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GLUCOSE TRANSPORT IN ISOLATED BRUSH-BORDER AND LATERAL–BASAL PLASMA-MEMBRANE VESICLES FROM INTESTINAL EPITHELIAL CELLS

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SUMMARY

Free-flow electrophoresis was used to separate microvilli from the lateral–basal plasma membrane of the epithelial cells from rat small intestine. The activities of the marker enzyme for the microvillus membrane, i.e. alkaline phosphatase (EC 3.1.31), was clearly separated from the marker for the lateral–basal plasma membrane, i.e. the (Na^+, K^+)-ATPase (EC 3.6.1.3). A microvillus membrane fraction was obtained with a high specific activity of alkaline phosphatase (an 8-fold enrichement over the starting homogenate). The lateral–basal plasma membrane fraction contained (Na^+, K^+)-ATPase (5-fold over homogenate) with some alkaline phosphatase (2-fold over homogenate).

Glucose transport was studied in both membrane fractions. The uptake of D-glucose was much faster than that of L-glucose in either plasma membrane. D-Glucose uptake could be accounted for completely by its transport into an osmotically active space. Interestingly, the characteristics of the glucose transport of the microvillus membrane were different from those of the lateral–basal plasma membrane. In particular: Na^+ stimulated the D-glucose transport by the microvillus membrane, but not by the lateral–basal plasma membrane. In addition, the glucose transport of the microvillus membrane was much more sensitive to phlorizin inhibition than that of the lateral–basal plasma membrane.

These experiments thus provide evidence not only for an asymmetrical distribution of the enzymes, but also for differences in the transport properties with respect to glucose between the two types of plasma membrane of the intestinal epithelial cell.

INTRODUCTION

The absorptive epithelium of the small intestine is a tissue specialized in the transcellular transfer of a number of important nutrients. In most in vitro studies of

Abbreviation: HEPES: *N*-2-hydroxyethylpiperazine-*N'*-2-ethane-sulfonic acid.

intestinal transport, tissue preparations have been employed such that the polarity of the epithelial cell remained intact. The functional polarity of the epithelial cell is undoubtedly related to the fact that it possesses two types of plasma membrane: (a) the brush border or microvillus membrane of the luminal surface containing disaccharidases, alkaline phosphatase(s) (EC 3.1.31), but not (Na^+, K^+)-ATPase (EC 3.6.1.3) [1–4]; and (b) the lateral–basal plasma membrane facing the serosal compartment and containing (Na^+, K^+)-ATPase, but not disaccharidases and alkaline phosphatase [4, 5].

The active transport step for glucose at the luminal cell side has been well characterized by various methods as a Na^+-dependent, phlorizin-sensitive process [6–9]. Evidence that the lateral–basal plasma membrane handles glucose in a different fashion from brush border membrane has been demonstrated in isolated epithelial cells [10]. In order to elucidate in more detail the contributions of the two different plasma membranes to transepithelial transport, it is essential to isolate and separate them. This means, in experimental terms, the preparation of a membrane fraction with a high alkaline phosphatase and low (Na^+, K^+)–ATPase activity (predominantly microvillus) and a second fraction with a high (Na^+, K^+)–ATPase and low alkaline phosphatase activity (predominantly lateral–basal plasma membrane). A gentle and rapid method for the separation of membrane particles of different surface charge is free-flow electrophoresis. Using this method, it has been possible to isolate the two different types of plasma membrane of the renal proximal tubule cell [11].

The present experiments were designed to isolate the brush border and the lateral–basal plasma membranes from intestinal epithelial cells and to compare the transport properties of the isolated membranes with respect to D- and L-glucose. The separation of the intestinal plasma membranes was accomplished by the same electrophoretic procedure that had been employed for the kidney.

METHODS AND MATERIALS

Preparation of a "crude" plasma-membrane fraction

Male Wistar rats, 180 g body weight, were sacrificed by a blow on the neck and the small intestine was removed immediately. Isolated intestinal cells were prepared according to a modification of the method of Stern [12]. The small intestine was rinsed thoroughly with ice-cold 0.154 M NaCl, then filled with a citrate solution, containing KCl (1.5 mM), NaCl (96 mM), sodium citrate (27 mM), KH_2PO_4 (8 mM), Na_2HPO_4 (5.6 mM), pH 7.3, and incubated for 15 min at 37 °C. The citrate solution was discarded and the intestine filled with the ice-cold buffer used for further fractionation (250 mM sucrose, 10 mM triethanolamine–HCl, pH 7.6). The filled gut was gently pressed to release cells into the medium. The epithelial cells were washed three times with the cold sucrose solution by collecting them by low-speed centrifugation and resuspension in fresh buffer. Then the cells were homogenized in a glass–teflon homogenizer and a "crude" plasma-membrane fraction was isolated according to Scheme I. The centrifugations were carried out in a refrigerated centrifuge (Sorvall RC-2B). The "crude" plasma-membrane fraction obtained in this manner was used for further separation in a Desaga FF IV free-flow electrophoresis [11].

Scheme 1. Isolation scheme for the preparation of a "crude" plasma membrane fraction from intestinal epithelial cells.

Intestinal epithelial cells of 4 rats	Homogenize in 50 ml of isolation medium with a glass-teflon homogenizer, 25 strokes at 1200 rev./min, fill up to 100 ml, centrifuge homogenate for 15 min at 2600 × *g*. Discard Pellet 1 (nuclei, brush-border fragments).
↓ Supernatant 1	Layer on top of 100 ml solution of 500 mM sucrose, 10 mM triethanolamine-HCl (pH 7.6). Centrifuge for 20 min at 10 000 × *g*. Save Supernatant 2 for resuspension.
↓ Fluffy layer of Pellet 2	Resuspend white fluffy top layer of Pellet 2 in Supernatant 2. Homogenize, 5 strokes at 1200 rev./min. Centrifuge for 20 min at 10 000 × *g*. Save Supernatant 3 for resuspension.
↓ Fluffy layer of Pellet 3	Resuspend white fluffy top layer in Supernatant 3. Homogenize, 5 strokes at 1200 rev./min. Centrifuge for 20 min at 20 000 × *g*. Discard Supernatant 4.
↓ Pellet 4	Resuspend white fluffy top layer of Pellet 4 in the buffer required for further experiments (50 ml). Homogenize, 10 strokes at 1200 rev./min. Centrifuge for 20 min at 20 000 × *g*.
↓ "CRUDE" PLASMA MEMBRANE FRACTION	Resuspend white pellet.

Determination of enzyme activities and protein

Commonly used tests were applied to measure protein and enzymes. They have been described previously [11].

Glucose transport

In order to eliminate interference from sucrose during the transport experiments, the various membrane fractions were treated as follows (the exceptions are noted).

The membranes were collected by centrifugation and resuspended in 50 ml buffer of 100 mM D-mannitol, 1 mM Tris–*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), pH 7.5. The membranes were then homogenized in a glass–teflon homogenizer with 10 strokes at 1200 rev./min and centrifuged at 30 000 × *g* for 20 min, resuspended, rehomogenized and centrifuged again.

The uptake of labeled D- or L-glucose by the membranes was measured with a Millipore-filtration technique in a manner similar to that previously described [9]. The membranes were incubated either with labeled D-glucose plus labeled L-glucose, or with labeled D-glucose alone. The uptake reaction was terminated by dilution of an aliquot from the reaction vessel (20 μl) with 1 ml of ice-cold buffer and immediate collection of the membranes on a Millipore filter (HA 025, 0.45 μm). The filters were rinsed with 3 ml of the same ice-cold buffer to remove the adhering medium. When the incubation was carried out with only one radioactive substrate then D-mannitol, labeled with a different isotope, was added to the buffer used for dilution, but not to the one used for rinsing. Radioactivity from the diluting buffer was occasionally found on the filter due to insufficient washing. These counts from D-mannitol were used to correct the uptake for unspecific retention of sugars by the filter as described

[13]. The buffer for dilution and washing consisted of 150 mM NaCl, 10 mM Tris–HEPES, pH 7.5, and 0.2 mM phlorizin. The temperature of incubation for the transport experiments was 25 °C.

The absolute amount of substrate uptake per mg protein among different experiments varied by a factor of about two. Therefore, the results of single experiments are presented in this paper except where noted otherwise. All experiments were repeated at least three times, with similar results.

Isotopes were counted by using standard techniques for doubly-labeled samples in a liquid scintillation spectrometer (Packard Tri Carb). The counts were corrected for background and spillage of ^{14}C into the ^{3}H channel. The specific activity of the radioactive substances was determined by counting an appropriate amount of labeled sugars under identical conditions as the samples from the uptake studies.

Materials

All chemicals used for the experiments were of the highest grade available. D-Mannitol, D-glucose, sucrose and triethanolamine were purchased from Merck (Darmstadt, Germany), phlorizin from Fluka (Buchs, Switzerland), and L-glucose, HEPES, Tris from Sigma Chemical Corp. (St. Louis, Mo.) All radioactive compounds were obtained from New England Nuclear (Boston, Mass.).

RESULTS

Enzymatic characterization of the membranes

In order to preserve the structural and morphological integrity of the intracellular organelles an isotonic isolation and separation medium was chosen. The "crude" plasma-membrane fraction, isolated by differential centrifugation, contained about 10% of the brush border membrane and 19% of the lateral–basal plasma membrane from the homogenate as measured by the marker enzymes alkaline phosphatase and (Na^+, K^+)-ATPase, respectively. Most of the alkaline phosphatase activity was lost in the first centrifugation pellet together with large brush-border fragments (Table Ia). As judged by phase-contrast microscopy, brush borders, but not individual microvilli were excluded from the "crude" plasma membrane. Only 7% of the mitochondria cosedimented with the "crude" plasma-membrane fraction as measured by cytochrome oxidase (EC 1.9.3.1). The enrichment of this "crude" fraction with plasma membrane is indicated by an increase of the specific activity of alkaline phosphatase and (Na^+, K^+)-ATPase (Table Ia).

On further fractionation of the "crude" membrane by electrophoresis a protein distribution was obtained as illustrated in Fig. 1A. The protein content peaked around Fraction 24 and showed a close coincidence with the Mg^{2+}-ATPase. This protein peak contained mitochondria as indicated by the levels of succinate dehydrogenase (EC 1.3.99.1) (data not shown). The distribution of the plasma-membrane enzymes is shown in Fig. 1B. Alkaline phosphatase activity migrated as a single peak with the least deflection towards the anode. The (Na^+, K^+)–ATPase exhibited a bimodal distribution. Most of the activity was associated with the protein peak. The residual (Na^+, K^+)–ATPase moved further towards the anode. The specific activity was highest in these latter fractions.

Fractions 16–22, 23–28 and 29–35 were pooled and the specific activity of

TABLE I

RECOVERIES OF MARKER ENZYMES

a. Recoveries of marker enzymes in the fractions according to Scheme 1. Values are expressed in percentage of the amount of enzyme in the homogenate and are the average from three experiments with a S.D. of ±7 %. Figures in parentheses represent the specific activity (μmoles of substrates turned over/mg protein per min.)

	Alkaline phosphatase	Cytochrome oxidase	(Na^+, K^+)-ATPase
Homogenate	100 (0.26)	100 (0.10)	100 (0.06)
Pellet 1			
(nuclei, brush border, intact cells)	74 (0.11)	25 (0.11)	13 (0.07)
Lower layer of Pellet 2+3	5 (0.56)	59 (0.25)	1 (0.01)
"Crude" plasma membrane	10 (0.75)	7 (0.04)	19 (0.14)

b. Recoveries of marker enzymes in the fractions obtained by free-flow electrophoresis. Values are expressed in percentage of the amount of enzyme in the injected material ("crude" plasma membrane). Figures in parentheses represent the specific activity (μmoles of substrates turned over/mg protein per minute).

	Alkaline phosphatase	(Na^+, K^+)-ATPase
"Crude" plasma membrane	100 (0.75)	100 (0.14)
Lateral–basal plasma membrane (Fraction 16–22)	6 (0.55)	17 (0.30)
Fraction 23–29	63 (0.84)	74 (0.18)
Brush border membrane (Fraction 29–35)	31 (1.91)	9 (0.11)

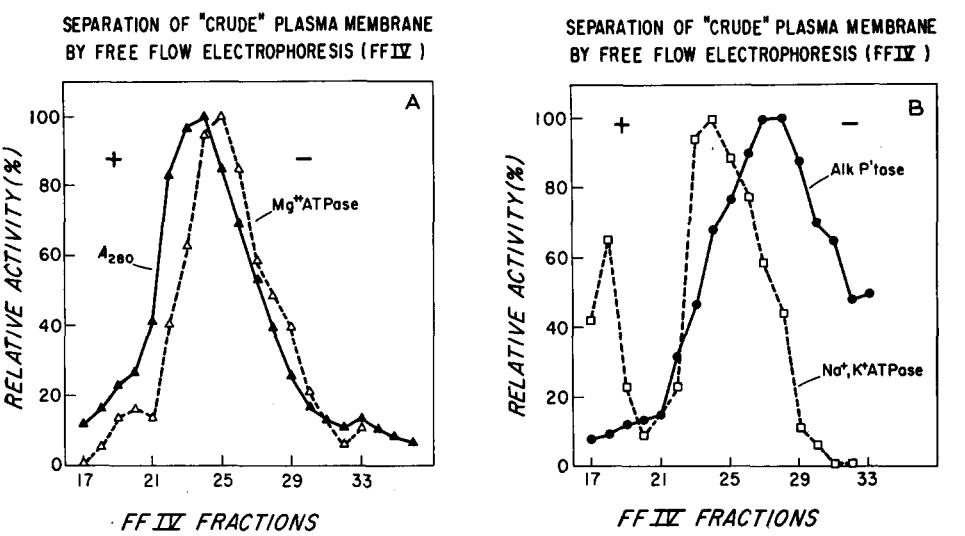

Fig. 1. Separation of the "crude" plasma-membrane fraction by free-flow electrophoresis (FF IV). The "crude" plasma membranes are injected above Fraction 70 (injection point not shown in the diagram). All of the material was deflected towards the anode. The enzymatic activities Mg^{2+}-ATPase, alkaline phosphatase (alk P'tase) and (Na^+, K^+)-ATPase as well as protein ($A_{280\ nm}$) were determinated for all fractions. The enzymatic activities (μmole/ml) or the protein (mg/ml) in each fraction were expressed as a percentage of the fraction with the highest activity (= 100 %) or the highest protein content, respectively.

the marker enzymes measured. After the electrophoretic separation, the combined fraction 16–22 contained membranes with high (Na^+, K^+)-ATPase and low alkaline phosphatase activity. On the other hand, alkaline phosphatase was enriched and (Na^+, K^+)-ATPase was reduced in the membranes obtained from fractions 29–35 (Table Ib). These results indicated a separation of the brush border and lateral–basal membranes during the electrophoresis, with the lateral–basal plasma membranes moving faster than the microvillus membranes towards the anode.

Transport properties of the membranes

When "crude" and "purified" plasma membranes were incubated with labeled D- and L-glucose a differential time course of uptake was detected for the two isomers (Table II). This is reflected in Table II by ΔGlc (D- minus L-glucose) with a fast initial rise and a slow decrease.

TABLE II

UPTAKE OF D- AND L-GLUCOSE BY ISOLATED PLASMA MEMBRANES

Uptake of D- and L-glucose by isolated plasma-membrane vesicles in pmoles/mg protein. D- and L-glucose uptake was measured for the "crude "plasma-membrane fraction and the various plasma-membrane fractions purified by electrophoresis. All fractions were obtained in the same electrophoretic separation. Incubation medium for all uptake experiments (final concentrations): D-[^{3}H] glucose (1 mM), L-[^{14}C]glucose (1 mM), Tris–HEPES (1 mM), pH 7.5, D-mannitol (100 mM) and NaCl (100 mM).

Time of incubation	"Crude" plasma membrane			Lateral–basal plasma membrane (Fractions 16–22)			Brush border membrane (Fractions 29–35)		
	D-Glc	L-Glc	ΔGlc	D-Glc	L-Glc	ΔGlc	D-Glc	L-Glc	ΔGlc
30 s	608	252	356	902	453	449	1002	288	714
90 s	870	358	512	1300	592	708	1505	498	1007
120 s	904	405	499	1495	755	740	2190	664	1526
25 min	1120	980	140	2434	1904	530	2038	1442	596

D-Glucose was taken up 2–3 times faster than L-glucose by all membrane fractions indicating a specific glucose transport system in all of them.

Experiments were carried out to determine whether glucose uptake represented transport into an intravesicular space or binding to the membrane. D-Glucose was at equilibrium after about 20 min of incubation. In the case of transport, the amount of glucose uptake after this time should be dependent on the available intravesicular volume. Therefore, the intravesicular space was decreased by increasing the medium osmolarity with cellobiose. The intravesicular space should be inversely proportional to the medium osmolarity as long as the impermeable solute (cellobiose) represents the main component of the medium osmolarity. As shown in Fig. 2 the D-glucose uptake was proportional to the inverse osmolarity and, thus, to the intravesicular space. Moreover, extrapolation to infinite medium osmolarity (zero intravesicular space) yielded no uptake. Therefore, under the condition of these experiments the measured glucose uptake could be completely accounted for by transport into an intravesicular osmotically active space.

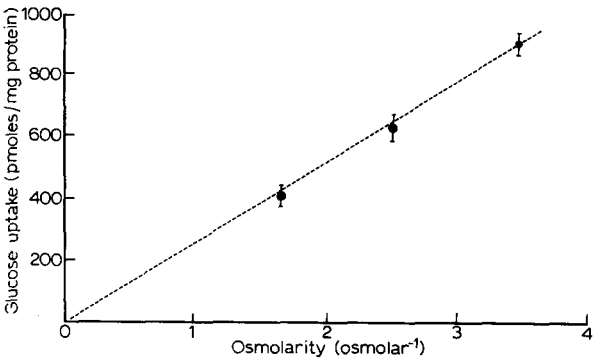

Fig. 2. Effect of medium osmolarity on D-glucose uptake. "Crude" plasma membranes were rehomogenized and suspended in 100 mM cellobiose, 1 mM Tris–HEPES, pH 7.5. Uptake was measured from a medium with the following final composition: NaCl (100 mM), Tris–HEPES (1 mM), D-[^{14}C]glucose (1 mM). Cellobiose was used to give the indicated osmolarity (shown as inverse osmolarity). The buffer for dilution or rinse consisted of 450 mM NaCl, 10 mM Tris–HEPES and 0.2 mM phlorizin. Time of incubation: 20 min. The vertical bars indicate the standard errors of the means from three experiments made with the same preparation of membranes.

An intact glucose carrier system in isolated microvillus membranes had been demonstrated earlier [9]. The D-glucose transport by the brush border membranes, isolated by free-flow electrophoresis, had the same features with respect to Na^+ stimulation and phlorizin inhibition as described for that membrane preparation. This indicates that during the isolation procedure by electrophoresis none of the characteristics of the glucose transport were lost. Na^+ increased D-glucose uptake more than 2-fold over that observed with K^+, and phlorizin decreased it to 24% of the control (Table III).

In contrast, the lateral–basal plasma membrane, isolated from the same sample of "crude" plasma membranes, was stimulated only slightly by Na^+ and also phlorizin was less effective as an inhibitor in this fraction (Table III). The L-glucose transport in both fractions was independent of Na^+ and insensitive to the addition of phlorizin.

DISCUSSION

A description of the molecular events involved in the intestinal transport of nutrients has been difficult because the systems studied extensively to date are very complex. One of the problems stems from the polarity of the intestinal epithelial cell.

The presented data demonstrate that free-flow electrophoresis can also be applied successfully to enterocytes. It was possible to purify brush border and lateral–basal plasma membrane as defined by the corresponding marker enzymes and the increase in the specific activity. The final yield of membranes was relatively low due to two inherent problems: most of the microvilli remain as large brush borders and are lost in the low-speed sediment together with nuclei. The major peak of (Na^+, K^+)-ATPase coincides with the Mg^{2+}-ATPase, a mitochondrial enzyme. This probably represents an attachment of the lateral–basal plasma membranes to the mito-

TABLE III

D-GLUCOSE UPTAKE BY BRUSH BORDER AND LATERAL–BASAL PLASMA MEMBRANE

D-Glucose uptake in pmoles/mg protein by brush border membrane and lateral–basal plasma membrane. Composition of the incubation medium: D-[^{14}C]glucose (1 mM), Tris–HEPES (1 mM), pH 7.5, D-mannitol (100 mM) plus the indicated additions. The percentage of stimulation (+) by NaCl and of inhibition (−) by phlorizin are indicated in parentheses. The figures are means ±S.E. of three experiments.

Additions	Lateral–basal plasma membrane (Fractions 16–22)			Brush border membrane (Fractions 29–35)		
	0.5 min	1.5 min	25 min	0.5 min	1.5 min	25 min
KCl (100 mM)	693±81	978±148	2159±518	491±117	620±147	1599±458
NaCl (100 mM)	848±151 (+22±12 %)	1317±301 (+34±19 %)	2198±404 (+9±4 %)	909±156 (+104±10 %)	1211±262 (+97±10 %)	1944±545 (+23±3 %)
NaCl (100 mM) +phlorizin (0.5 mM)	757±146 (−27±2 %)	1104±172 (−17±5 %)	2226±502 (−7±3 %)	264±118 (−71±6 %)	384±127 (−63±3 %)	1123±82 (−5±2 %)

chondria, a phenomenon first observed by Quigley and Gotterer [14]. However, it is obvious that the (Na^+, K^+)-ATPase is absent from the brush border membrane in agreement with the results of Fujita et al. [4] for intestine and Heidrich et al. [11] for kidney.

This electrophoretic method offers two advantages over the published methods [4, 5, 14] involving exclusively centrifugations: (1) the time for the membrane preparation is relatively short (7 h) so that membrane isolation and the transport experiments could be carried out within the same day; (2) both types of plasma membranes are purified under identical conditions. The first point is especially important since it had been noted previously that isolated brush border membranes become unspecifically "leaky" within 24 h [9]. In these experiments isolated epithelial cells and not mucosal scrapings were used as source for the plasma membranes. This ensured that the membrane fraction with high (Na^+, K^+)-ATPase activity is derived from epithelial cells. That mucosal scrapings are contaminated by other than epithelial cells is indicated by a 3–4-fold higher specific activity of alkaline phosphatase and sucrase in isolated cells compared to scrapings (unpublished results).

It has been demonstrated by several groups that isolated plasma membranes reseal and that they transport sugars into an osmotically active space [9, 15, 16]. The transport experiments in this paper confirm these observations. It was demonstrated here for the "crude" plasma membrane that the D-glucose uptake could be accounted for completely by transport into an osmotically active space. Thus, in this respect, the D-glucose transport behaved as in purified brush border membranes [9]. The final uptake of D- and L-glucose was about twice as high in the purified plasma-membrane fractions after electrophoresis (Table II). This increase corresponds roughly to the purification of the corresponding enzyme markers (Table Ib) for this step and strongly suggests that the glucose uptake represents transport into an osmotically active space rather than binding also for the "purified" membranes.

The comparison of transport characteristics for D-glucose between brush border membranes and lateral–basal plasma membranes is important because direct experimental data on transport across the lateral–basal plasma membrane are lacking. It has generally been assumed, on the basis of indirect evidence, that this transport pathway is energy independent, but rapid enough to mediate the outward transfer of sugar in the physiological process of absorption [8, 17]. The glucose transport in the membrane fraction with high alkaline phosphatase and low (Na^+, K^+)-ATPase activity exhibited the same characteristics as those found in the microvillus membranes isolated by the method of Hopfer *et al.* [9]. It exhibited stereospecificity for D-glucose, Na^+ stimulation and phlorizin inhibition to the same extent as in those highly purified microvillus membranes. The membranes with high (Na^+, K^+)-ATPase and low alkaline phosphatase activity (lateral–basal plasma membranes), isolated in the same electrophoresis step, showed only a very small Na^+ stimulation and very little phlorizin inhibition. This Na^+ stimulation and phlorizin inhibition can be accounted for by contamination with microvillus membrane as measured by the marker enzyme alkaline phosphatase. These results, therefore, suggest that D-glucose transport in the lateral–basal plasma membrane is not dependent on Na^+ and is much less sensitive towards phlorizin inhibition than the microvillus membrane. Thus, these data provide evidence for a polarity of the transport functions on a subcellular basis: Na^+-dependent D-glucose transport at the luminal side; Na^+-independent

transport at the lateral–basal side. The data support the concept that the transcellular "active" transport of D-glucose consists of an uphill carrier-mediated influx at the brush border membrane and a downhill efflux at the lateral–basal plasma membrane.

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