Relationship of Fluid Transport Through the Dentin to the Incidence of Dental Caries

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The rate of fluid movement in the teeth of 28-day-old rats is inversely related to the incidence of dental caries after 13 weeks of the same diets. The early hypomineralization found in the dentin at 28 days is a result of altered metabolism imposed by a reduced rate of fluid transport in the rats fed a cariogenic diet.

It is generally accepted that three conditions are essential in the oral cavity for dental caries to occur: a suitable microbe, a substrate for the microbe, and a susceptible tooth. Susceptibility or resistance to dental caries is usually thought of in terms of structure, which is determined, for the most part, during calcification. However, it is also plausible that susceptibility to dental caries may be related to altered metabolism within the tooth.

To maintain a rapid rate of metabolism through the Embden-Meyerhof pathway in the avascular dentin, as reported by Bosia, Arese, and Pezolli,[1] a rapid fluid transport system is essential for nutrient uptake and waste removal from the tooth structure. Any reduction in the rate of fluid transport through the dentin may have a deleterious effect on the health of the tooth. Under these conditions, the rate of metabolism would be altered and the end product of metabolism (lactic acid) might accumulate in the structure. The purpose of these experiments was to determine if the rate of fluid transport is related to the incidence of dental caries, to determine if the rate of fluid transport can be altered by systemic means, and to find what effect this alteration would have upon the incidence of dental caries.

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Materials and Methods

Male, 21-day-old Sprague-Dawley rats* were fed either a high sucrose cariogenic diet or Purina laboratory chow.† Citrulline,‡ urea,§ and acriflavine hydrochloride|| were obtained.

Pentobarbital sodium (Nembutal) was the anesthetic used.

EXPERIMENT 1.—The rate of fluid movement (FM) through the dentin beneath the occlusal grooves was determined on the 28th day of life in rats fed either laboratory chow or a cariogenic diet, and in the anesthetized and unanesthetized state. Groups of ten or more rats were given an injection of the fluorescent dye, acriflavine hydrochloride (50 mg/kg) intraperitoneally. The rate at which the fluorescent dye moved in and disappeared from the odontoblastic processes was used as a criterion for the occurrence of FM. The unanesthetized rats fed laboratory chow and the rats fed the cariogenic diet were killed at 15, 30, 45, and 60 minutes, and at 45, 60, 70, 90, 135, and 180 minutes, respectively. The rats were decapitated quickly and the upper and lower jaws were removed, frozen, and coded. The frozen jaws were sectioned,[2] and viewed microscopically under ultraviolet light.[3] Particular attention was paid to the dentin beneath each occlusal groove to determine whether acriflavine hydrochloride was present in the odontoblastic processes. When the dye was present, these

* Simonsen Laboratories Inc., Gilory, Calif.
† Ralston-Purina Co., St. Louis, Mo.
‡ Nutritional Biochemical Corp., Cleveland, Ohio.
§ J. T. Baker Chemical Co., Phillipsburg, N.J.
|| Sigma Chemical Co., St. Louis, Mo.

processes fluoresced a brilliant green. To quantitate the results, a count of the fluorescing and nonfluorescing areas was made and a ratio was expressed:

$$\frac{\text{fluorescing occlusal grooves}}{\text{(fluorescing + nonfluorescing grooves)}} = \text{FMR},$$

where FMR is fluid movement ratio.

EXPERIMENT 2.—The systemic effects of urea and citrulline on FM were studied in rats maintained on the cariogenic diet. On the 28th day, these compounds were infused through the subclavian vein or the internal carotid artery. The chemicals were dissolved in saline in the concentrations shown in Table 1. The dose administered for each solution was 0.5 ml/100 gm body weight. When the infusion was through the subclavian vein, the vein was exposed and the compounds were injected through a half inch (27G) needle attached to a 1 ml disposable tuberculin syringe. For infusions through the arterial system, the left common, external, and internal carotid arteries were exposed. To infuse through the internal carotid artery, the external carotid artery was ligated close to the bifurcation of the common carotid artery. The common carotid artery was ligated just anterior to the manubrium. Then polyethylene tubing (Clay Adams, PE 10) attached to a half inch (27G) needle was slipped into the common carotid artery up to the internal carotid artery. The total dose of each compound was administered gradually over a 35 minute period. After ten minutes, the rats were decapitated and the jaws processed as described in experiment 1.

EXPERIMENT 3.—This study consisted of two phases. In the first phase, the incidence of dental caries at 111 days of age in 25 rats maintained on a cariogenic diet, was compared to the incidence in 23 rats maintained on laboratory chow. In the second phase, the relationship between FM through the dentin to the incidence of dental caries was studied. By use of the FM-promoting capacity of urea and citrulline, various multiple doses dissolved in saline were administered subcutaneously or intraperitoneally for 91 days. Group 1, the control group of 20 rats, received 0.5 ml/100 gm body weight of 0.9% saline subcutaneously three times a day. Group 2, 17 rats, received 20 mg urea/100 gm body weight intrapertioneally twice a day. Group 3, 14 rats, recevied 80 mg urea/100 gm body weight subcutaneously three times a day. Group 4, 14 rats, received 240 mg urea/100 gm body weight subcutaneously three times a day. The doses of urea and citrulline were dissolved in 0.9% saline (0.5 ml/100 gm body weight). The rats were placed

TABLE 1
COMPARISON OF THE EFFECTIVENESS OF UREA AND CITRULLINE TO STIMULATE FLUID MOVEMENT BY INTRA-ARTERIAL OR INTRAVENOUS INFUSION

Compound	Dose (mM)	Fluid Movement: Common Carotid Artery*	Fluid Movement: Subclavian Vein	Significance
Noninfused	...	0.11 ± 0.02 (14)†	...	...
Saline	155.0	0.14 ± 0.02 (21)	0.14 ± 0.02 (18)	NS‡
Urea	6.25	0.25 ± 0.05 (12)	...	<0.03 §
	12.5	0.42 ± 0.03 (12)	...	<0.001
	25.0	0.66 ± 0.04 (11)	...	<0.001
	25.0	...	0.22 ± 0.04 (5)	0.05
	50.0	...	0.48 ± 0.07 (5)	<0.001
	100.0	...	0.76 ± 0.03 (10)	<0.001
Citrulline	6.25	0.31 ± 0.04 (7)	...	<0.001
	12.5	0.46 ± 0.04 (9)	...	<0.001
	25.0	0.66 ± 0.04 (9)	...	<0.001
	12.5	...	0.21 ± 0.02 (13)	0.02
	25.0	...	0.49 ± 0.03 (16)	<0.001
	50.0	...	0.76 ± 0.02 (8)	<0.001

* Administered through the left common carotid artery with the external carotid artery ligated.

† Assay performed as described except that no infusion was done.

‡ The change in FM because of saline infusion by either arterial or venous infusion was not statistically significant compared with FM in the noninfused rats.

§ Level of statistical significance compared with the appropriate saline infusion control.

in raised cages and fed the high sucrose diet ad libitum during the experimental period.

The rats were weighed twice a week for the first eight weeks, and once a week thereafter. At the end of the experiment, an autopsy was performed and the jaws were removed. The teeth were coded, examined, and scored for dental caries by the method of Shaw et al.[4] The adrenal glands, testes, ventral prostate, thymus, thyroid, submaxillary glands, spleen, kidneys, and pituitary gland were dissected free of extraneous tissue, and weighed on a torsion balance.

EXPERIMENT 4.—In this experiment an attempt was made to ascertain the mechanism whereby the daily systemic administration of urea resulted in a reduction in the incidence of dental caries. Forty 21-day-old male rats were fed a cariogenic diet for six weeks. Group 1, 20 rats, was injected subcutaneously three times a day with urea (240 mg/100 gm body weight). Group 2, 20 rats, received saline in the same volume three times a day. The injections were continued for three weeks while the rats were fed the cariogenic diet. At the end of the third week, blood and saliva samples were taken at 45, 120, and 180 minutes after an injection of urea or saline. The blood (0.5 ml) was drawn with a heparinized syringe from each rat through a heart puncture. The individual blood samples were centrifuged, and the plasma was saved. Immediately after the blood collection, the rats were given an injection of pilocarpine (0.1 ml of 10%) subcutaneously to induce salivation. Approximately 0.5 to 1 ml of saliva was collected from each rat. The urea content of each plasma and saliva sample was determined by the method of Chaney and Marbach.[5]

On the last day of the experiment, the rats were bled to death through heart punctures two hours after the last injection of urea or saline. The blood from each group of rats was pooled and centrifuged. The plasma from the saline and urea injected rats was bioassayed for FM-promoting activity, as discussed under experiment 2, at doses of 0.5, 1, and 2 ml plasma. When necessary, saline was added to the plasma to maintain the dose volume at 2 ml.

Results

The fluorescent dye appeared to move in and disappear from the odontoblastic processes at a faster rate in the unanesthetized rats fed the laboratory chow than in the unanesthetized rats fed the cariogenic diet (Fig 1). The peak response in the latter group was obtained at 45 minutes, and a comparable level of FM was obtained at 15 minutes in the other rats. The response in the rats fed the cariogenic diet appeared to be blunted. Pentobarbital sodium anesthesia dramatically enhances the suppressive effect of the cariogenic diet on the rate of FM.

The relationship of the FM ratio to the incidence of dental caries is shown in Figure 2. The FM ratio in a 28-day-old rat demon-

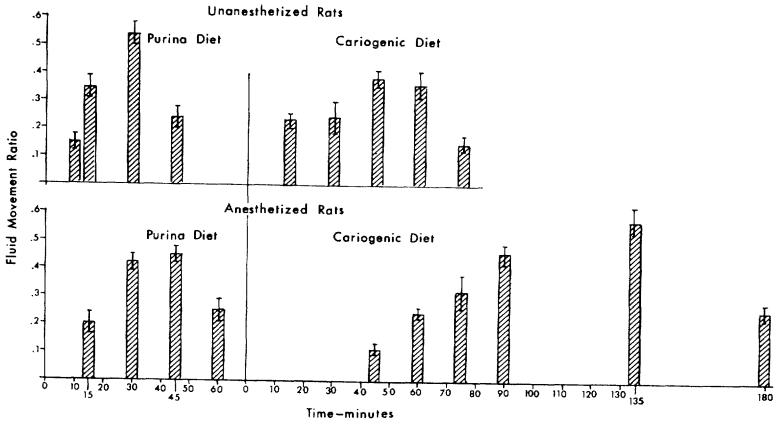

FIG 1.—Effect of diet, and diet with pentobarbital sodium anesthesia on fluid movement through odontoblastic processes of 28-day-old rats.

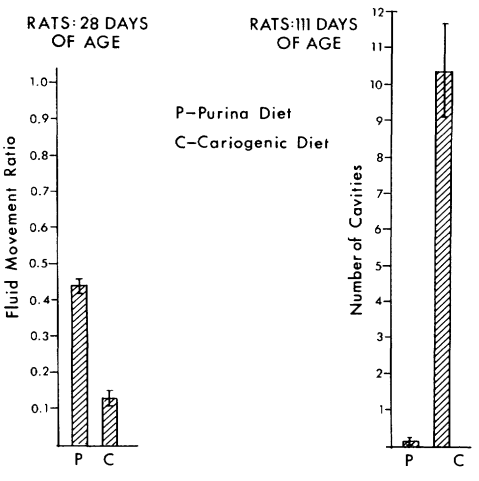

FIG 2.—Inverse relationship between fluid movement and incidence of dental caries in 28- and 111-day-old rats maintained on a cariogenic or Purina laboratory chow diet.

strates an inverse relationship to the incidence of dental caries at 111 days of age. A high FM ratio is associated with a low incidence of decay, and vice versa. These differences are statistically significant at the <0.001 level.

The capacity for urea and citrulline to stimulate FM when infused into the rats fed the high sugar diet is shown in Table 1. The FM response is related to dose. Infusion through the internal carotid artery is two to four times more effective than through intravenous infusion. At higher doses, the FM response obtained with either compound is equal to or greater than that found in rats fed the laboratory chow.

The effect of long-term systemic administration of urea and citrulline on the incidence of dental caries is shown in Table 2. A highly significant reduction in dental caries was obtained with the higher doses of urea. The decrease in caries incidence obtained with citrulline was equivalent to that obtained with the lowest dose of urea, but it was not statistically significant. The decrease in the number of carious lesions was found to be a function of the total dose of urea.

The effect of urea and of citrulline on body weight at autopsy, and on the weight of various glands and organs, is summarized in Table 3. With the larger doses of urea, the body and thymus weights were reduced. Increased weight of the adrenal glands, testes, spleen, kidneys, and pituitary gland also was found in rats receiving higher doses of urea. The statistical significance of the observed differences is given as compared with the control rats.

The concentrations of urea in the blood plasma and saliva from rats receiving subcutaneous injections of urea three times a day for three weeks, are shown in Table 4. Forty-five minutes after an injection of urea, there was a sharp rise in the blood plasma concentration of urea, from 0.185 to 0.296 mg/ml. There was a corresponding rise in urea concentration in the saliva, from 0.121 to 0.253 mg/ml. The concentration of urea in the saliva was always less than that in the blood. Two hours after an injection, the blood urea dropped down to almost the control level. After three hours, the urea concentration both in the blood and saliva was within the control range. Table 5 shows the FM-promoting capacity of plasma obtained one hour after the last injection of urea in 28-day-old rats that were maintained on a high sugar diet for one week. The data show that as little as 0.5 ml of this plasma was capable of stimulating a significant rise in FM, and 2 ml of plasma from the saline injected rats was ineffective.

TABLE 2
EFFECT OF SUBCUTANEOUS INJECTION OF UREA AND CITRULLINE ON INCIDENCE OF DENTAL CARIES IN MALE RATS

Chemical	Dose mg/100 gm Body Weight	Injections Per Day	Caries Score	Percent Reduction	Statistical Significance	No. Animals
Saline	...	3	33.3 ± 4.2*	...	...	20
Urea	20†	2	22.5 ± 2.0	33	0.02	17
	80	3	9.0 ± 2.3	73	0.002	13
	240	3	3.5 ± 0.7	90	0.001	12
Citrulline	60	2	23.9 ± 4.9	29	0.16	14

* Standard error of the mean.
† Administered intraperitoneally.

TABLE 3

EFFECT OF CHRONIC ADMINISTRATION OF UREA AND CITRULLINE ON VARIOUS ORGANS AND GLANDS IN MALE RATS

Chemical	Dose mg/100 gm	Injections Per Day	Body Weight* (gm)	Adrenal† Glands (mg)	Testes (mg)	Ventral Prostate (mg)	Thymus (mg)	Thyroid (mg)	Submaxillary Gland (mg)	Spleen (mg)	Kidney (mg)	Pituitary (mg)
Saline	...	3	409 ± 11	10.0 ± 0.5	781 ± 39	109 ± 5	193 ± 39	3.7 ± 0.5	164 ± 28	166 ± 29	716 ± 15	2.4 ± 0.4
Urea	20	2	412 ± 10	10.4 ± 0.7	839 ± 33	130 ± 4	203 ± 17	...	...	...	705 ± 25	...
			NS‡	NS	NS	0.08	NS				NS	
	80	3	373 ± 27	11.9 ± 0.7	844 ± 22	115 ± 5	148 ± 9	3.5 ± 0.7	180 ± 3	247 ± 17	797 ± 90	2.7 ± 0.4
			$P = 0.16$	$P = 0.11$	$P = 0.05$	NS	$P = 0.03$	NS	$P = 0.05$	$P = 0.001$	$P = .02$	NS
	240	3	322 ± 10	21.1 ± 1.1	960 ± 36	105 ± 3	134 ± 15	3.7 ± 0.2	174 ± 6	692 ± 224	914 ± 24	3.3 ± 0.2
			$P = 0.001$	$P = 0.001$	$P = 0.002$	NS	$P = 0.01$	NS	NS	$P = 0.001$	$P = 0.001$	$P = 0.001$
Citrulline	60	2	416 ± 13	12.4 ± 0.8	809 ± 24	130 ± 2	148 ± 7	4.0 ±0.2	177 ± 5	170 ± 7	803 ± 7	2.6 ± 0.2
			NS	$P = 0.10$	NS	0.04	$P = 0.02$	NS	NS	NS	$P = 0.01$	NS

* Body weight at autopsy.
† Organ and gland weights expressed as mg/100 gm of body weight.
‡ Level of statistical significance; NS, not significant.

Discussion

The data show that the rate of FM through the odontoblastic processes is significantly effected by the dietary regime (Fig 1). In contrast to the rate observed in rats fed Purina laboratory chow, a cariogenic diet significantly suppresses the rate of FM. Pentobarbital sodium anesthesia potentiates the suppressive effect of the high sugar diet. Correlation of the FM ratio to the incidence of dental caries demonstrates that a high incidence of caries is associated with a suppressed FM. The common denominator between these two parameters is the cariogenic diet. A high FM ratio is correlated with an exceptionally low incidence of decay (Fig 2).

Previous studies[3] have shown that the suppressive effect of a cariogenic diet on FM is discernible as early as two days after the rats have been placed on this diet. However, in acute experiments that last only 45 minutes, the suppressive effect can be overcome by the systemic administration of urea or citrulline through intravenous or intra-arterial infusion (Table 1). With these chemicals, it is possible to establish FM at a level equal to or greater than that observed in rats fed laboratory chow. From the data, it is apparent that the intra-arterial infusion is significantly more effective in re-establishing FM than the intravenous infusion. This suggests that these compounds exercise their effect on FM by activating a mechanism in the central nervous system.

Application of the beneficial systemic effect of urea and citrulline as observed in the acute experiments to a chronic experiment that required daily administration over a period of 13 to 16 weeks resulted in a significant reduction in the incidence of dental caries (Table 2). Urea was significantly more effective than citrulline, because urea is a terminal metabolic product that is not metabolized further, but citrulline can be diverted into other metabolic pathways. With urea, the decrase in caries was a function of the dose. The correlation between suppressed FM and increased dental decay suggests that the rate of fluid transport is important for resistance to dental caries.

The beneficial systemic effect of urea is in agreement with the findings of McClure,[6] who reported a significant decrease in the

TABLE 4
COMPARISON OF UREA CONCENTRATION IN THE PLASMA AND SALIVA AT VARIOUS TIMES IN RATS RECEIVING EITHER SALINE OR UREA BY INJECTION THREE TIMES PER DAY

Materials Injected Subcutaneously	Bleeding Time Postinjection (minutes)	Urea Concentration (mg%)	
		Plasma	Saliva
Saline control	45	18.5 ± 0.5 (20)	12.1 ± 0.4 (20)
	120	15*	
Urea†	45	29.6 ± 1.5 (14)	25.3 ± 1.4 (14)
	120	20.0†	
	180	17.3 ± 0.5 (20)	11.7 ± 0.3 (20)

* Urea concentration in the pooled plasma at the time the rats were bled.
† Dose of urea 240 mg/kg body weight, three times per day.

incidence of dental caries in rats fed a diet containing 1 to 2% urea. However, certain differences in experimental design should be noted. With urea in the diet, administration was correlated with food consumption, and in our experiment urea was administered three times a day when food consumption was low. No urea was administered during the night when food consumption is high.

The beneficial systemic effect of urea is emphasized by examination of the blood and saliva urea levels in the rats that received the highest dose of urea 45 minutes to three hours after injection (Table 4). There was an initial sharp rise in urea concentration in both fluids, and in 2 to 3 hours the level returned to the control level found in saline-injected rats. These data show that during a 24 hour period there were three relatively brief periods when the urea concentration in the biologic fluids was elevated, and it remained low during the night when the rats ate the major portion of their food. The intermittent rise in urea level in the oral cavity is considered to have little or no effect on conditions at the bottom of the food plug where decay occurs, because the time for urea to penetrate to the bottom of the plug is too short.[7] The rats were housed in raised cages to further reduce the availability of extraneous urea in the oral cavity.

The idea that urea must exercise its effect through a systemic mechanism rather than through a surface reaction is confirmed with the following observations. Others have shown that a reduction in food consumption is often associated with a decrease in dental caries. However, rats under stress may have a reduction in food consumption but have an increase in dental decay.[8] A reduction in growth rate also is associated with an increase in dental decay.[9] In our experiment, the rats receiving the highest dose of urea were under stress, as indicated by the increased adrenal and pituitary weights and the reduced thymus and body weights (Table 3). The kidneys were hypertrophied because of the increased stress from rapidly eliminating the urea load from the body fluids. In spite of stress,

TABLE 5
PLASMA LEVEL OF FLUID MOVEMENT-PROMOTING PRINCIPLE AFTER SUBCUTANEOUS INJECTION OF SALINE OR UREA

Materials Injected Intravenously	Dose (ml) Plasma + Saline	Fluid Movement	Level of Significance
Saline Control	0 + 2	0.14 ± 0.02 (18)	...
Plasma* Experimental	2 + 0	0.16 ± 0.02 (16)	NS†
Plasma‡	0.5 + 1.5	0.23 ± 0.03 (10)	0.01§
	1.0 + 1.0	0.29 ± 0.03 (10)	0.002
	2.0 + 0.0	0.30 ± 0.03 (10)	0.002

* Plasma obtained from saline injected rats by heart puncture.
† Level of significance compared with saline infused rats.
‡ Plasma obtained from urea injected rats by heart puncture.
§ Experimental plasma compared with control plasma.

a significant decrease in the incidence of dental caries was achieved.

More evidence for the systemic effect of urea becomes apparent when the FM response of 28-day-old rats to plasma obtained from the urea- and saline-injected rats is compared (Table 5). The intravenous infusion of 2 ml of control plasma from the saline-injected rats had no significant effect on the suppressed FM ratio. In contrast, as little as 0.5 ml of plasma from the urea-injected rats caused a significant rise in the FM ratio. The urea concentration in 0.5 ml of plasma was 0.1 mg. This concentration of urea is seven times less than the minimum effective dose required to stimulate FM when infused intravenously into the assay rat. The urea concentration in the highest dose (2 ml) used is only 54% of the minimum effective dose of urea. The inevitable conclusion from these data is that a hormonal factor, not urea, was the responsible factor in the plasma that stimulated FM. Evidence submitted elsewhere for publication shows that urea and other compounds of the ornithine cycle, and carbamyl aspartate of the pyrimidine cycle, exercise their effect on FM through the hypothalamus. Under the influence of urea, the hypothalamus secretes the hypothalamic, parotid hormone-releasing factor, which stimulates the parotid gland. The latter gland secretes a parotid hormone that stimulates FM by acting on the odontoblasts. Surgical interruption of this endocrine axis, such as parotidectomy, renders these compounds ineffective in stimulating FM. Their effectiveness is dependent on an intact endocrine axis. Evidence for the existence of the hypothalamic-parotid gland endocrine axis has been published previously.[10]

The tooth is a unique structure, even when compared with bone, to which it shows certain structural similarities. Bone is a relatively vascularized structure, whereas dentin is avascular, and is dependent on a rapid fluid transport system for nutrient uptake and waste removal. Bone is surrounded by body fluid, and teeth are exposed to the atmosphere. The aerobic environment may be of importance in influencing metabolic pathways in the teeth.[11,12] Beneath the occlusal food plug, a more anaerobic environment can exist that could adversely affect the metabolic pathways. In germfree animals[13] on a high sucrose diet, the hypomineralization found in the enamel and dentin beneath the food plug might be explained as a consequence of reduced fluid transport, along with a shift to a more anaerobic metabolism and the accumulation of lactic acid in the dentin. These changes prepare the tooth for a successful attack by the unfavorable external environment.

The authors suggest that the early changes in teeth which eventually lead to carious lesions are the result of altered metabolism that includes a reduced rate of fluid transport and external conditions imposed by the food plug and its microbial inhabitants. These conclusions do not minimize the importance of the external conditions, but rather give added importance to them by showing how external conditions may influence metabolism within the teeth. Teeth will not decay in the absence of the microbe and its products on the external surface. The external factors cannot be ignored, but for dental caries to be controlled, it might be well to consider how the altered internal metabolic processes contribute to the problem of dental caries. Consideration of both the internal and external factors is a more complete approach to the prevention of dental caries. Teeth decay because of an initial alteration in the internal metabolism. Reduction in the rate of fluid transport, as found in the teeth of animals maintained on the high sucrose diet, may be of primary importance in understanding the alterations in metabolism and pathologic sequelae.

Conclusions

The rate of fluid transport was suppressed significantly in rats maintained on a high sucrose diet as compared with rats fed Purina laboratory chow. Fluid transport may be stimulated by the systemic administration of urea or citrulline.

The systemic administration of urea to rats on a high sucrose diet significantly reduces the incidence of dental caries. This reduction in dental caries may be the result of stimulation of the dentin fluid transport system produced by the hypothalamic-parotid endocrine axis.

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