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Inhibition of Protein Degradation in Regenerating Rat Liver by Ethanol Treatment

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PÖSÖ, H. *Inhibition of protein degradation in regenerating rat liver by ethanol treatment.* PHARMAC. BIOCHEM. BEHAV. 13: Suppl. 1, 83–88, 1980.—The effect of ethanol on the activity of ornithine decarboxylase (ODC), tyrosine aminotransferase (TAT), alanine aminotransferase (ALAT) and lactate dehydrogenase (LD), as well as on protein concentration, was studied in regenerating rat liver after partial hepatectomy. It was found that administration of an ethanol-containing liquid diet for 5 days after partial hepatectomy caused a significant accumulation of proteins in the liver. The activities of ODC and TAT were stimulated by ethanol treatment in the beginning of the regeneration. In control livers, partial hepatectomy decreased the activity of ALAT, but ethanol prevented this decrease. No differences in the activity of LD was found between ethanol and control groups after partial hepatectomy. When the half-lives of ODC and TAT were measured 24 hr after partial hepatectomy by using cycloheximide, it appeared that ethanol caused a significant stabilization of both enzymes. It is concluded that ethanol caused inhibition of degradation of ODC and TAT and it is suggested that this could be a general phenomenon, and could markedly contribute to the pathological accumulation of proteins in the liver after chronic ethanol consumption.

Ethanol Ornithine decarboxylase Tyrosine aminotransferase Alanine aminotransferase Lactate dehydrogenase Protein degradation Liver regeneration

CHRONIC alcohol consumption has been shown to cause accumulation of proteins in the liver [2, 11, 18]. In part, this accumulation has been explained by the inhibitory effect of ethanol on protein secretion from the liver cells, since albumin and transferrin has been found to accumulate in the cytoplasm after ethanol treatment [3]. One cause of this accumulation could be the inhibition of glycosylation of the exportable proteins in the liver cell [30,32], and thus inhibition of their secretion. Experimental evidence also supports the theory that ethanol inhibits the polymerization of tubulin, thus inhibiting the formation of the microtubules which could lead to the inhibition of the secretion of proteins from the liver [17].

Since only part of the considerable accumulation of proteins after chronic ethanol treatment can be explained by the inhibition of the excretion of proteins, the experiments of the present study were undertaken to see if ethanol has any inhibitory effect on the degradation of proteins which are not excreted. The experiments were performed in partially hepatectomized rats where ethanol (similar to intact rats) causes protein accumulation [8]. Four enzyme activities were used as markers of protein metabolism. Two of these enzymes, ornithine decarboxylase (ODC) and tyrosine aminotransferase (TAT) possess short half-lives, 15 min and 2–4 hr, respectively [12]. The other two, alanine aminotransferase (ALAT) and lactate dehydrogenase (LD) represent more stable proteins: the half-life for ALAT is 18 hr–3.5 days and that for LD is 16 days [12]. Part of these results has been published previously [24,26].

METHOD

Chemicals

D,L-(1-^{14}C)ornithine and L-(U-^{14}C)leucine were obtained from the Radiochemical Centre (Amersham, U.K.). Cycloheximide (Actidione®) was the product of Boehringer Mannheim (Mannheim, FRG).

Animals

Female rats of the mixed stain [7] bred at the laboratories of Alko (Helsinki, Finland) were used. At the start of the experiment, the mean weight of the rats was 245±27 g. Rats 3–4 months of age were used. Until the start of the experiments, rats were housed 6–8 per cage in a room maintained at 22–24°C and 53–57% relative humidity, with a day–night schedule of 12:12 hours. During the experiments, rats were housed individually. Before the experiments, rats were fed with ordinary laboratory chow (Astra-Ewos, Södertälje, Sweden) and tap water ad lib. During the experiments, rats were force-fed with a nutritionally adequate liquid diets [14], 4 times per day, for 1 to 6 days. In the control diet, carbohydrate was substituted for ethanol. The constant presence of ethanol in the blood of the ethanol-treated rats was ver-

TABLE 1

EFFECT OF ETHANOL ON PROTEIN CONCENTRATION IN THE LIVER AFTER PARTIAL HEPATECTOMY*

Days after Operation	Presence of Ethanol	n	Total Protein mg/g lipid-free liver wet wt.	Cytoplasmic Protein mg/ g liver wet wt.	Total Protein mg/liver
Unoperated	−	5	175 ± 25	106 ± 6	1390 ± 360
1	−	8	152 ± 17	90 ± 8	530 ± 80
1	+	8	157 ± 11	78 ± 8†	520 ± 100
2	−	7	150 ± 12	86 ± 5	800 ± 80
2	+	6	162 ± 14	95 ± 4†	790 ± 80
3	−	6	181 ± 23	92 ± 11	1260 ± 200
3	+	5	174 ± 14	92 ± 5	1080 ± 210
5	−	5	172 ± 12	88 ± 2	1280 ± 130
5	+	5	197 ± 9‡	78 ± 11	1670 ± 150‡

*Rats were force-fed with either control or ethanol-containing liquid diet 1 day before and 1 to 5 days after partial hepatectomy. The results are shown as the mean ± S.D. †=$p<0.01$, and ‡=$p<0.001$ for the statistical difference from the corresponding control group.

ified with measurements of blood ethanol concentration, and the amount of the ethanol-containing diet was adjusted accordingly. During the experiments, water was freely available.

Experimental Procedure

Rats were fasted for 24 hr and thereafter fed with either control or ethanol diet. The daily dose of ethanol was 10, 3.7, 5.4, 9.6, 7.7 and 11.4 g per kg body weight on days 1, 2, 3, 4, 5 and 6, respectively. Partial hepatectomy was performed under light diethylether anaesthesia [10] 24 hr after the beginning of the diet administration. About 67% of the liver was resected. The pieces of the liver were weighed and frozen for future analysis of ALAT and LD activities. In the morning of the 1st, 2nd, 3rd and 5th days after the operation, 5 to 8 rats from the control and ethanol groups were killed by decapitation. A group of unoperated rats served as additional controls. The livers were quickly removed, rinsed with ice-cold Tris-HCl buffer (pH 7.1) and weighed. Livers were homogenized in two volumes of a 25 mM Tris-HCl buffer (pH 7.1) which contained 0.2 mM EDTA and 1 mM dithiotreitol. Part of the homogenate was used for the measurement of total proteins. The rest was centrifuged at 4°C with $105{,}000 \times g_{max}$ for 30 min. The supernatant was used for the determination of the enzyme activities and the protein concentration.

Before the half-lives of ODC and TAT were measured, rats were first fasted for 24 hr, then given one dose of ethanol or control diet, and partially hepatectomized 3 hr later. The treatment with the diets was continued for 1 day. The whole dose of ethanol was 5 g per kg body weight. Cycloheximide (1.5 mg per kg body weight) was given intraperitoneally 15–3 hr before decapitation. The livers were treated exactly as described above and the activities of ODC and TAT were measured.

Analytical Procedure

Activities of ODC, TAT, ALAT and LD were measured as cited [4, 5, 13]. Protein was measured with bovine plasma albumin as standard [15]. Blood ethanol concentrations were measured gas chromatographically (Perkin-Elmer F 40) using a head-space technique [6].

Statistics

The Student's *t*-test for unpaired data or paired data (when indicated) was used to compare the ethanol and control groups. Linear correlations were measured with the least squares method.

RESULTS

Effect of Ethanol on Protein Content of the Liver after Partial Hepatectomy

Protein concentrations were measured from the $105{,}000 \times g$ supernatant fraction and from the total homogenate. In accord with an earlier study [31] in control livers, partial hepatectomy slightly decreased the protein content (per g liver tissue) in the beginning of the regeneration (Table 1). This may be due to the rapid accumulation of lipids [26], since the liver weight increases steadily [21,26]. Towards the end of the experiment this transient decrease was overcome and the protein concentration did not differ from that in the unoperated rat liver. No changes in the concentration of supernatant proteins was found (Table 1).

A decrease similar to that found in the controls was observed in the concentration of proteins in the livers of the ethanol-treated rats in the beginning of the regeneration. At the end of the experiment, the livers of the ethanol group contained more protein than the control livers (Table 1). No changes in the cytoplasmic protein concentration was found, suggesting that there may be concomittant accumulation of water [11].

Effect of Ethanol on the Activities of Ornithine Decarboxylase and Tyrosine Aminotransferase in the Kidneys and in Regenerating Liver after Partial Hepatectomy

We have previously shown that a single dose of ethanol markedly inhibits the stimulated activity of both ODC and

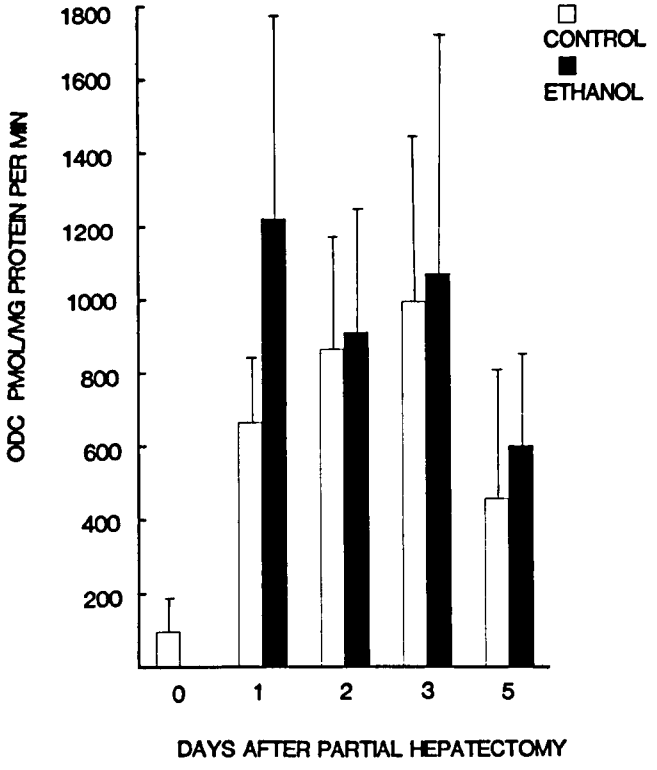

FIG. 1. Effect of ethanol on the activity of ornithine decarboxylase in regenerating rat liver after partial hepatectomy. Experimental conditions as in Table 1. The results are shown as the mean of 5 to 8 rats with the vertical bars showing the standard deviations. The bar at the day 0 represents unoperated control rats. Control and ethanol group were statistically different on day 1 ($p<0.05$).

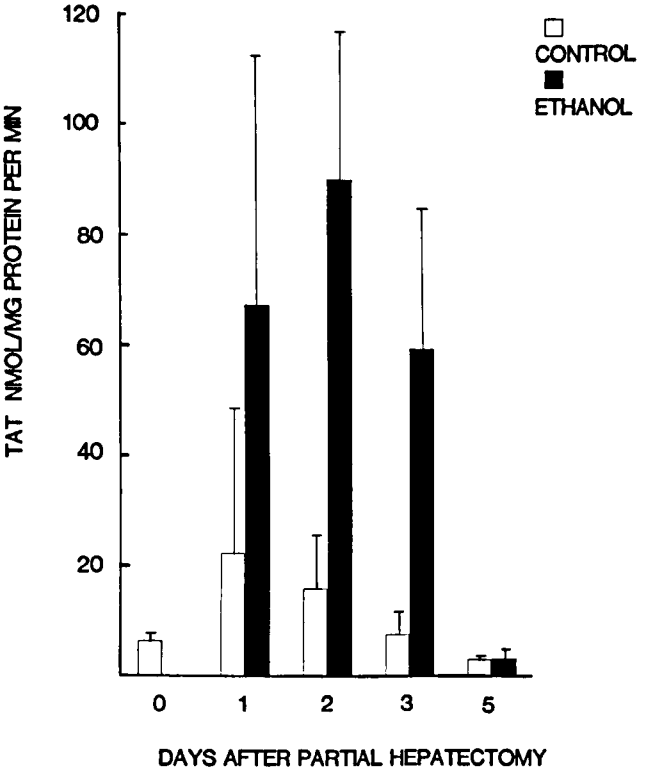

FIG. 2. Effect of ethanol on the activity of tyrosine aminotransferase in regenerating rat liver after partial hepatectomy. Experimental conditions as in Table 1 and Fig. 1. The results are shown as the mean of 5 to 8 rats with the vertical bars showing the standard deviations. Control and ethanol group were significantly different on days 1 ($p<0.05$), 2 ($p<0.001$) and 3 ($p<0.001$).

TAT in rat liver 4 hr after partial hepatectomy [23–25]. In striking contrast to this inhibition, it was found in the present study that continued ethanol treatment stimulated both ODC and TAT 1 day after partial hepatectomy (Figs. 1 and 2). In the case of ODC this stimulation persisted for 1 day, but for TAT the significant stimulation lasted for 3 days. However, the enzyme activities in the ethanol-treated rats were never lower than in the corresponding controls, indicating that the longer treatment with ethanol abolished the inhibitory action of ethanol seen immediately after partial hepatectomy [23–25], and in isolated hepatocytes [16].

We also measured the effect of ethanol on the activity of kidney ODC. In our previous studies [23,25], it was shown that partial hepatectomy stimulates the activity of ODC in the kidneys and that this stimulation is most probably due to the stress of the operation [25]. Also, it has been shown that a single dose of ethanol inhibits the stimulation of ODC in the kidneys [23,25]. Similarly as in the liver, chronic ethanol treatment stimulated the activity of ODC in the kidney (Fig. 3), and the stimulation persisted even longer than in the liver.

Effect of Ethanol on the Activity of Alanine Aminotransferase and Lactate Dehydrogenase in the Regenerating Liver Remnant after Partial Hepatectomy

As control we tested the effect of ethanol on two enzymes with relatively longer half-lives than those of ODC and TAT, namely ALAT and LD. In control livers it was found that partial hepatectomy significantly decreased the activity of ALAT (Fig. 4). The reason for this phenomenon cannot be determined from these experiments, but it may be related to the slow protein catabolism of regenerating rat liver [29]. To control for this possibility we measured the activity of ALAT from the pieces of the liver which were removed during partial hepatectomy. When the two ALAT activities of the same liver (at the time of partial hepatectomy and 1–5 days after the operation) were compared, it was found that the values after partial hepatectomy were always significantly lower (paired t-test, results not shown) than the values measured at the time of the operation.

As seen in Fig. 4, ethanol prevented this decrease in the activity of ALAT during 2 postoperative days. Later on, the

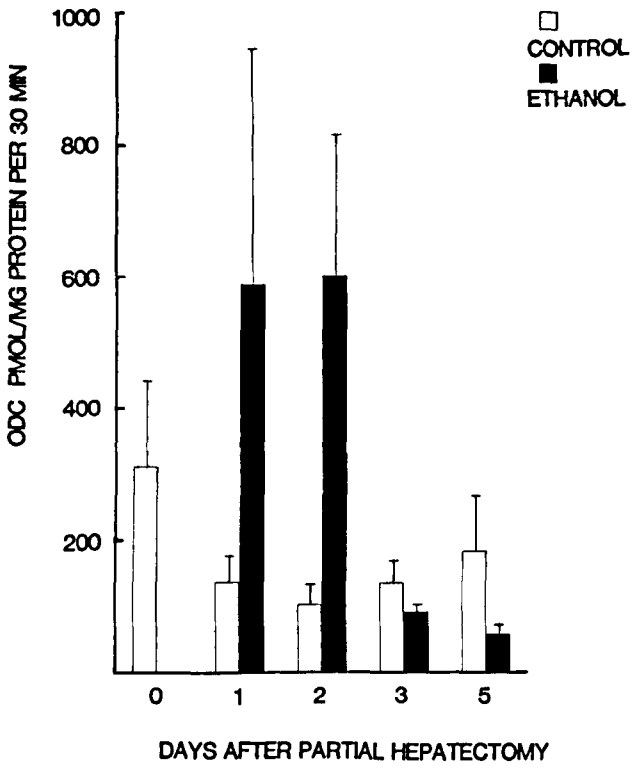

FIG. 3. Effect of ethanol on the activity of ornithine decarboxylase in rat kidney after partial hepatectomy. Experimental conditions as in Table 1 and Fig. 1. The results are shown as the mean of 5 to 8 rats with the vertical bars representing the standard deviations. Control and ethanol group differed significantly on days 1 ($p<0.01$), 2 ($p<0.001$), 3 ($p<0.01$) and 5 ($p<0.01$).

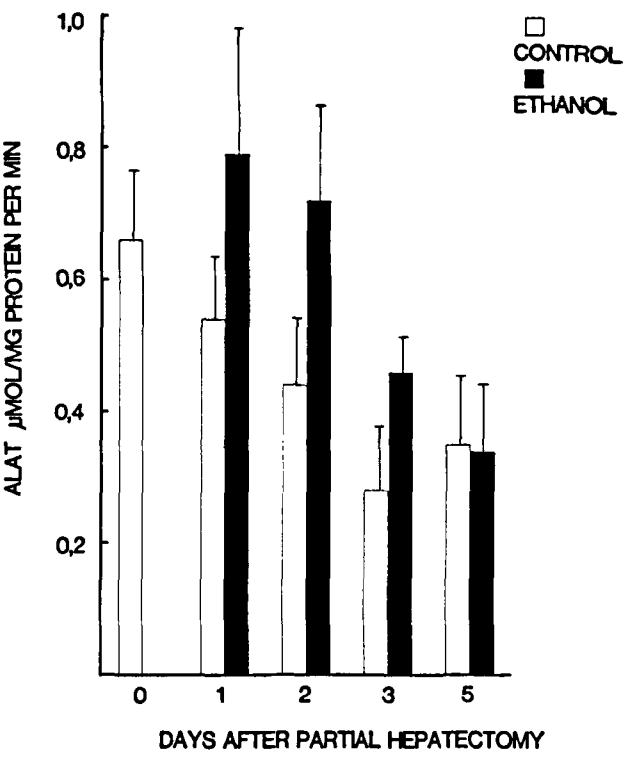

FIG. 4. Effect of ethanol on the activity of alanine aminotransferase in regenerating rat liver after partial hepatectomy. Experimental conditions as in Table 1 and Fig. 1. The results are shown as the mean of 5 to 8 rats with the vertical bars representing the standard deviations. Control and ethanol group were statistically different on days 1 ($p<0.01$), 2 ($p<0.01$) and 3 ($p<0.01$).

values of ethanol livers were found to decrease to the same level as in the controls. Similarly, as in the controls, ALAT activities measured on days 3 and 5 after partial hepatectomy were significantly lower (paired t-test, results not shown) than the values of the same livers measured at the time of the operation.

Lactate dehydrogenase increased linearly with time both in the ethanol-treated ($r=0.990$; $df=22$; $p<0.001$) and in the control ($r=0.991$; $df=24$; $p<0.001$) livers during the 3 first days after partial hepatectomy and thereafter stayed at a constant level which was significantly higher than the LD activity in the liver removed during the partial hepatectomy (paired t-test, results not shown). The changes in the activity of LD are similar to those reported earlier [31].

Effect of Ethanol on the Half-Life of Ornithine Decarboxylase and Tyrosine Aminotransferase in Regenerating Rat Liver after Partial Hepatectomy

The increase in the activity of the short half-life enzymes, ODC and TAT, as well as the increasing effect of ethanol on the activity of ALAT after partial hepatectomy, led us to study more closely if ethanol has any effect on protein degradation. Therefore, we measured the half-lives of ODC and TAT 1 day after partial hepatectomy in ethanol-treated and in control rats and found that ethanol stabilized the activity of both ODC and TAT after cycloheximide treatment. The half-life of ODC was changed from 17 min to 33 min ($p<0.01$) and that of TAT from 97 min to 140 min ($p<0.01$).

DISCUSSION

Hepatomegaly is a common manifestation of alcoholic liver disease [28]. Fat accumulation can partially explain the increase in the liver mass, but it has also been shown that ethanol treatment leads to accumulation of proteins in the liver [2, 3, 11]. This accumulation most probably increases cell volume since the amount of intracellular water is increased [11]. Until this time the only explanation for this pathological accumulation of proteins has been the possible retention of exportable proteins in the liver after acute and chronic ethanol consumption. However, as pointed out by Israel *et al.* [11], the colloidal osmotic pressure following the accumulation of export proteins accounts only for two per cent of the increase in the intracellular water. Furthermore,

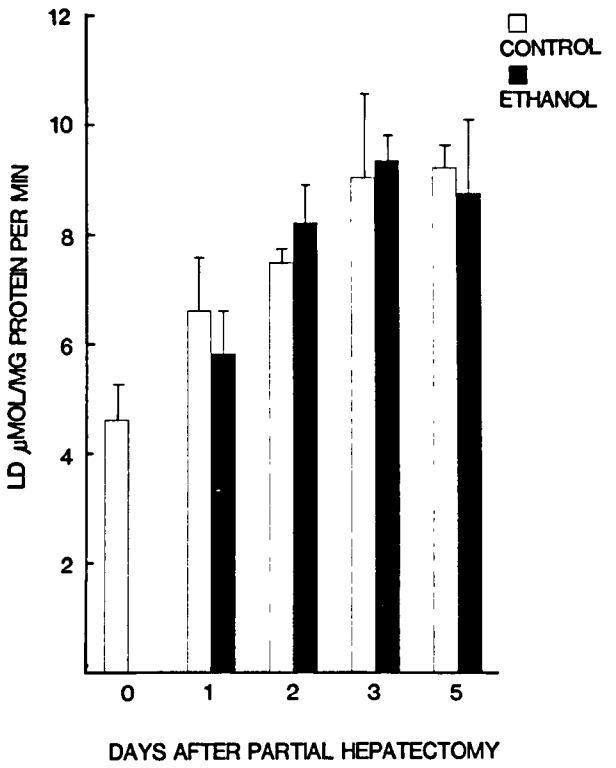

FIG. 5. Effect of ethanol on the activity of lactate dehydrogenase in regenerating rat liver after partial hepatectomy. Experimental conditions as in Table 1 and Fig. 1. The results are shown as the mean of 5 to 8 rats with the vertical bars showing the standard deviations.

in two recent studies, no effect of ethanol on the secretion of proteins was found [20,27]. This simply means that there has to be other mechanism(s) for protein accumulation or even other accumulating compounds leading to the increased colloidal osmotic pressure which is the driving force for the increase in the intracellular volume after alcohol consumption.

The results of the present study suggest an entirely new mechanism contributing to the accumulation of proteins. Ethanol seems to inhibit the degradation of two enzyme proteins, at least in regenerating rat liver after partial hepatectomy. Although the stabilization of proteins was shown only for ODC and TAT (Figs. 1 and 2), it might be a general phenomenon since the activity of ALAT was also increased in the ethanol-treated livers (Fig. 4). Additionally, five days after partial hepatectomy, the livers from ethanol-treated rats contained more protein than the control livers (Table 1). The present results are thus consistent with an earlier study where the half-life of collagen was found to be longer in ethanol-fed rats than in controls [9]. Also, the decay rate of the activity of TAT after cycloheximide treatment has been shown to be slower in the presence of ethanol in perfused rat liver [19]. However, to prove that the stabilization is a general phenomenon, a new and elegant method recently developed to study degradation of proteins in regenerating rat liver should be used [18].

The molecule responsible for the protein stabilization, as well as the mechanism of this stabilization are both totally open questions. Ethanol elimination in regenerating rat liver is quite similar to that in intact liver [21]. Also the ethanol-induced redox change does not differ from the ethanol-induced change in unoperated rats [22]. Thus in the regenerating rat liver the effect could be mediated either via ethanol, acetaldehyde or the ethanol-induced redox change. In the present study, the stimulated activity of ODC was found in the kidneys of ethanol-treated rats. The activity of alcohol dehydrogenase in the kidney is lower than in the liver [33] and accordingly less acetaldehyde and NADH is formed in this tissue, thus suggesting that the effect could be a direct effect of ethanol. However, more direct evidence is needed to confirm this suggestion.

One possible mechanism leading to the stabilization of proteins after ethanol-treatment could be the inhibition of proteolysis by ethanol in the regenerating liver. In regenerating rat liver, the half-life of proteins is doubled and this has been suggested to play an important role in the recovery of liver proteins [29]. Recently it was shown that the decreased rate of protein degradation could be due to lowered level of lysosomal cathepsins in the liver [31]. Since ethanol inhibits protein synthesis it may as well inhibit the synthesis of cathepsins and thus additionally slow down protein degradation. This possibility is very interesting, since it has been shown that in addition to degradation of intracellular proteins, proteolytic activity is needed to convert proalbumin to albumin which is then secreted from the cells [1]. Thus the possible interference of ethanol with intracellular proteolysis could explain the accumulation of both intracellular proteins and the acculation of albumin.

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