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Interaction of Cationic Drugs with Nafion on Electrode Surfaces and Its Applicability to Determination of Electroinactive Drugs

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The interaction of several electroinactive cationic drugs (hexamethonium, quinidine, neostigmine, nicotine and acetylcholine) with Nafion and the applicability of Nafion-coated electrodes to determination of the drugs have been investigated. The addition of a drug to an electrolyte solution containing (ferrocenylmethyl)trimethylammonium (FA^+) induced a decrease in the peak current and a shift in the apparent half-wave potential of a cyclic voltammogram for FA^+. The decrease in the peak current has been used for determination of the drugs at low concentrations less than 10^{-3} mol dm^{-3}. The ion-selectivity coefficients of Nafion for the drugs have been determined by an electrochemical method. These drugs showed quite large values and the values seemed to be dependent on the hydrophobicity of the drugs.

Du Pont's Nafion, a perfluoro sulfonated ion-exchange polymer, has been receiving a great deal of attention as a modifier for polymer-coated electrodes mainly because of its outstanding chemical stability and excellent ionic conductivity. Among extensive studies[1] dealing with mechanisms of charge and mass transportation as well as catalytic properties, some attempts[2-4] have recently been made to use the Nafion-coated electrodes for determination of electroactive compounds.

Nafion has two important aspects which are useful for electroanalytical purposes. One is its preconcentrating ability[5,6] of cations by electrostatic interaction between the cations and negatively charged sulfonato group in Nafion. Thus, amperometric determination of quite low concentration of electroactive materials becomes possible on the preconcentrated electrodes. Szentirmay and Martin[2] demonstrated that 10^{-8} mol dm^{-3} of methyl viologen could be detected on the Nafion-coated electrode by cyclic voltammetry. This detection limit is about three orders lower than that observed on a bare electrode. The lowering of the effective diffusion coefficient in the Nafion matrix is apparently unfavorable for the electroanalytical purpose, but the favorable preconcentrating effect more than compensates the unfavorable lowering. The other is ionic selectivity. Previous investigations[7,8] have proved that Nafion favors hydrophobic organic cations as compared with hydrophilic inorganic cations. This phenomenon is caused by hydrophobic interaction between fluorocarbon network of the polymer and substrates. There is a possibility for selective determination of organic cations in the presence of hydrophilic cations.

The important advantage of the electrochemical determination over the spectroscopic one widely used currently is that only the vicinity of the electrode surface is necessary in the former method. Therefore, the preconcentrated layer can be very thin; in extreme case, a monolayer is enough to detect the adsorbed species.[9-11] This situation is usually difficult in the spectroscopic method. We report here the indirect determination of electroinactive cationic drugs with Nafion-coated electrodes, taking advantages of the strong interaction of such drugs with Nafion. Indirect electrochemical determination[12,13] is one of the attractive methods since it removes the limitation of direct electrochemical determination. The present method is based on the competitive partitioning effect between an electroactive compound and cationic drugs into Nafion layer, and has been applied to several ammonium drugs using (ferrocenylmethyl)trimethylammonium ion (FA^+) as an electroactive chemical agent. Since the drugs have shown strong affinity with Nafion, the electroinactive drugs less than 10^{-3} mol dm^{-3} level have been determined by voltammetry.

Eperimental

A Nafion solution (Nafion 117, 1100 EW, 5% solution) was obtained from Aldrich Co. and was diluted with methanol to give a 0.025% solution. (Ferrocenylmethyl)trimethylammonium (FA^+) perchlorate was prepared from the iodide salt of FA^+ (Tokyo Kasei Co.) by using aqueous solution of $NaClO_4$ and was recrystallized three times from water. Five drugs were obtained from the commercial sources: hexamethonium chloride (*N,N'*-hexamethylenebis(trimethylammonium chloride), autonomic ganglionic blocking drug), Wako chemicals; acetylcholine chloride (parasympathomimetic drug), Kanto Chemicals; neostigmine bromide ([3-(dimethylcarbamoyloxy)phenyl)trimethylammoniumbromide, parasympathomimetic drug), Wako Chemicals; nicotine (autonomic ganglionic blocking drug), Kanto Chemicals; quinidine sulfate (antiarrhythmic drug), Nakarai Chemicals. The structures of these drugs are shown drugs are shown in Fig. 1.

Cyclic voltammetry was carried out with a PAR model 273. The cell was a water-jacket type and kept at 25 °C. The working electrode was a 3 mm diameter glassy carbon (GC) disk (GC-20, Tokai Carbon Co.) mounted in a Teflon rod and was polished finally with alumina powder (50 nm average particle size, Buehler Co.) to give a mirror-like surface. A

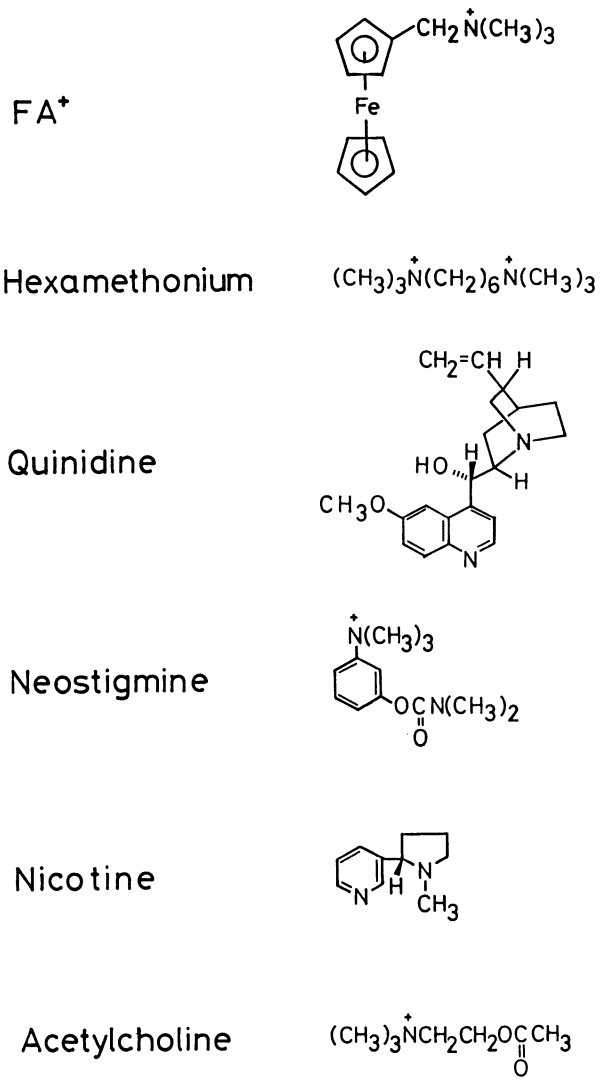

Fig. 1. Structures of drugs.

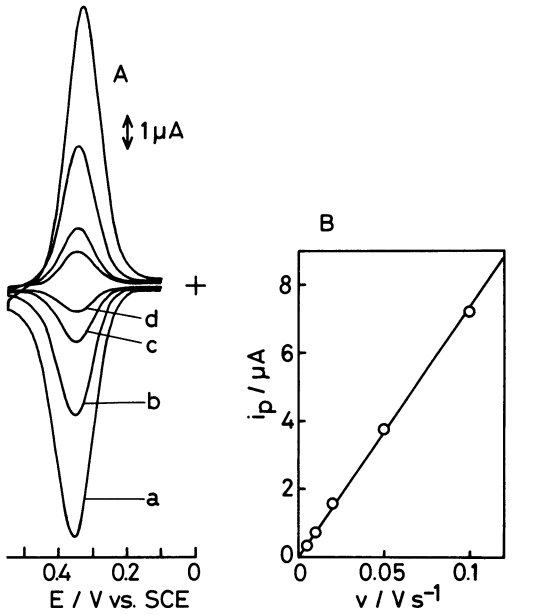

Fig. 2. (A) Cyclic voltammograms of FA^+ on the Nafion-coated GC in 0.1 mol dm^{-3} Na_2SO_4. Scan rate: a, 0.1; b, 0.05; c, 0.02; d, 0.01 V s^{-1}. Concentration of FA^+ in the solution: 7.2×10^{-6} mol dm^{-3}. (B) Relationship between the anodic peak current (i_p) and scan rate (v).

Pt wire was used as a counter electrode and the potentials were referenced to a saturated calomel electrode (SCE). Nafion coating was prepared by pipetting 2 mm^3 of the 0.025% Nafion solution onto a clean GC surface and allowing the solvent to evaporate at a room temperature. This process was repeated several times using methanol without Nafion to avoid island-like surface coatings. These procedures are important to obtain stable coatings and reproducible results.

Results and Discussion

Voltammetry of FA^+ on the Nafion-Coated Electrode. Figure 2A shows the cyclic voltammograms for FA^+ on the Nafion-coated electrode at different scan rates. The shape indicates near-ideal behavior of a thin film.[14] The peaks are almost symmetrical with very little diffusional tails at the anodic region. The peak current is clearly proportional to the scan rates when the scan rate is less than 200 mV s^{-1} as shown in Fig. 2B. The peak width at half-peak height is 120 mV which is larger than the theoretical value, 90 mV,[14] predicted for a surface species, which may indicate that the entrapped FA^+ exists under various circumstances. The small separation between the anodic and cathodic peaks (ca. 15 mV at 50 mV s^{-1}) would be mainly caused by the residual resistance of the polymer film.

The surface concentration of $-SO_3^-$ sites on the electrode is estimated to be 3.4×10^{-9} mol equivalent cm^{-2} from the loading amount of FA^+ entrapped in the Nafion and ion-exchange selectivity coefficient for FA^+.[2] Thus, the average thickness of the film is calculated to be ca. 24 nm by using the density of wet Nafion (1.58 g cm^{-3}) in Na^+ form.[15] This value agrees reasonably with the value (ca. 40 nm) estimated from the amount of the Nafion on the surface. Although the thickness of the Nafion film on the GC surface might be uneven, FA^+ in the outside solution equilibrates fairly rapidly. It takes only ca. 10 min to reach near equilibrium for the cationic exchange between Na^+ and FA^+ after the Nafion-coated electrode is dipped into the electrolyte solution containing 7.2x10^{-6} mol dm^{-3} FA^+. This can be compared with the results reported by Szentirmay and Martin[2] with a 1 μm thick Nafion film. Almost a day was necessary to reach ion-exchange equilibrium between Na^+ and methyl viologen.

The apparent half-wave potential of FA^+ observed on the Nafion-coated electrode is 0.34 V vs. SEC, which is ca. 50 mV negative from the value on a bare GC electrode. This difference in the half-wave potential indicates the stabilization[16-19] of the oxidized species by electrostatic interaction with the negativity charged sulfonato group. This phenomenon contrasts well

with that observed on a perfluorinated carboxylic acid polymer.[17]

Determination of Cationic Drugs on the Nafion-Coated Electrode. The concentration of FA^+ inside the Nafion membrane is susceptible to the presence of cations. Figure 3 shows the cyclic voltammograms observed on the Nafion-coated GC electrode with 7.2×10^{-6} mol dm^{-3} FA^+ and different concentrations of hexamethonium in the electrolyte solution. The addition of hexamethonium resulted in a decrease in the peak current for FA^+. This phenomenon clearly demonstrates that some amount of FA^+ initially trapped inside the Nafion membrane is replaced by hexamethonium added to the electrolyte solution. The extent of the decrease in the peak current is dependent on the concentration of hexamethonium in the solution and thus the determination of the concentration becomes possible by using this phenomenon. As shown in Fig. 3, the presence of 1.0×10^{-6} mol dm^{-3} hexamethonium in solution induces a clear decrease in the peak current for FA^+. We have used several other cationic drugs (acetylcholine, neostigmine, nicotine and quinidine) to clarify the influence of these drugs on the voltammetric response of FA^+ and to investigate the applicability of the Nafion-coated electrodes for determination of these drugs. The drugs induced the decrease in the oxidation peak current for FA^+ as was observed for hexamethonium. Figure 4 demonstrates the relationship between the $i_p/i_{p,o}$ value (where i_p and $i_{p,o}$ are the peak currents with and without drugs, respectively) and the concentration of the several drugs in the electrolyte solution. All drugs investigated here show a clear increase in the $i_p/i_{p,o}$ value in the fairly low concentration ranges of the drugs. Therefore, the concentration of a drug can be determined by using these curves. The detection limits of the drugs in the present procedure are as follows: hexamethonium, 2×10^{-7}; quinidine, 5×10^{-7}; neostigmine, 2×10^{-6}; nicotine, 1×10^{-5}; acetylcholine, 5×10^{-5} mol dm^{-3}.

In these measurements, the cyclic voltammograms were recorded after 5 min of immersion of the Nafion-coated electrode into the sample solution containing a specific amount of a drug. Hexamethonium, acetylcholine and neostigmine equilibrated fairly rapidly and a near equilibrium state was obtained after 5 min of immersion. Only small changes in the voltammetric peak current were observed on the subsequent scans. However, ca. 30 min was necessary to reach a near equilibrium state for quinidine. These differences in the equibration time seem to be mainly dependent on the size of the cation. For analytical purposes it is not necessary to obtain equilibrium response. The same measurements were carried out using a number of Nafion-coated GC electrodes. The results were quite reproducible and the variations of the relative peak current under different runs were within 5%. When the Nafion-coated GC electrode used for these measurements was transferred to the electrolyte solution containing 7.2×10^{-6} mol dm^{-3} FA^+ without any drug, the anodic peak current recovered to its original value. The Nafion film on the GC surface seems to be quite stable and the electrode can be used at least for several times.

Influence of the Added Drugs on the Apparent Half-wave Potentials and Voltammetric Shapes. The apparent half-wave potential ($E_{1/2,app}$) of FA^+ on the Nafion-coated GC is dependent on the loading level of

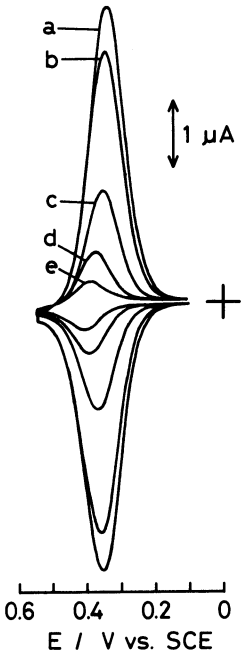

Fig. 3. Cyclic voltammograms of FA^+ in the presence of hexamethonium on the Nafion coated GC in 0.1 mol dm^{-3} Na_2SO_4. Concentration of FA^+ in the solution: 7.2×10^{-6} mol dm^{-3}. Concentration of hexamethonium in the electrolyte solution: a, 0; b, 1.0×10^{-6}; c, 1.0×10^{-5}; d, 1.0×10^{-4}; e, 1.0×10^{-3} mol dm^{-3}. Scan rate: 0.05 V s^{-1}.

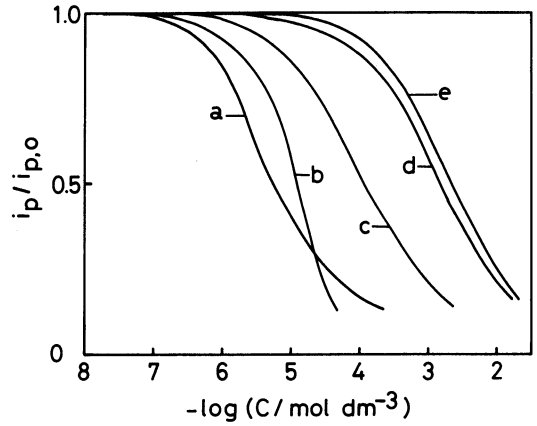

Fig. 4. Relationship between the relative peak current and concentration of drug. a: Hexamethonium. b: Quinidine. c: Neostigmine. d: Nicotine. e: Acetylcholine. Concentration of FA^+ in the solution: 7.2×10^{-6} mol dm^{-3}. Scan rate: 0.05 V s^{-1}.

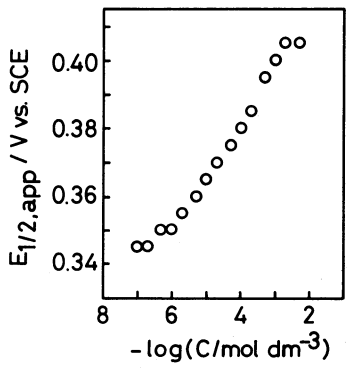

Fig. 5. Relationship between $E_{1/2,app}$ and concentration of hexamethonium in the electrolyte solution.

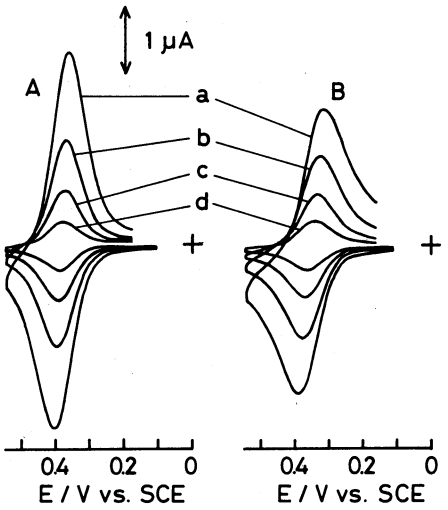

Fig. 6. Cyclic voltammograms of FA^+ on the Nafion coated GC in the presence of 2.0×10^{-4} mol dm^{-3} hexamethonium (A) and 2.0×10^{-5} mol dm^{-3} quinidine (B) in pH 7.0 phosphate buffer. Scan rate: a, 0.2; b, 0.1; c, 0.05; d, 0.02 V s^{-1}. Concentration of FA^+ in the solution: 7.2×10^{-6} mol dm^{-3}.

hexamethonium. Figure 5 shows the relationship between the $E_{1/2,app}$ value and the concentration of hexamethonium in the electrolyte solution. This concentration dependence corresponds well to that of the relative peak current shown in Fig. 4. The half-wave potential (0.34 V vs. SCE) observed on the Nafion-coated GC without hexamethonium is negative compared with the value on a bare GC (0.392 V vs. SCE). The $E_{1/2,\,app}$ value shifts to positive potentials with increasing concentration of hexamethonium and surpasses the value on a bare GC at hexamethonium concentration higher than 10^{-3} mol dm^{-3}. At the low concentration of hexamethonium, the $E_{1/2,\,app}$ value is governed by the stabilization[15-19] of the oxidized FA^+ by the favorable electrostatic interaction of the divalent cation (FA^{2+}) with the sulfonato groups in Nafion film. However, the incorporation of hexamethonium into the hydrophilic phase in the Nafion film provides a more hydrophobic environment relative to the original state.[19] This environmental change obviously destabilizes the highly charged species, which implies a positive shift of the $E_{1/2,app}$ of FA^+ entrapped in the Nafion film. Therefore, the shift in $E_{1/2,\,app}$ is caused by the compensation of extra stability due to electrostatic interaction by the increasing hydrophobicity of the ionic cluster.[19] This trend was also observed for other drugs.

The incorporation of bulky drugs also caused changes in the cyclic voltammetric shapes of FA^+. In Fig. 6 are shown the cyclic voltammograms of FA^+ on the Nafion-coated GC electrodes in the presence of hexamethonium (2.0×10^{-4} mol dm^{-3}) and quinidine (2.0×10^{-5} mol dm^{-3}). The peak heights at 50 mV s^{-1} are about two-tenth of those in the absence of the drugs. A significant difference in the cyclic voltammetric shape was observed. The voltammogram in the presence of quinidine in solution shows obvious diffusional tails after the anodic and cathodic peaks. In addition, the peak separation in this case was ca. 50 mV at 100 mV s^{-1}, which can be compared to ca. 20 mV observed in the original voltammogram. The peak separation gradually increased with increasing concentration of quinidine. These changes in the voltammetric shape are probably caused by the decrease in the apparent diffusion coefficient of FA^+ in the Nafion film since the anodic peak current is no longer linearly dependent on the scan rate. The incorporation of bulky quinidine into the ionic cluster will decrease the hydrodynamic mobility of FA^+ in the hydrophilic region.[19] Therefore, the cyclic voltammetric shape alters from the thin-layer behavior to the diffusion-controlled situation. On the other hand, such an effect on the cyclic voltammetric shape in the presence of hexamethonium in solution is small, suggesting no severe influence of entrapped hexamethonium on the ionic movement of FA^+ in the hydrophilic region. Similarly only small changes in the shape were observed when acetylcholine, neostigmine and nicotine were added to the electrolyte solution. The above may be a reason for the difference in curvature for quinidine in Fig. 4 as compared with those for other drugs.

Ion-Selectivity Coefficient for the Drugs. The present procedure for determination of cationic drugs is based on the competitive partitioning effect between FA^+ and the drug into Nafion, and thus the detection limits are largely dependent on the strength of the interaction. In order to obtain quantitative feature of the drug-Nafion interaction, the determination of steady-state concentration was also carried out. The strength of the interaction can be expressed by the ion-exchange selectivity coefficient, K^{D}_{Na},[2] which is the equilibrium constant for the following ion-exchange reaction:

$$D^{n+} + n(SO_3^-Na^+)_{Naf} \rightleftharpoons [(SO_3^-)_nD^{n+}]_{Naf} + nNa^+ \quad (1)$$

and given by

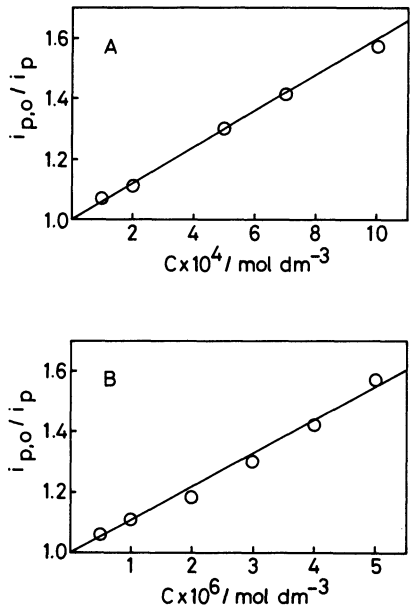

Fig. 7. Relationship between the $i_{p,o}/i_p$ value and concentration of acetylcholine (A) and quinidine (B) in pH 7.0 phosphate buffer. Concentration of FA^+ in the electrolyte solution: 1.0×10^{-5} mol dm^{-3}. Scan rate: 0.05 V s^{-1}.

Table 1. Ion-Selectivity Coefficients for Ammonium Drugs

Drug	Ion-selectivity coefficient
Acethylcholine	5.6×10^2
Hexamethonium	2.0×10^5
Qunidine	1.0×10^5
Neostigmine	2.8×10^4
Nicotine	3.7×10^3
FA^+	7.3×10^4[a)]

a) From Ref. 2.

$$K^D_{Na}=x_D(C_{Na})^n/(x_{Na})^nC_D \quad (2)$$

where C_{Na} and C_D are the concentrations of Na^+ and a drug in an aqueous solution and x_{Na} and x_M are the equilibrium ionic fractions of $-SO_3^-$ sites occupied by each ion, respectively. Thus, for monocationic drugs the following simple equation is derivated:

$$x_{FA,o}/x_{FA}=K^D_{Na}C_D/(C_{Na}+K^{FA}_{Na}C_{FA})+1 \quad (3)$$

where $x_{FA,o}$ and x_{FA} represent the x values for FA^+ without and with a drug, respectively. The $x_{FA,o}/x_{FA}$ value is directly proportional to the ratio of the peak current, $i_{p,o}/i_p$ observed at a steady-state. Figure 7 shows the plots for $i_{p,o}/i_p$ vs. concentrations of quinidine and acetylcholine at low concentration ranges. The plots show quite good linearity and the K^D_{Na} values can be determined from the slopes of the plots by using the K^{FA}_{Na} value (7.3×10^4) reported by Szentirmay and Martin.[2)] Since the experimental points deviated from the line at high concentration ranges, the measurements were restricted in the relatively low concentration ranges. The deviation may be indicative of a structural change of Nafion. Hexamethonium is a dicationic drug and the plot does not show a linear relationship. The K^D_{Na} value in this case has been determined by a curve fitting between the experimental and theoretical current ratios. Table 1 shows the ion-selectivity coefficients. It is obvious that these drugs have strong affinity with Nafion. The K^D_{Na} value seems to be related with the hydrophobicity of the drugs. In a series of monocationic drugs, relatively hydrophobic quinidine shows a large value, whereas hydrophilic actylcholine shows a small value.

Since all of these drugs employed here are electroinactive in a practical potential range, the determination by a direct electrochemical method is difficult. In addition, neither acetylcholine nor hexamethonium has a chromophore in the molecule and thus the determination by absorption spectroscopy will also be difficult. It should be emphasized that the simple method proposed here can be used for determination of these drugs and will be applicable to many cationic drugs.

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