Dietary modifications of the biliary bile acid glycine:taurine ratio and activity of hepatic bile acid-CoA:amino acid *N*-acyltransferase (*EC* 2.3.1) in the rat

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Effects of dietary manipulations on the biliary bile acid glycine:taurine (G:T) ratio and the activity of hepatic bile acid-CoA:amino acid *N*-acyltransferase (*EC* 2.3.1) in the post-mitochondrial fraction of liver homogenates were examined in the rat. The G:T ratio in rats fed on the diet containing 100 g pectin/kg (2·18) was markedly higher than that in the animals fed on the diet containing 100 g cellulose/kg (0·09). The diets containing either 10 g cholesterol/kg or 5 g sodium cholate/kg, especially the latter, also increased the G:T ratio (0·77 and 2·33 respectively) compared with a control diet free of these steroids (0·34). When the saturating concentrations of taurine (20 mM) and glycine (100 mM) were the substrates, dietary pectin relative to cellulose significantly increased the activity of both taurine- and glycine-dependent bile acid-CoA:amino acid *N*-acyltransferase, but neither dietary bile acid nor cholesterol influenced it. In spite of the marked difference in the G:T ratio among the rats given various types of experimental diet, the bile acid-CoA:amino acid *N*-acyltransferase reaction produced taurine- but little glycine-conjugated bile acid when both taurine and glycine coexisted at physiological concentration ranges in the assay media. Dietary manipulations modified the hepatic taurine concentrations and the changes were inversely correlated with those in the G:T ratio. However, hepatic concentration of taurine (1·67–4·82 μmol/g) in rats given various types of experimental diet was comparable with or even higher than the reported Michaelis constant (K_m) value of *N*-acyltransferase for this compound (0·8–2·5 mM). In contrast, glycine concentrations (1·81–2·58 μmol/g) were much lower than the K_m value for this amino acid (35–40 mM) under various dietary conditions. Thus, neither the substrate specificity of the bile acid conjugation enzyme nor the alteration in the hepatic concentration of taurine or glycine accounted for the change in the G:T ratio in the present study.

Bile acid conjugation: Dietary fibre: Cholesterol: Rat

Bile acid-CoA:amino acid *N*-acyltransferase (*EC* 2.3.1) catalyses condensation of bile acids with amino acids to form taurine- or glycine-conjugated bile acids in the liver. There is a weight of comparable studies regarding the specificity toward the amino acid substrates of this enzyme among various animal species (Vessey *et al.* 1977, 1990; Killenberg & Jordan, 1978; Vessey, 1978, 1979; Czuba & Vessey, 1981*a,b*; Johnson *et al.* 1991; Kwakye *et al.* 1991). Considerable species-to-species difference in the bile acid glycine:taurine (G:T) ratio (Haslewood & Wooton, 1950; Jacobsen & Smith, 1968; Elliot, 1984) has been explained by the difference in the substrate specificity of the enzyme among

the animal species. Killenberg & Jordan (1978) have purified the rat liver enzyme in the cytosolic fraction near to homogeneity, but the taurine- and glycine-dependent activities were not separated during the purification process indicating that a single enzyme is catalysing bile acid conjugation in the liver. The Michaelis constant (K_m) value of the purified rat enzyme for taurine (0·8 mM) was 40–50 times lower than that for glycine (40 mM). Thus, the rat enzyme is exclusively specific for taurine, and the substrate specificity accounts for the predominance of taurine-conjugated bile acids in this animal species (Haslewood & Wooton, 1950; Jacobsen & Smith, 1968; Elliot, 1984).

However, we recently demonstrated that dietary manipulations profoundly influence the bile acid G:T ratio in rats. Thus, water-soluble dietary fibres (pectin, guar gum and konjak mannan; Ide & Horii, 1989; Ide et al. 1989, 1990; Ide & Sugano, 1991) and a protein source low in sulphur amino acids (soya-bean protein; Ide & Sugano, 1991) elicited a large increase in the G:T ratio in biliary bile acids accompanying a decrease in the concentration of hepatic taurine. Also, dietary cholesterol increased the ratio irrespective of the dietary fibre source, pectin or cellulose (Ide & Horii, 1989). However, when the glycine concentration was determined in the liver of rats given a water-soluble dietary fibre (pectin), it was comparable with that of taurine (Ide et al. 1989). Thus, in terms of the substrate specificity of the bile acid conjugation enzyme, the alteration in the hepatic taurine concentration could not account for a huge increase in glycine conjugation at least in rats given pectin. These results raise the interesting possibility that the bile acid conjugation enzyme specific for glycine, that is distinct from the taurine-specific enzyme, may be induced under certain dietary conditions in the rat, and cause a large increase in the glycine-conjugation of bile acids. No such information has hitherto been available. Thus, in the present study, we examined the effects of dietary manipulations on the biliary bile acid G:T ratio, hepatic taurine and glycine concentrations and the activity of the bile acid conjugation enzyme in rat liver.

EXPERIMENTAL

Animals and diets

Male Wistar rats obtained from the Imamichi Institute of Animal Reproduction, Ibaraki, Japan, at 4 weeks of age were used in the present study. The animals were housed individually in a room with controlled temperature (20–22°), humidity (55–65%) and lighting (lights on from 07.00 hours to 19.00 hours), and fed on a commercial non-purified diet (Type NMF; Oriental Yeast Co., Tokyo, Japan). After 5–7 d of acclimatization to housing conditions, rats were given purified experimental diets. We followed the guide of our Institute for the care and use of laboratory animals.

In the first experiment (Expt 1), rats were randomly divided into two groups and fed on experimental diets containing either 100 g cellulose/kg or 100 g pectin/kg as dietary fibre sources for 26 d (seven and eight rats for the cellulose and pectin groups respectively). In the second experiment (Expt 2), three groups consisting of seven rats each were fed on one of the 20 g cellulose/kg diets containing either 10 g cholesterol/kg or 5 g sodium cholate/kg, or free of these steroids (control) for 29 d. The experimental diets were given to the animals in powdered form in both Expts 1 and 2. The food intakes were recorded every 2 d during the entire experimental period. Body weights of animals at the initiation of these experiments were 74–93 g. The basal composition of the experimental diet was (g/kg): casein 200, maize starch 150, maize oil 50, mineral mixture 35, vitamin mixture 10, choline bitartrate 2, and sucrose to 1 kg. The compositions of mineral and vitamin mixtures were the same as those recommended by the American Institute of Nutrition (1977). Dietary fibre sources, cholesterol and sodium cholate were added to the experimental diets at the expense of sucrose. At the termination of the experimental period, rats were

anaesthetized with intraperitoneal injection of Nembutal (50 mg/kg, Abbott Laboratories, North Chicago, IL, USA) and bile ducts were cannulated with PE-10 tubing (Becton Dickinson Co., Parsippany, NJ, USA) and bile was drained into a test tube cooled on ice.

Assay of hepatic bile acid-CoA: amino acid N-acyltransferase

After 1 h biliary drainage, rats were bled from the inferior vena cava and livers were quickly excised. Samples of each liver (about 3 g) were homogenized in 20 ml 0·25 M-sucrose and the homogenate was centrifuged at 8000 g at 4° for 10 min. Because of the uncertainty in the subcellular localization of bile acid-CoA: amino acid N-acyltransferase in rat liver (cytosol, microsomes, peroxisomes or lysosomes; Bremer, 1955; Schersten et $al.$ 1967; Killenberg, 1978; Kase et $al.$ 1986; Kase & Björkhem, 1989), we employed the post-mitochondrial fraction of the liver homogenate which contained all of these organelles for the enzyme assay. As a spectrophotometric assay of the enzyme activity developed by Killenberg (1978) using the crude enzyme source gave an extremely high blank value, we employed a radioassay reported by Kase and colleagues (Kase et $al.$ 1986; Kase & Björkhem, 1989). The incubation mixture contained 200 μM-[2,4-^{3}H(N)]cholyl-CoA (2220 disintegrations/min per nmol), amino acid substrates (taurine or glycine, the concentrations are indicated in the text) and 10–15 μg enzyme protein in 0·25 ml 100 mM-potassium phosphate buffer (pH 8·0). The reaction was started by the addition of the bile acid-CoA substrate, incubated at 37° for 10 min, terminated, hydrolysed, and extracted (Kase et $al.$, 1986; Kase & Björkhem, 1989). Glycine- and taurine-conjugated bile acids in the extract were fractionated using a column (5 × 18 mm) of piperidinohydroxydextran gel (Shimadzu, Corp., Kyoto, Japan; Ide & Horii, 1987). The radioactivities in these fractions were measured by a liquid scintillation counter. [2,4-^{3}H(N)]Cholic acid was purchased from Du Pont Co., Wilmington, DE USA, and [2,4-^{3}H(N)cholyl-CoA with a specific activity of 2·5 μCi/μmol and unlabelled cholyl-CoA were synthesized, and purified by the method of Shah & Staple (1967).

Analyses of bile and amino acids

Biliary bile acids were extracted, fractionated and determined enzymically (Ide & Horii, 1987; Ide & Sugano, 1991). Hepatic taurine and glycine concentrations were measured using an amino acid analyser (Ide et $al.$ 1989).

Statistical analysis

Statistical analyses of the data were carried out according to the methods described by Snedecor & Cochran (1989). Thus, the Levene's test was adopted to inspect the constancy of variance of the observations. In cases where variances were found to be heterogeneous with this method, observations were transformed logarithmically. Subsequent Levene's test showed that the logarithmic transformations successfully rendered the variances of these data to be constant, and these transformed values were used for the subsequent statistical examinations. The data in Expt 2 were analysed by one-way analysis of variance to establish the significance of the effect of dietary steroids before the examination of the significant differences of means. Examinations of significant differences of means with a pooled estimate of variance for Expts 1 and 2 were established appropriately according to the methods described by Snedecor & Cochran (1989) for the comparison of two samples (Expt 1) and for one-way classifications (Expt 2) respectively.

RESULTS

An experimental diet which contains an excessive amount of water-soluble dietary fibre having high water-holding capacity, but not water-insoluble fibre, sometimes reduces food intake and thus retards the growth of animals (Ide et $al.$ 1990). However, in the present

Table 1. *Effect of dietary modifications on biliary bile acid secretion and concentrations of liver taurine and glycine in the rat**

(Mean values with their standard errors)

| | Bile flow (ml/h) | | Biliary bile acid | | | | Liver amino acids (μmol/g) | | | |
| | | | Secretion (μmol/h) | | Glycine: taurine ratio | | Taurine | | Glycine | |
Diet	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Expt 1										
Cellulose (100 g/kg)	0·81[a]	0·02	21·5[a]	1·2	0·09[a]	0·01	4·82[a]	0·47	2·21[a]	0·08
Pectin (100 g/kg)	1·25[b]	0·07	45·2[b]	3·3	2·18[b]	0·34	1·88[b]	0·10	2·11[a]	0·13
Expt 2										
Control	1·03[a]	0·07	38·0[a]	2·2	0·34[a]	0·05	2·71[a]	0·34	2·08[a]	0·14
Cholesterol (10 g/kg)	0·80[a]	0·06	27·5[b]	2·2	0·77[b]	0·14	2·29[ab]	0·10	1·81[a]	0·19
Sodium cholate (5 g/kg)	1·32[b]	0·12	59·7[c]	6·1	2·23[c]	0·15	1·67[b]	0·12	2·58[b]	0·13

[a,b,c] Mean values within each experiment with unlike superscript letters were significantly different, $P < 0.05$.
* For details of diets and procedures, see pp. 94–95.

study the cumulative food intake in rats fed with pectin (493 (SE 3) g/26 d) was the same that in the animals fed with cellulose (509 (SE 10) g/26 d), irrespectively of the fact that the experimental diets were given in the form of powder (Expt 1). In addition, the growth of the animals was the same between the two groups (204 (SE 3) g and 201 (SE 4) g/26 d for rats fed cellulose and pectin respectively). Neither dietary cholesterol nor bile acid affected the food intake compared with the control diet that was free of these steroids (560–625 g/29 d; Expt. 2). The growth of animals was also comparable among the three groups of rats (190–245 g/29 d). Body weights at the termination of the experiments was 260–310 and 270–315 g for Expts 1 and 2 respectively.

As shown in Table 1, rats fed with pectin relative to those fed with cellulose showed increased bile flow. Pectin compared with cellulose markedly increased biliary bile acid excretion. As the increase was essentially attributed to the glycine-conjugated bile acids, the bile acid G:T ratio in bile became much higher in rats fed with pectin than in the animals fed with cellulose. In Expt 2, the rate of the bile flow in rats given sodium cholate was significantly higher than those in the other two groups. Bile acid feeding markedly increased biliary bile acid excretion, while cholesterol feeding rather decreased it. However, both cholesterol and bile acid feeding, especially the latter, significantly increased the bile acid G:T ratio. Dietary pectin relative to cellulose significantly decreased the taurine concentration in the liver, whereas liver glycine concentration was comparable (Expt 1). In Expt 2, bile acid feeding significantly decreased liver taurine concentration. Dietary cholesterol also tended to decrease this variable. Liver glycine concentration in rats fed with sodium cholate was significantly higher than those in the other groups.

Table 2 summarizes the activity of cholyl-CoA:amino acid (glycine or taurine) *N*-acyltransferase in the post mitochondrial fraction of the liver homogenate. When the enzyme activity was assayed in the presence of the saturating concentration of taurine (20 mM) no significant amounts of glycine-conjugated bile acids were produced in the various groups both in Expts 1 and 2. Similarly, in the presence of the saturating concentration of glycine (100 mM) the enzyme reaction did not produce significant amounts of taurine-conjugated bile acids. Dietary pectin relative to cellulose significantly increased the bile acid-CoA:amino acid *N*-acyltransferase activity, especially the glycine-dependent

Table 2. *Effects of dietary modification on the activity of bile acid-CoA:amino acid*
N-acyltransferase in the rat*

(Mean values with their standard errors)

	Diets									
	Expt 1				Expt 2					
	Cellulose (100 g/kg)		Pectin (100 g/kg)		Control		Cholesterol (10 g/kg)		Sodium cholate (5 g/kg)	
Amino acid substrate	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Bile acid-CoA:amino acid *N*-acyltransferase activity (nmol/min per mg protein)										
20 mM taurine										
Glycine-conjugation	ND		ND		ND		ND		ND	
Taurine-conjugation	43·4[a]	3·1	52·9[b]	3·4	39·6[a]	2·4	45·8[a]	2·5	45·0[a]	3·2
100 mM glycine										
Glycine-conjugation	45·2[a]	3·1	71·7[b]	3·5	53·8[a]	5·2	57·2[a]	4·9	56·5[a]	6·0
Taurine-conjugation	ND		ND		ND		ND		ND	
4 mM taurine + 4 mM glycine										
Glycine-conjugation	1·36[a]	0·74	0·79[a]	0·40	2·67[a]	0·73	3·42[a]	0·81	3·23[a]	0·85
Taurine-conjugation	32·5[a]	2·0	41·7[b]	2·8	34·5[a]	2·6	39·6[a]	2·6	36·9[a]	2·3
2 mM taurine + 4 mM glycine										
Glycine-conjugation	0·23[a]	0·14	0·94[b]	0·22	3·11[a]	0·89	2·39[a]	0·48	4·50[a]	1·70
Taurine-conjugation	26·7[a]	1·35	36·5[b]	1·7	24·1[a]	2·0	29·7[a]	2·4	26·0[a]	2·7

ND, not detected.

[a,b] Mean values within each experiment with unlike superscript letters were significantly different, $P < 0.05$.

* For details of diets and procedures, see pp. 94–95.

activity (Expt 1). In the second experiment (Expt 2), neither taurine- nor glycine-dependent activities were modified by the type of experimental diet. In spite of the observation that dietary manipulations strikingly modified the bile acid G:T ratio in the bile (Table 1), the response of the enzyme reaction was rather in favour of the formation of taurine- than glycine-conjugated bile acids in various dietary groups when both taurine and glycine coexisted at physiological concentration ranges (2 or 4 mM) in the assay media. Moreover, the reduction in the concentration of taurine relative to glycine in the assay medium decreased taurine-conjugation, but did not increase the glycine-conjugation of bile acids. The enzyme activities were assayed with varying concentrations of taurine and glycine, and the K_m values for these amino acid substrates were determined in Expt 1. The K_m values for taurine (0·83 and 1·02 mM for rats fed with cellulose and pectin respectively) and glycine (38 and 40 mM for rats fed with cellulose and pectin respectively) were not essentially influenced by the difference in the dietary fibre source.

DISCUSSION

The rat has long been regarded as a species that conjugates bile acids exclusively with taurine (Haslewood & Wooton, 1950; Jacobsen & Smith, 1968; Elliot, 1984). This consensus needs to be re-evaluated in the light of our previous (Ide & Horii, 1989; Ide *et al.* 1989, 1990; Ide & Sugano, 1991) and present findings (Table 1) that rat bile contained considerable amounts of glycine-conjugated bile acids under certain dietary conditions. Also, a recent study (Zang *et al.* 1992) presented evidence that the rat can synthesize considerable amounts of glycine-conjugated bile acids. We have previously demonstrated

that water-soluble dietary fibres including pectin elicited a large increase in the bile acid G:T ratio (Ide & Horii, 1989; Ide *et al.* 1989, 1990; Ide & Sugano, 1991), while water-insoluble fibres rather decreased it (Ide *et al.* 1990). This finding was essentially reproduced in the present study. Thus, the G:T ratio in rats fed with pectin compared with those fed with cellulose was markedly elevated (Table 1, Expt 1). The ratio in rats fed on a diet containing 100 g cellulose/kg in Expt 1 appeared to be considerably lower than that in the animals fed on a diet containing 20 g cellulose/kg that was free from cholesterol or bile acids (control group) in Expt 2. Also, we confirmed the previous finding (Ide & Horii, 1989) that dietary cholesterol increased the bile acid G:T ratio, and further found that dietary bile acid elicited a considerable increase in the ratio (Expt 2).

As the dietary variables (dietary fibres in Expt 1 and cholesterol and sodium cholate in Expt 2) were added to the experimental diets at the expense of sucrose, the energy densities of the experimental diets and thus the total amounts of energy consumed during the entire experimental period may have been different from each other among the rats fed on the various experimental diets. Both cellulose and pectin added to the experimental diets at the level of 100 g/kg in lieu of sucrose in Expt 1 are indigestible and unabsorbable in the small intestine, and therefore the energy densities of experimental diets containing these dietary fibres are considered to be indistinguishable. However, it has been reported that significant amounts of dietary fibre which escape digestion and absorption in the small intestine are fermented to small organic compounds including volatile fatty acids, which are absorbed in the large intestine and serve as an energy source (Cummings *et al.* 1986). Thus, differences in the fermentability between cellulose and pectin may to some extent modify the energy densities of experimental diets. No information is available regarding the effect of the differences in the energy densities of experimental diets on bile acid conjugation. However, diets containing water-soluble fibres increased while those containing water-insoluble fibres rather decreased bile acid G:T ratio compared with a fibre-free diet which is more dense in energy compared with the fibre-containing diets (Ide *et al.* 1990). This observation suggests that the difference in the energy densities of the experimental diets may not be a crucial factor in regulating the bile acid conjugation reaction in the rat.

The specificity toward amino acid substrates of bile acid-CoA:amino acid *N*-acyltransferase appears to be a determinant in regulating the partitioning of bile acid between taurine or glycine. Thus, the crude and purified enzymes from fish, chicken and dog (all of these animals are exclusive taurine-conjugators) utilize taurine but not glycine as a substrate for the conjugation of bile acids (Vessey, 1978; Czuba & Vessey, 1981*a*,*b*; Vessey *et al.* 1990; Kwakye *et al.* 1991). On the other hand, the enzyme of the rabbit, an exclusive glycine-conjugator, utilizes glycine preferentially over taurine (Vessey, 1978). The bile acid conjugation enzyme purified from the rat liver cytosols has a much higher affinity for taurine (K_m, 0·8–2·7 mM) than for glycine (K_m, 35–40 mM; Killenberg & Jordan, 1978; Kwakye *et al.* 1991). Although the dietary manipulations profoundly alter the bile acid G:T ratio in rats (Ide & Horii, 1989; Ide *et al.* 1989, 1990; Ide & Sugano, 1991; Table 1), the observations that the rat enzyme utilizes taurine preferentially over glycine were essentially confirmed in the present study in which the post mitochondrial fraction was employed as the enzyme source. Accordingly, in the presence of both taurine and glycine at physiological concentrations, the rat enzyme synthesized taurine- but little glycine-conjugated bile acid in all groups of rats fed on various experimental diets (Table 2).

Another important determinant of the bile acid G:T ratio is the availability of taurine and glycine in the liver. The predominant proportion of biliary bile acids in the guinea pig is conjugated with glycine (Haslewood & Wooton, 1950; Jacobsen & Smith, 1968; Elliot, 1984). However, when liver cytosol of this animal species was assayed for the *N*-acyltransferase, the affinity for taurine was 50–100 times higher than that for glycine

(Vessey, 1978). The conflict may be resolved by a limited amount of taurine (0–1·2 μmol/g) but a huge amount of glycine (13·8–22·9 μmol/g; Vessey, 1978) being available in guinea pig liver. This was not the case in the rats given various types of experimental diet in the present study. Apparently there is an inverse correlation between liver taurine concentration and bile acid G:T ratio (Table 1). However, the taurine concentration observed in the present study (1·67–4·82 μmol/g) appears to be similar to or even higher than the K_m value (0·8–2·7 mM) for taurine of the bile acid conjugation enzyme determined currently and reported by others (Killenberg & Jordan, 1978; Kwakye et al. 1991). In contrast, the hepatic glycine concentration (1·81–2·58 μmol/g, Table 1) was much lower than the K_m value for this amino acid (35–40 mM; Killenberg & Jordan, 1978). Thus, the observed data for the hepatic concentration of these amino acids could not account for the dietary modulation of the bile acid G:T ratio in the rat.

The current observation that neither substrate specificity of bile acid-CoA:amino acid N-acyltransferase nor the availability of amino acid substrates could account for the dietary manipulation of the bile acid G:T ratio indicates the existence of an alternative mechanism that regulates the G:T ratio at a cellular level. Experiments using perfused rat liver (Hardison & Proffitt, 1977; Ogura & Ogura, 1986; Sweeney et al. 1991) have demonstrated that infusion of bile acids evokes a marked increase in glycine-conjugation of biliary bile acids accompanying a decrease in hepatic taurine concentrations. This situation resembles that in the present study where glycine-conjugation was increased. Accordingly, the dietary conditions which expand the bile acid pool or synthesis in the rat (bile acid, cholesterol and pectin feeding; Myant & Mitropoulous, 1977; Ide et al. 1990) all increased the bile acid G:T ratio accompanying a decrease in hepatic concentration of taurine. However, Ogura & Ogura (1986) reported that the concentration of taurine in rat liver perfused with cholic or chenodeoxycholic acids was similar to that of glycine at the end of the perfusion. Moreover, they also showed that simultaneous infusion of taurine or cysteine greatly suppressed the increase in the glycine-conjugation without influencing taurine concentration in the liver. To account for these observations they suggested the existence of a distinct hepatic taurine pool that is reserved for bile acid conjugation reaction in the rat liver. Thus, the dietary conditions which induce glycine-conjugation may specifically decrease the hepatic concentration of taurine in this pool.

Zhang et al. (1992) recently reported that substantial deconjugation of glycine- but not taurine-conjugated chenodeoxycholic acid occurs during intestinal absorption in the rat. They also observed that 30–40 % of radiolabelled chenodeoxycholic acid administered in the intestinal lumen was conjugated with glycine in the bile of rats with biliary fistulas. From these observations they suggested that newly synthesized conjugated bile acids in vivo contained considerable amounts of glycine-conjugates, and resistance of taurine-conjugated bile acids to deconjugation in the intestine rather than a difference in the synthesis of taurine- and glycine-conjugated bile acids in the liver may account for the low bile acid G:T ratio usually observed in the rat. Thus, dietary manipulations of the bile acid G:T ratio observed in the present study may represent modifications of the deconjugation process in the intestinal lumen rather than those of the conjugation reaction in the liver. Our previous study (Ide & Horii, 1989) which showed that bile acid G:T ratios in the intestinal lumen were almost the same as those in the bile under various dietary conditions, however, does not necessarily support this consideration.

In conclusion, the bile acid G:T ratio can readily be modified by dietary manipulations in the rat. Neither substrate specificity of amino acid substrates of bile acid-CoA:amino acid N-acyltransferase nor the availability of amino acid substrates could account for these changes. Thus, an alternative unknown mechanism should be considered for the observed changes in the bile acid G:T ratio.

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