, 60, 90, and 120 min. Mescaline was rapidly taken up by all of the organs examined. In general, the organs of younger rats accumulated much larger amounts than those of adult animals. Brain concentrated the lowest amounts in comparison with other tissues. In the brain, the uptake was the highest in 1-day-old rats and decreased with age. The disappearance of mescaline in various organs was comparatively slower in younger animals than in 20-day or older rats. Rats immediately after birth and up to 20 days of age metabolized mescaline less efficiently than adults. From the data, it appears that the blood-brain barrier for mescaline develops gradually with age but is not completely impermeable in adults.

Age-dependent differences in the distribution, metabolism, and excretion of foreign chemical compounds have been recognized. Several factors may contribute; for example, it is well established that fetal and neonatal animals are generally inefficient in their ability to dispose of drugs or to convert them to their inactive metabolites. At early ages, the development of the blood-brain barrier (b-b-b)[3] is incomplete (1) and this may greatly alter the uptake of drugs in the central nervous system.

A marked accumulation of mescaline in the mouse fetal brain after its injection to the mother on day 15 of gestation was attributed to incomplete development of the b-b-b (2). In adult animals, peripherally administered mescaline does cross the b-b-b, although to a small extent (3). The fate of injected mescaline in the brain and several organs during postnatal growth is little understood. The present report examines the distribution, metabolism, and elimination of ^{14}C-mescaline in the rat at different stages of ontogenic development.

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[3] The abbreviations used are: b-b-b, blood-brain barrier; TLC, thin-layer chromatography; TMPA, 3,4,5-trimethoxyphenylacetic acid.

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Methods and Materials

The [8-^{14}C]mescaline hydrochloride (sp. act. 4.53 mCi/mmol) was obtained from New England Nuclear Corp. (Boston, Mass.) and had a purity of 97%. The chemical and radiochemical purity was verified in our laboratory by column and thin-layer chromatography (TLC) described below:

Column Chromatography. A 3-ml aqueous solution containing 0.1 μCi of ^{14}C-mescaline was passed over a 50-mm long column (4.2-4.5 i.d.) containing Dowex 50W-X4 (200-400 mesh). The column was washed successively six times with 5-ml portions of distilled water. The hallucinogen was then eluted successively, first with 7 ml and then 4 times with 3 ml each of 50% aqueous ethanol containing 5% concentrated NH_4OH; each fraction was collected separately. The effluent, 6 water washes and 5 eluates were each individually dried under vacuum and re-extracted in 0.5 ml of 50% ethanol. An aliquot of 0.1 ml of each fraction was individually added to 10 ml of Bray's scintillation fluid (4) for measurement of ^{14}C. About 95-97% of the radioactivity was recovered in a fraction eluted with 7 ml of ethanol-ammonia solution.

TLC. A 25-μl solution of ethanol-ammonia eluate

containing 95-97% ^{14}C was applied to a Polygram Sil G thin layer chromatographic plate (Brinkmann Instrument Inc., Westbury, N.Y.) and developed in a solvent system composed of *n*-butanol/acetic acid/water (4:1:1 v/v). Scanning with a Packard model 7201 radiochromatogram scanner displayed a single spot with an R_F value of 0.63 identical to that of the authentic nonlabeled mescaline.

Experimental. Rats of the Sprague-Dawley strain were used in the present investigation. All animals were born in the Institute's animal house and their time of birth was noted within 12 hr. The mean body weights were: 5 g at day 1, 11 g at day 4, 16 g at day 8, 26 g at day 12, 40 g at day 20, and 180 g at day 60. Six to eight animals were included per group in 1- and 4-day-old rats and 4-6 per group in the 8-, 12-, 20-, and 60-day-old animals. ^{14}C-Mescaline was prepared in 0.9% sodium chloride solution and injected ip in volumes of 0.1 to 1.0 ml; the dose was 50 nCi/g of body weight. Animals were killed by decapitation 30, 60, 90, and 120 min after the administration of the hallucinogen. Plasma was separated from the oxalated blood. The whole brain, liver, kidneys, lung, spleen, and heart were quickly dissected out and frozen on Dry Ice. Samples were extracted for mescaline on the same day; in some cases they were stored at −20° for 18-20 hr before assays were made. The procedure reported previously (5, 6) for the extraction of radioactivity and subsequent separation of unchanged ^{14}C-mescaline from its principal deaminated metabolite, 3,4,5-trimethoxyphenylacetic acid (TMPA), was used. Briefly, all tissues were homogenized in chilled 0.4 N perchloric acid. The extracts were adjusted to pH 5.8 with 5 N potassium carbonate solution and the salt of potassium perchlorate was removed by centrifugation in the cold. Aliquots of 0.5 ml were added to 10 ml of Bray's scintillation solution (4) for measurement of total ^{14}C. For the separation of unchanged mescaline from TMPA, the remaining extract was placed on 50-mm long columns (4.2-4.5 mm i.d.) containing Dowex 50W-X4 (200-400 mesh) previously buffered with 0.1 M phosphate buffer (pH 5.8). The amine was eluted with 10 ml of 50% aqueous ethanol containing 5% concentrated ammonia. Aliquots (1 ml) of effluent containing TMPA and eluate containing mescaline were added to 10 ml of scintillation solution (4). The radioactivity was counted in a Mark II liquid scintillation system using ^{133}Ba as an external standard. The efficiency of the counting system ranged between 86 and 90%. The overall recovery of radioactivity through the extraction and isolation procedures ranged between 74 and 83%. All data have been corrected for counting efficiency and for recovery.

The levels of unchanged mescaline and TMPA are reported as the mean (μg/g (wet weight tissue) or ml) ± SD.

Results

The plasma concentrations of unchanged ^{14}C-mescaline are shown in fig. 1. In 1- and 4-day-old rats, the 30-min levels were 0.98 ± 0.21 and 0.93 ± 0.19 μg/ml, respectively, and remained unchanged even up to 120 min. In the 8- and 12-day-old rats the peaks levels of mescaline were observed 60 min after its injection and declined thereafter. In 20- and 60-day-old rats, the levels of mescaline were highest at 30 min and declined to about 8% of the peak concentration at 120 min.

The concentration of unchanged mescaline in the brain of 1-day-old rats at 30 min after injection was 0.92 ± 0.07 μg/g (fig. 2); at 60 min it rose to 1.08 ± 0.1 μg/g. At 120 min the level of the unchanged drug was not significantly different from the 30-min level. Although the brain levels of mescaline in 4-day-old rats were somewhat lower than those in 1-day-old animals, the patterns were not different in these two age groups. A marked decrease in the uptake of mescaline was evident in the 8-day-old and older animals. Thus, the 30-min levels in 8-, 12-, 20-, and 60-day-old rats were 0.29 ± 0.05, 0.25 ± 0.05, 0.15 ± 0.009, and 0.09 ± 0.01 μg/g, respectively. In 8- and 12-day-old rats, brain concentrations of mescaline continued to increase and reached their highest at 60 min; the disappearance was slow in 8-day-old rats compared to that in 12-day-old rats. On the other hand, in 20- and 60-day-old rats, the concentration of mescaline began to decline from the 30-min time point and reached negligible levels at 120 min. The 30-min brain to plasma ratio of mescaline in 60-day-old rats was 17% of that in 1-day-old rats.

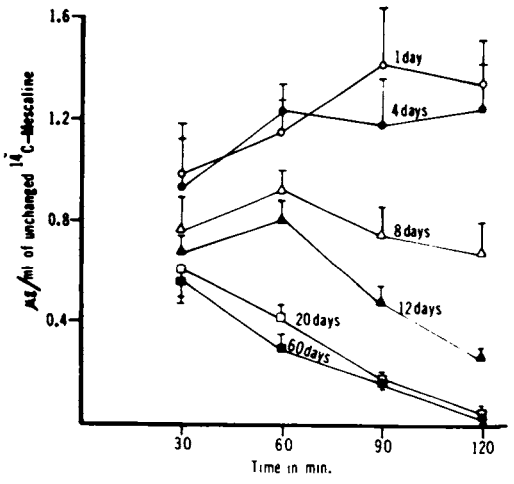

FIG. 1. *Concentrations of unchanged ^{14}C-mescaline in the plasma after ip ^{14}C-mescaline (50 nCi/g body weight) administration to rats from 1 day postpartum to adulthood.*

Each point represents the mean of 4-8 animals; *vertical bars* designate the SD.

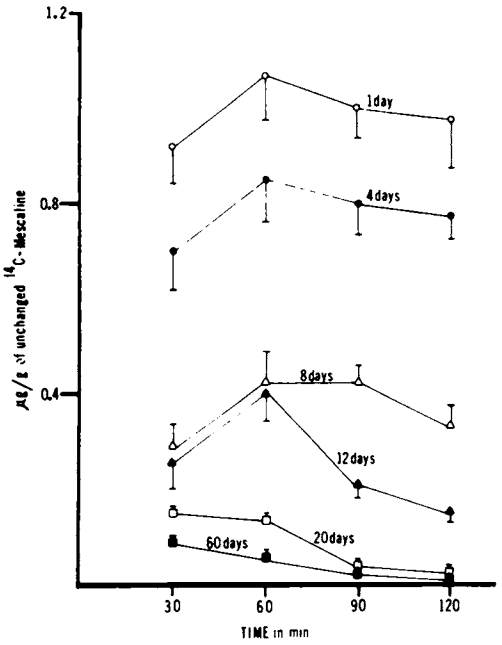

FIG. 2. *Concentrations of unchanged ^{14}C-mescaline in the brain.*

See fig. 1 for details.

Hepatic concentrations of mescaline in 1- and 4-day-old rats 30 min after the administration of the hallucinogen were 3.41 ± 0.31 and 3.20 ± 0.24 μg/g, respectively (fig. 3); the initial accumulation of mescaline declined to less than 50% in the 8-day and older rats. A decrease in the concentration of mescaline occurred from the 30-min time point in the 20-day or older animals, whereas in the 1- and 4-day postpartum groups any decrease in concentration noted was from the 60-min time point. The 30-min liver to plasma ratio of mescaline in 60-day-old rats was 64% of that in 1-day-old rats.

Mescaline contents in the hearts of 1- and 4-day-old rats at 30 min were 1.98 ± 0.20 and 1.68 ± 0.17 μg/g, respectively (fig. 4); in the 1-day-old group, the concentration of mescaline further increased and reached a level of 2.36 ± 0.17 μg/g at 60 min. Thus, the levels of mescaline in 1-day-old rats were approximately three times more than those in 60-day-old rats. The amounts of mescaline taken up in the hearts of 8-day and older rats were considerably lower than in those of younger rats. The disappearance was slower in 1-, 4-, and 8-day-old animals than in older rats. The decrease in the 30-min heart to plasma ratio of mescaline in 60-day-old rats *vis-à-vis* the 1-day-old rats was similar to that observed for the liver.

In 1- and 4-day-old rats, the 30-min levels of mescaline in the spleen were 1.76 ± 0.09 and 1.52 ± 0.12 μg/g and these levels further increased, reaching their highest at 60 min; the levels declined to values of 1.48 ± 0.1 and 1.18 ± 0.06 μg/g, respectively, at 120 min (fig. 5). Mescaline con-

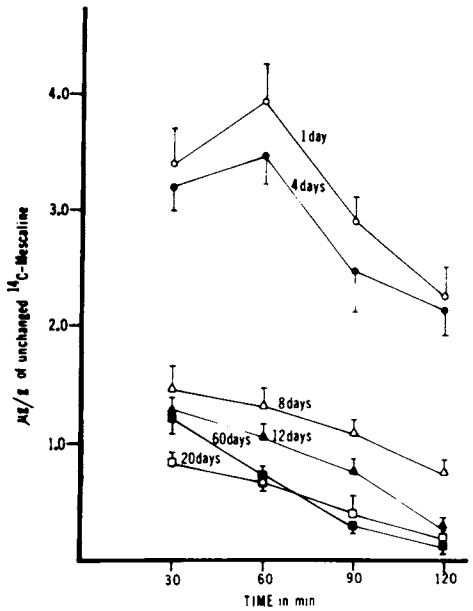

FIG. 3. *Concentrations of unchanged ^{14}C-mescaline in the liver.*

See fig. 1 for details.

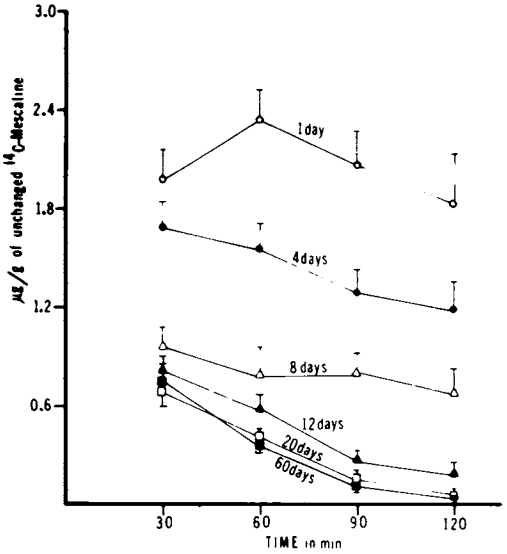

FIG. 4. *Concentrations of unchanged ^{14}C-mescaline in the heart.*

See fig. 1 for details.

centrations in spleens of 8- and 60-day-old rats at 30 min were approximately 50 and 16% of those in 1-day-old rats for the corresponding time period. The elimination of mescaline in the 12-, 20-, and 60-day-old rats was comparatively faster than in the postpartum days 1 and 4. The 30-min spleen to plasma ratio of mescaline in 60-day-old rats was 40% of that in 1-day-old rats.

The pattern of distribution of mescaline in the lung was somewhat different. The 30-min levels in 1- and 4-day-old rats (2.52 ± 0.17, 2.75 ± 0.35 μg/g) were only slightly higher than those in 8-, 12-, 20-, or 60-day-old rats (2.26 ± 0.17, 2.33 ± 0.12, 2.06 ± 0.26, 1.88 ± 0.20 μg/g) for the corresponding time period (fig. 6). In 1-day-old rats the 60-min levels further increased, followed by a slow decline over an additional 60-min period. In the rats 4 days old and over, the removal was relatively rapid but significant amounts of mescaline were still present at 120 min. The 30-min lung to plasma ratio of mescaline in 60-day-old rats was higher than in 1-day-old rats.

Thirty-minute levels of mescaline in the kidney of 1- and 4-day-old rats were 3.77 ± 0.26 and 3.42 ± 0.27 μg/g, respectively (fig. 7). These levels declined to 2.10 ± 0.17 and 1.46 ± 0.25 μg/g at 120 min. In 8-, 12-, and 20-day-old animals, the levels at 30 min were 2.25 ± 0.21, 1.61 ± 0.17, and 1.96 ± 0.11 μg/g, respectively. At 120 min, the level in the 8-day-old group was 0.92 ± 0.20 μg/g and those in the 12- and 20-day-old groups were 0.57 ± 0.11 μg/g and 0.44 ± 0.07 μg/g. In 60-day-old rats, the concentrations of mescaline at 30 min were 2.09 ± 0.18 and at 120 min 0.48 ± 0.17. The 30-min kidney to plasma ratios of mescaline in 1- and 60-day-old rats were similar.

Table 1 reports the concentrations of ^{14}C-TMPA in absolute amounts and as per cent of total ^{14}C. In 1- and 4-day-old rats the 30- to 120-min lowest and highest brain levels of ^{14}C-TMPA were 0.044 and 0.073 μg/g, respectively and accounted for 5-8% of the total ^{14}C. As the age advanced, the absolute brain levels decreased

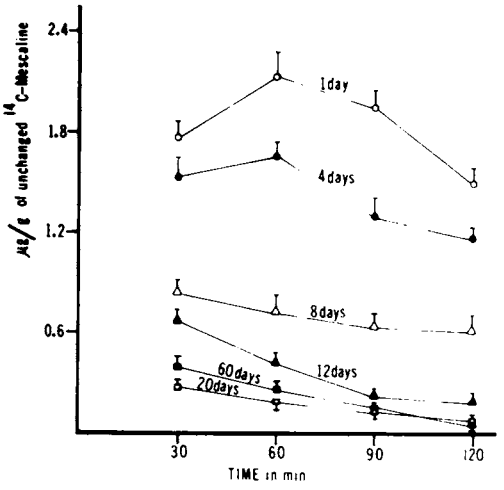

FIG. 5. *Concentrations of unchanged ^{14}C-mescaline in the spleen.*

See fig. 1 for details.

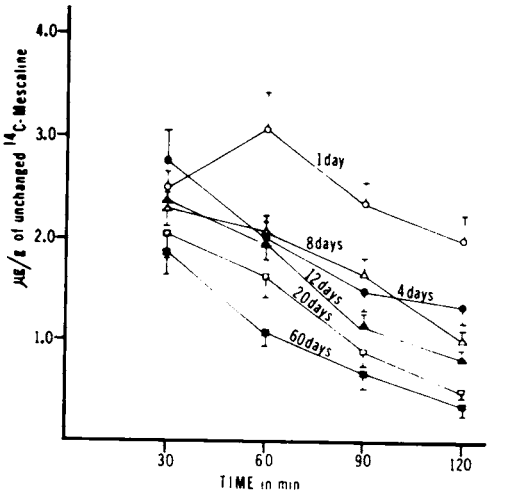

FIG. 6. *Concentrations of unchanged ^{14}C-mescaline in the lung.*

See fig. 1 for details.

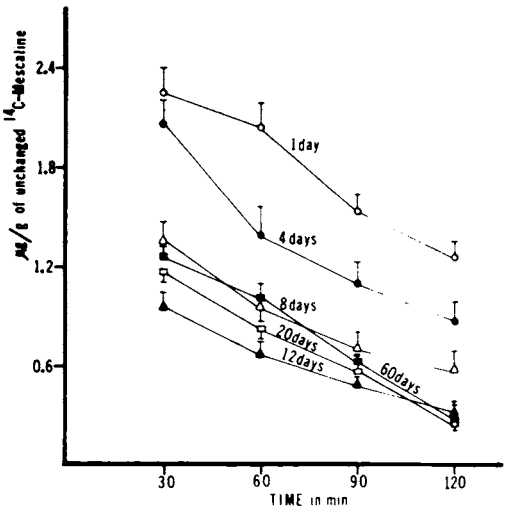

FIG. 7. *Concentrations of unchanged ^{14}C-mescaline in the kidney.*

See fig. 1 for details.

TABLE 1

Brain and hepatic levels of ^{14}C-TMPA in developing rats injected ip with ^{14}C-mescaline (50 nCi/g body weight) and killed at various time intervals

Data are expressed as μg per g of tissue ± SD, and as per cent of total ^{14}C. Each value represents the mean of 4–8 separate observations.

Time of Death	^{14}C-TMPA at											
	1 Day		4 Days		8 Days		12 Days		20 Days		60 Days	
min	μg/g	%	μg/g	%	μg/g	%	μg/g	%	μg/g	%	μg/g	%
Brain												
30	0.051 ± 0.008	5	0.044 ± 0.006	6	0.017 ± 0.004	6	0.019 ± 0.003	7	0.04 ± 0.007	22	0.027 ± 0.004	24
60	0.054 ± 0.012	5	0.073 ± 0.015	8	0.065 ± 0.010	13	0.025 ± 0.003	6	0.052 ± 0.01	28	0.016 ± 0.002	23
90	0.066 ± 0.006	6	0.063 ± 0.008	7	0.061 ± 0.009	12	0.032 ± 0.005	14	0.013 ± 0.003	27	0.006 ± 0.0008	27
120	0.071 ± 0.012	7	0.046 ± 0.005	6	0.045 ± 0.011	12	0.025 ± 0.006	14	0.006 ± 0.001	23	0.002 ± 0.0003	27
Liver												
30	0.91 ± 0.13	21	0.69 ± 0.12	18	0.35 ± 0.06	19	0.37 ± 0.06	23	0.46 ± 0.04	35	0.92 ± 0.17	43
60	0.95 ± 0.14	19	1.06 ± 0.26	23	0.54 ± 0.09	30	0.40 ± 0.05	28	0.51 ± 0.06	43	0.75 ± 0.09	51
90	0.66 ± 0.09	18	0.77 ± 0.07	24	0.47 ± 0.07	30	0.38 ± 0.05	34	0.38 ± 0.07	48	0.40 ± 0.04	57
120	0.57 ± 0.10	20	0.64 ± 0.10	23	0.34 ± 0.05	31	0.15 ± 0.03	33	0.13 ± 0.03	38	0.09 ± 0.01	44

but ^{14}C-TMPA recovered as per cent of total ^{14}C increased; *e.g.* in 8- and 12-day-old rats the levels were 0.017–0.065 μg/g and ranged from 6–14% of total ^{14}C, and in 20- and 60-day-old rats the levels were 0.002–0.052 μg/g and ranged from 22–28% of total ^{14}C. Another interesting observation was that in the older group of animals (20–60 days old) the 90- and 120-min levels were lower than the 30- and 60-min levels. Although the absolute levels of ^{14}C-TMPA in the liver were higher than those in the brain, a similar trend toward age-related decreases in the absolute levels and increases in the per cent of total radioactivity was observed. Furthermore, the decrease in absolute levels in relation to time of death was observed at all ages.

Discussion

In the present study the accumulation of ^{14}C-mescaline was examined in some organs of the rat from birth to adulthood. The rapid tissue distribution of mescaline was evidenced by the fact that 30 min after ip injection, all the organs examined showed significant amounts of mescaline. The organs of younger animals accumulated much larger amounts than those of the adult rats. In general, the uptake of mescaline during ontogenic development showed a tendency to decrease with age. Although the main site of action of mescaline is the brain, this organ accumulated the lowest amounts of mescaline in comparison with the peripheral tissues examined. In the time study, the initial higher concentrations of ^{14}C-mescaline in the brains of 1- to 12-day-old rats as compared to those in 20- or 60-day-old animals imply that more mescaline entered the brain from the periphery. The prolonged time curve of disappearance of mescaline in the younger animals is perhaps due to deficient mechanisms that remove mescaline from the brain. A definitive and marked decline in brain to plasma ratios was observed with advancing age. The tissue to plasma ratios for the liver, heart, and spleen were also reduced but the reduction was comparatively much smaller. On the other hand, the tissue to plasma ratios for the kidney and the lung were either unchanged or increased. A generalized decrease in tissue permeability with development should give a similar decrease for tissue to plasma ratios for all tissues. The marked decrease in brain to plasma ratios attendant with advancing age could be due to the development of the b-b-b in addition to the generalized decrease in tissue permeability. Support for this suggestion is derived from the reported maturation between the 7th and 9th day postnatally in rats of the b-b-b for the neurotoxic action of 6-hydroxydopamine, which is structurally similar to mescaline (7). Our data indicate that the development of the b-b-b for mescaline occurs in a bimodal manner. A marked reduction in uptake on the 8th day indicates that in the first stage a rapid development of the b-b-b occurs between 4 and 8 days after birth. The second stage appears between 12 and 20 days after birth. In the adult animals, the barrier is not completely impermeable since small amounts of mescaline do penetrate the b-b-b.

In addition to incomplete development of the b-b-b and deficient mechanisms for the removal of drugs in the neonatal subjects, the low conversion of a parent drug to its inactive metabolite may greatly influence drug-host interactions on an age

continuum. Numerous studies have demonstrated lower levels of drug-metabolizing enzymes in young *vs.* adult animals (8–10). Glowinski *et al.* (11) have shown that although catechol-O-methyltransferase and monoamine oxidase enzymes are present in the fetus and newborn rats, their activities are less than 20% of those observed in adult animals. Earlier studies by Friedhoff and Goldstein (12) have shown that in adult rats mescaline is converted primarily to the corresponding acid, TMPA, by oxidative deamination, and that pretreatment with iproniazid exerts a marked inhibitory action on the formation of this acid from mescaline. Although the absolute levels of TMPA in the brain and liver decreased with development, the relationship was reversed when TMPA levels were expressed as per cent of total ^{14}C (table 1). An increased accumulation of mescaline and low metabolic activity in the tissues of younger animals *vs.* a decreased accumulation and greater metabolic activity in the tissues of older animals may account for the reciprocal relationship observed in the present study.

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