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Pharmacokinetics and Bioavailability of Intravenous and Topical Nitroglycerin in the Rhesus Monkey: Estimate of Percutaneous First-Pass Metabolism

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Abstract □ [^{14}C]Nitroglycerin was administered intravenously and topically to three rhesus monkeys and the pharmacokinetics were determined. The rhesus monkey is an animal model for which percutaneous absorption is similar to that in the human. After intravenous administration the decline in plasma nitroglycerin concentration was biexponential with an initial half-life of 0.8 min (2–5 min postadministration) and a terminal half-life of 18 min (5–60 min postadministration). After topical application in an ointment, plasma concentrations of unchanged nitroglycerin were first detectable at 0.25 hr postapplication. Peak plasma nitroglycerin concentrations occurred between 4–6 hr, and nitroglycerin was still detectable at 24 hr postapplication. Plasma levels fit a biexponential curve with an α-phase half-life of 3.0 hr, a β-phase half-life of 4.3 hr, and a lag time of 0.5 hr. The absolute bioavailability of topical nitroglycerin was 56.6 ± 5.8%. The differences in bioavailability estimates between unchanged nitroglycerin and total carbon-14 is considered to be the amount of nitroglycerin which is metabolized as it is absorbed through the skin (percutaneous first-pass effect). This value for topical nitroglycerin was quite small, only 16–21% depending on the method of comparison.

Keyphrases □ Nitroglycerin—percutaneous absorption pharmacokinetics in the rhesus monkey, comparison of intravenous and topical administration bioavailability □ Pharmacokinetics—nitroglycerin in the rhesus monkey, comparison of intravenous and topical administration bioavailability □ Bioavailability—topical nitroglycerin in the rhesus monkey, comparison with intravenous administration, pharmacokinetics □ Percutaneous absorption—topical nitroglycerin, determination of first-pass effect

Nitroglycerin is a drug shown to be effective in angina pectoris (1–4) which may be effective in other cardiac diseases (5). The usual dosage form is a sublingual tablet, which has the disadvantage of a short duration of action. Nitroglycerin is also administered orally; however, first-pass metabolism during absorption is estimated to be large (6, 7). A third route of administration is transdermal delivery. Transdermal administration results in a longer duration of action than sublingual administration and may bypass the first-pass metabolism seen with oral administration. The result is a dosage form that delivers nitroglycerin over an extended time period. Recent publications attest to the clinical effectiveness of topically applied nitroglycerin (4, 5). Thus, it is important to determine the pharmacokinetic parameters of nitroglycerin after topical administration. This information can help in understanding the effectiveness of the drug and perhaps can be used to improve nitroglycerin therapy.

The animal model chosen for the study of the transdermal delivery of nitroglycerin was the rhesus monkey. Percutaneous absorption of several compounds in the rhesus monkey have been shown to be similar to that in the human (8–11).

EXPERIMENTAL

Female rhesus monkeys weighing ~4–6 kg were used. The monkeys were lightly anesthetized with 50–100 mg ketamine[1] for placement in the metabolism chairs. The monkeys were restrained in the metabolism chairs for 24 hr after drug administration (length of topical application) and then returned to metabolism cages for continued blood and urine collection. The monkeys had free access to food and water in the metabolism cages; they were hand fed and watered while confined to the metabolism chairs. In the topical application studies, the wrists of the monkey were taped to the sides of the chair to prevent the monkey from removing the applied dose. Each animal had an indwelling catheter in the saphenous vein for blood collection; a saline drip was connected to the cathether while blood samples were not being collected.

[^{14}C]Nitroglycerin was prepared from uniformly labeled glycerol and had a specific activity of 128 μCi/mg. The synthetic material was purified by chromatography on silica gel (toluene) followed by further purification using high-performance liquid chromatography (HPLC) on a μ-Bondapack C-18 column (water–methanol, 60:40) at a flow rate of 2 ml/min. The chemical and radiochemical purity, as determined by HPLC and TLC, was 99%.

The intravenous dosage form was 1.92 mg of labeled nitroglycerin with a specific activity of 250 μCi in 0.5 ml of ethanol. Administration was by bolus injection in the saphenous vein of the noncatheterized leg. The topical dosage form was 2% nitroglycerin ointment[2] containing 19.0 mg of labeled nitroglycerin (specific activity 210 μCi) spread over a 50-cm^2 area of skin. [^{14}C]Nitroglycerin was added to the ointment using the procedure of Lindsay *et al.* (12). Topical administration was to the inner upper arm. The area was lightly clipper shaved, which does not affect percutaneous absorption in the rhesus monkey (8). [The application site also has been determined not to affect the percutaneous absorption of nitroglycerin in the rhesus monkey (13).] The topical application was occluded with aluminum foil and adhesive tape. The ointment was left in place for 24 hr, and then the site was washed with soap and water. There was a 1-week period between drug administrations.

At each sampling time, ~3 ml of blood was drawn and placed in a

[1] Ketaset; Bristol Laboratories, Syracuse, N.Y.
[2] Nitro-BID; Marion Laboratories, Kansas City, Mo.

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Table I—Plasma Levels of Nitroglycerin Following Intravenous and Topical Administration

Time, hr	Nitroglycerin Concentration, ng/ml[a]	
	Intravenous	Topical
0.0	<0.1	<0.1
0.02	80.7 ± 5.5	—[b]
0.03	200.7 ± 31.0	—
0.05	90.3 ± 9.9	—
0.08	35.1 ± 5.7	—
0.25	17.6 ± 2.8	0.3 ± 0.03
0.5	9.0 ± 0.8	0.7 ± 0.3
0.75	3.5 ± 0.6	1.0 ± 0.4
1.0	1.8 ± 1.0	1.9 ± 0.5
2.0	<0.1	2.9 ± 0.4
3.0	<0.1	4.6 ± 1.1
4.0	—	6.8 ± 0.3
6.0	<0.1	6.7 ± 0.4
8.0	—	5.4 ± 0.3
12.0	—	3.6 ± 0.4
24.0	<0.1	0.7 ± 0.2
48.0	<0.1	<0.1
72.0	<0.1	<0.1

[a] Mean ± *SEM* for three monkeys. [b] — Plasma samples not drawn.

Table II—Plasma Levels of Carbon-14 Following Intravenous and Topical Administration

Time, hr	Concentration, ng equivalents/ml[a]	
	Intravenous	Topical
0.0	0	0
0.02	386 ± 54	—[b]
0.03	289 ± 48	—
0.05	292 ± 15	—
0.08	259 ± 3	—
0.25	338 ± 27	8 ± 4
0.5	353 ± 6	24 ± 12
0.75	340 ± 7	34 ± 17
1.0	333 ± 10	57 ± 25
2.0	314 ± 13	177 ± 41
3.0	295 ± 13	315 ± 69
4.0	—	426 ± 64
6.0	220 ± 24	616 ± 69
8.0	—	762 ± 46
12.0	—	917 ± 45
24.0	62 ± 7	754 ± 54
48.0	37 ± 2	374 ± 25
72.0	28 ± 2	249 ± 28

[a] Mean ± *SEM* for three monkeys. [b] — Plasma samples not drawn.

heparinized tube. The tube was centrifuged immediately (5 min from start-up to finish); the plasma was removed, placed in a vial containing 50 μl of 1.0 *M* silver nitrate for protein precipitation, and mixed. The samples were then frozen in a mixture of isopropyl alcohol and solid carbon dioxide. The majority of each plasma sample (1.0 ml) was utilized for nitroglycerin assay; a small aliquot (0.1 ml) was utilized for carbon-14 assay.

Blood sampling times following intravenous administration were 0, 1, 2, 3, 5, 15, 30, and 45 min and 1, 2, 3, 6, 24, 48, and 72 hr. Blood was withdrawn following topical administration at 0, 15, 30, and 45 min and 1, 2, 3, 4, 6, 8, 12, 24, 48, and 72 hr. Urine samples for carbon-14 analysis were collected at 0–24, 24–48, and 48–72 hr.

A GC method modified from Yap *et al.* (14) was used to analyze the nitroglycerin content in the monkey plasma. Nitroglycerin and the internal standard, isosorbide dinitrate, were extracted from plasma with hexane. The extract was then evaporated, reconstituted with ethyl acetate, and analyzed by GC[3] using an electron-capture detector. A glass column (1.2 m × 2 mm) was packed with 3% OV-1 liquid phase on Chromosorb W-HP, 100–200 mesh[4]. The injection port, column, and detector temperatures were 200°, 110°, and 200°, respectively. The carrier gas was 5% methane–argon and had a flow rate of 25 ml/min. The retention times for nitroglycerin and isosorbide dinitrate were 1.8 and 6.0 min, respectively.

Bioavailability of nitroglycerin was determined by the ratios of the areas under the concentration–time curves (AUC $0 \rightarrow \infty$) for intravenous and topical plasma nitroglycerin and plasma carbon-14. The trapezoidal area was measured to the last data point, and the remaining area was determined using the terminal slope of the concentration–time curve. The ratio of the topical AUC to the intravenous AUC is the absolute bioavailability of nitroglycerin following topical administration. Bio-

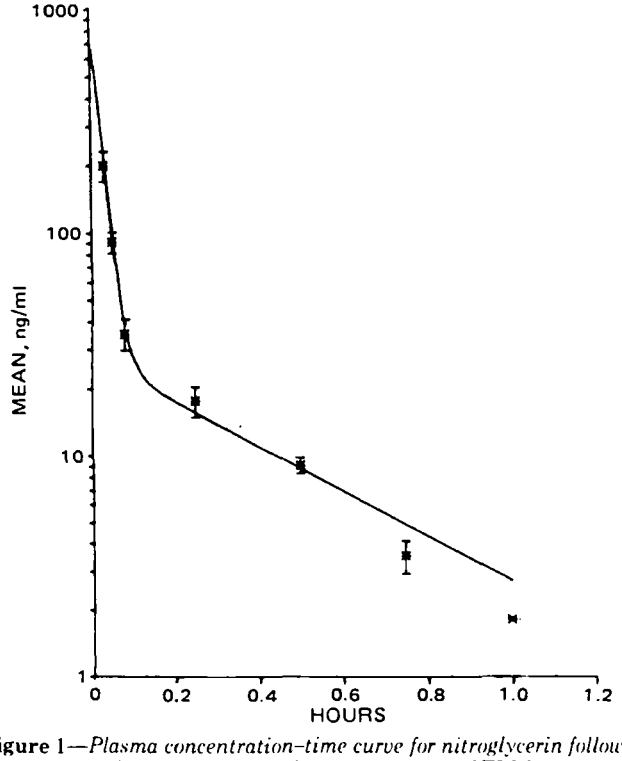

Figure 1—*Plasma concentration–time curve for nitroglycerin following intravenous administration. The bars represent the* SEM *for each mean value.*

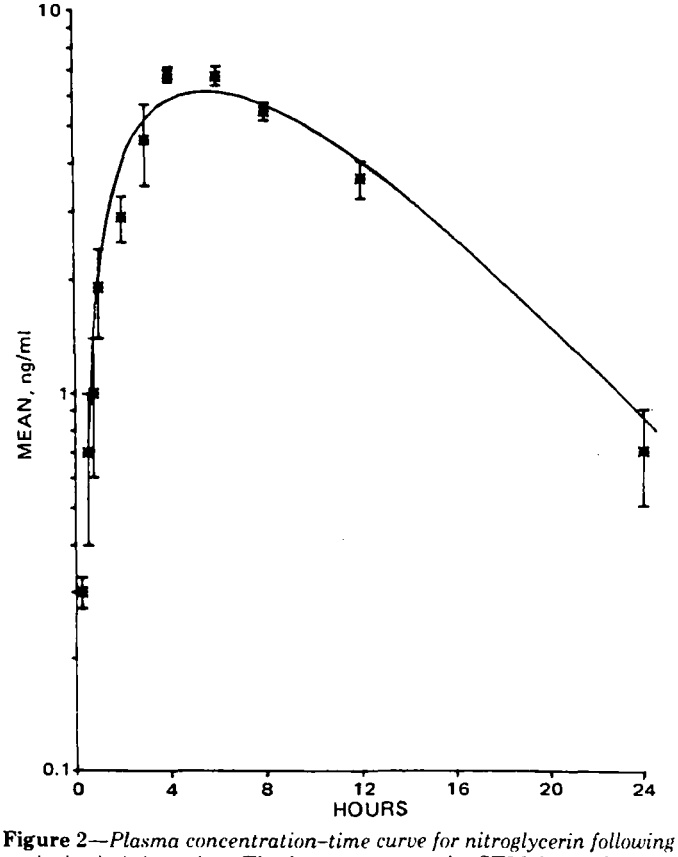

Figure 2—*Plasma concentration–time curve for nitroglycerin following topical administration. The bars represent the* SEM *for each mean value.*

[3] Hewlett-Packard Model 5710A; Avondale, Pa.

[4] Ohio Valley Specialty Chemical Inc., Marietta, Ohio.

Table III—Urinary Excretion of Carbon-14 Following Intravenous and Topical Administration of [^{14}C]Nitroglycerin

Time, hr	Excretion, % of dose[a]	
	Intravenous	Topical
0–24	50.9 ± 4.2	30.9 ± 3.9
24–48	2.4 ± 0.3	7.0 ± 0.2
48–72	0.3 ± 0.2	0.9 ± 0.03
0–72	53.7 ± 3.9	38.8 ± 3.7

[a] Mean ± *SEM* for three monkeys.

availability of nitroglycerin was also determined from the urinary excretion of carbon-14 following topical and intravenous administration. Blood levels were fit to the appropriate pharmacokinetic model using NONLIN (15).

The absolute bioavailability of nitroglycerin and carbon-14 was estimated by:

$$\text{percentage} = \frac{\text{AUC topical/topical dose}}{\text{AUC intravenous/intravenous dose}} \times 100 \quad \text{(Eq. 1)}$$

where the AUC is expressed as ng hr ml^{-1}. Bioavailability was estimated from urinary excretion using:

$$\text{percentage} = \frac{\text{total [}^{14}\text{C] excretion topical administration}}{\text{total [}^{14}\text{C] excretion intravenous administration}} \times 100 \quad \text{(Eq. 2)}$$

RESULTS

Table I and Fig. 1 show the plasma concentrations of unchanged nitroglycerin following intravenous administration. The decline in plasma nitroglycerin concentration was biexponential with an initial half-life of 0.8 min (2- to 5-min interval) and a terminal half-life of 18 min (5- to 60-min interval). The first data point (0.02 min) was not used in the NONLIN analysis.

Table I and Fig. 2 show the plasma concentrations of unchanged nitroglycerin following topical administration of the ointment. Nitroglycerin was first detected at 0.25 hr postapplication. Peak plasma nitroglycerin concentration occurred between 4 and 6 hr and nitroglycerin was still detectable at 24 hr postapplication. The NONLIN analysis best fit a biexponential curve with an α-phase half-life of 3.0 hr, a β-phase half-life of 4.3 hr, and a lag time of 0.5 hr.

Table II gives the plasma concentrations of carbon-14 following intravenous and topical administration of [^{14}C]nitroglycerin. After intravenous administration, plasma concentrations of carbon-14 fluctuated for the first 3 hr, probably due to metabolite formation. Levels then declined and by 72 hr had decreased 10-fold. After topical administration, plasma carbon-14 concentrations did not peak until 12 hr postadministration. Urinary excretion of carbon-14 was also determined (Table III). Within 72 hr 53.7 ± 3.9 (*SEM*) % of the intravenous dose and 38.8 ± 3.7% of the topical dose were excreted. Most of the radioactivity was excreted in the first 24 hr. Radioactivity not accounted for in urinary excretion probably was excreted by some other route (*e.g.*, feces).

Based on the data given in Tables I–III, the topical bioavailability of nitroglycerin was estimated by three methods; these are summarized in Table IV. The absolute bioavailability of topical nitroglycerin, determined from the ratio of the nitroglycerin AUC values following intravenous and topical administrations, was 56.6 ± 2.5% for the three monkeys. Bioavailability determined from the ratio of the plasma carbon-14 AUC values was 77.2 ± 6.7%, and the estimate from the urinary excretion of carbon-14 was 72.7 ± 5.8%.

The difference in bioavailability estimates between unchanged nitroglycerin and total carbon-14 is the amount of nitroglycerin which is metabolized as it is absorbed through the skin (percutaneous first-pass effect). By comparison of the plasma nitroglycerin AUC with the plasma carbon-14 AUC, the estimate of first-pass percutaneous metabolism is 20.6%; by comparison to urine carbon-14 excretion, the estimate is 16%. Therefore, we can conclude that the percutaneous first-pass effect is quite small, only 16–21% depending on the method of comparison.

DISCUSSION

Yap and Fung (16) studied the pharmacokinetics of nitroglycerin in rats after intracardiac, oral, and topical administrations. Nitroglycerin had a half-life of ~4 min after intracardiac administration and showed "flip-flop" kinetics after oral administration. The oral bioavailability was

Table IV—Bioavailability of Topical Nitroglycerin[a]

Pharmacokinetic Parameter	Monkey			Mean Bioavailability[b]
	1	2	3	
Dose, mg				
Intravenous	1.92	1.92	1.93	
Topical	18.0	19.7	19.4	
Plasma nitroglycerin AUC, ng hr ml^{-1}				
Intravenous	22.35	18.48	16.60	
Topical	127.98	99.75	93.82	
Bioavailability, %	61.1	52.5	56.2	56.6 ± 2.5
Plasma total radioactivity AUC, μg hr ml^{-1}				
Intravenous	6.77	6.92	8.22	
Topical	48.65	63.32	54.44	
Bioavailability, %	76.6	89.2	65.9	77.2 ± 6.7
Urinary total radioactivity				
Intravenous, % dose excreted	60.2	46.6	53.6	
Topical, % dose excreted	39.1	32.2	45.1	
Bioavailability, %	65.0	69.1	84.1	72.7 ± 5.8

[a] Determined from the plasma nitroglycerin AUC, plasma carbon-14 AUC, and urinary excretion of carbon-14. [b] Mean ± *SEM*.

1.6%, supporting the concept of extensive first-pass metabolism (17). No detectable levels of nitroglycerin after topical administration were found (16). However, it was suggested later (18) that this may have been due to site-dependent absorption. Site-dependent absorption also occurs in the rhesus monkey and the human (19); however, this does not occur with the topical administration of nitroglycerin in the rhesus monkey (13).

In the human, information on the topical administration of nitroglycerin is limited. In one subject, nitroglycerin was not detected until 20 min after topical application (20). When the study was stopped (60 min) nitroglycerin was still detected. This suggested a sustained delivery of nitroglycerin since administration of a sublingual tablet afforded measurable plasma nitroglycerin levels at 3 min and no detectable amounts at 16 min. Maier-Lenz *et al.* (21) similarly reported that the half-life of nitroglycerin with sublingual administration was 8 min, but with topical administration a lag time of 14 min existed and measurable levels of nitroglycerin were present through a 3-hr period. Clinically, the effectiveness of topical nitroglycerin in patients with angina has been demonstrated for up to 8 hr after administration (22).

The data in this study agree with the literature reports on sublingual and topical nitroglycerin administration. Intravenously administered nitroglycerin disappeared from the blood very rapidly with a terminal half-life of 18 min. With topical administration there was a lag time of 0.5 hr with detectable levels of nitroglycerin occurring through a 24-hr period with peak levels at 4–6 hr postapplication. Topical nitroglycerin ointment can be considered a sustained-release dosage form, especially when compared with a sublingual tablet.

The pharmacokinetics of topical nitroglycerin administration are of interest since the short half-life seen with intravenous administration was not present. Instead, the plasma concentration–time curve was a broad curve with an α-phase half-life of 3 hr and a β-phase half-life of 4.3 hr. It seems reasonable that these phases of the curve are part of the complex absorption process and that any true elimination phase for nitroglycerin is lost among the absorption kinetics. It should be stated also that the AUC values following topical administration may be overestimated due to the inability to accurately calculate β. The degree of error for the total is dependent on the degree of error in β and the fraction of the total AUC composed of the fraction from 24 hr. However, this degree of error probably is small since the extrapolated area fraction involves only ~5% of the total AUC.

The absolute bioavailability of nitroglycerin following topical administration was 56.6%. There are no literature values for comparison. The mean bioavailability in male volunteers of isosorbide dinitrate from an ointment was ~30% of the bioavailability from the sublingual tablet (23). Plasma levels of isosorbide dinitrate were present up to 120 min following sublingual administration and up to 32 hr following topical administration. These estimates of 30–60% are certainly greater than the very low bioavailability estimates for oral preparations.

Nitroglycerin bioavailability measurements using the plasma AUC (total carbon-14) method were similar to those obtained by total urinary excretion of the radioactive label. Estimates from carbon-14 analysis were only 16–21% above the absolute bioavailability, a difference probably due to percutaneous first-pass effect. Use of plasma carbon-14 AUC and total urinary carbon-14 excretion to estimate percutaneous absorption has

been reported previously (24). The methods seem appropriate for estimates of transdermal absorption if a specific analytical procedure is not available and percutaneous first-pass metabolism is not extensive.

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N-Acetyl-D-Mannosamine Analogues as Potential Inhibitors of Sialic Acid Biosynthesis

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Abstract □ The 1,3,6-tri-*O*-acetyl and 1,3,6-tri-*O*-acetyl-4-*O*-mesyl analogues of *N*-acetyl-D-mannosamine and the corresponding *N*-trifluoroacetyl derivative have been synthesized, and their effects on the proliferation of Friend erythroleukemia cells in culture have been evaluated. The acetamido series showed a dependency on the 4-substituent for optimum cytotoxicity while the trifluoroacetamido series did not. Thus, the 1,3,4,6-tetra-*O*-acetyl and 1,3,6-tri-*O*-acetyl-4-*O*-mesyl analogues of *N*-acetyl-D-mannosamine were 10-fold and 42-fold more active, respectively, than 2-acetamido-1,3,6-tri-*O*-acetyl-2-deoxy-α-D-mannopyranose as inhibitors of cellular replication. The corresponding trifluoroacetamido analogues were essentially equiactive and had a potency equivalent to that of the 4-*O*-mesyl derivative in the acetamido series.

Keyphrases □ *N*-Acetyl-D-mannosamine—synthesis of acetamido and trifluoroacetamido analogues, effects on the proliferation of Friend leukemia cells □ Analogues—of *N*-acetyl-D-mannosamine, acetamido and trifluoroacetamido series, synthesis, effects on the proliferation of Friend leukemia cells □ Antileukemic agents—potential, acetamido and trifluoroacetamido analogues of *N*-acetyl-D-mannosamine, tested against Friend leukemia cells

2 - Acetamido - 1,3,4,6 - tetra - *O* - acetyl - 2 - deoxy - β-D-mannopyranose (I), the peracetylated analogue of *N*-acetyl-D-mannosamine, a metabolic precursor in the biosynthetic pathway for sialic acid (1) (Fig. 1), and the corresponding trifluoroacetamido analogue (V) were recently reported to be inhibitors of the growth of Friend erythroleukemia cells in culture (2). Both analogues were equipotent inhibitors of the incorporation of [^{3}H]*N*-acetyl-D-mannosamine into the glycoprotein-bound sialic acid of Friend erythroleukemia cells (2); however, different enzymatic sites appeared to be involved. Compound I caused an accumulation of radioactivity from [^{3}H]*N*-acetyl-D-mannosamine in *N*-acetylneuraminic acid and a decrease in cytidine monophosphate-*N*-acetylneuraminic acid in the ethanol-soluble metabolites of cells, while V caused an accumulation of [^{3}H]cytidine monophosphate-*N*-acetylneuraminic acid. In addition, both I and V produced an increase in the amount of neuraminidase-hydrolyzable sialic acid-like material on the surface of treated cells, presumably as a result of their metabolic utilization and incorporation into cellular macromolecules (3).

Since intracellular deacetylation must precede metabolism of these analogues along the sialic acid biosynthetic pathway, we presumed that I would ultimately give the noncytotoxic metabolite, *N*-acetyl-D-mannosamine; it was therefore surprising to find that I was relatively active as an inhibitor of cellular replication. Consideration of the hydroxyl groups which are required for the conversion of *N*-acetyl-D-mannosamine to *N*-acetylneuraminic acid-9-phosphate (1) (Fig. 1), reveals that such metabolic conversion only necessitates the removal of acetyl groups from the 1, 3, and 6 positions of I, thereby suggesting that the 4-*O*-acetyl group remains intact and is responsible for the

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