

EFFECTS OF L-CARNITINE ON VENTRICULAR ARRHYTHMIAS IN DOGS WITH ACUTE MYOCARDIAL ISCHEMIA AND A SUPPLEMENT OF EXCESS FREE FATTY ACIDS

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The effects of L-carnitine on ventricular arrhythmias were evaluated in dogs with acute myocardial ischemia and a supplement of excess free fatty acids (FFA).

Acute myocardial ischemia was induced by ligation of left anterior descending coronary artery. After 80 minutes of coronary occlusion, high plasma FFA was induced by intravenous injection of heparin 200 u/kg and Intralipid® 5 ml/kg as a bolus. After additional 60 minutes, beating hearts were removed from animals and tissue levels of free carnitine, short and long chain acyl carnitine, FFA and adenosine triphosphate (ATP) were determined. L-carnitine 100 mg/kg was administered intravenously 5 minutes before coronary artery ligation. Electrocardiograms were recorded continuously by a Holter electrocardiographic recorder during the experiment and ventricular arrhythmias were quantified by an arbitrary scoring system.

In ischemic and excess FFA supplemented myocardium, free carnitine and ATP decreased, whereas long chain acyl carnitine and FFA increased. And these metabolic changes tended to be reduced by L-carnitine. Pretreatment of L-carnitine also reduced the grade of ventricular arrhythmias induced both by acute myocardial ischemia and by supplement of excess FFA.

These results suggest that the administration of L-carnitine may be beneficial to prevent serious arrhythmias in ischemic heart disease, presumably by restoring the impaired FFA oxidation.

HIGH concentrations of free fatty acids (FFA) have been reported to provoke some arrhythmias in ischemic heart disease.¹⁻³

Key Words:

L-carnitine
Ventricular arrhythmias
Acute myocardial ischemia
Excess free fatty acids
Long chain acyl carnitine

Several explanations have been proposed concerning the mechanism, such as nonspecific detergent action on biomembranes which leads to cation loss⁴ followed by the changes of action potential⁵⁻⁷ and uncoupling of oxidative phosphorylation in the mitochondria⁸⁻¹¹ which leads to the reduced production of adenosine triphosphate (ATP). However, recently, a great attention has been focused on the accumulation of inter-

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mediates subsequent to impaired oxidation of FFA as a cause of the toxic cardiac effects of excess FFA.¹²⁻¹⁵

Carnitine, a water-soluble naturally occurring amino acid, is essential for fatty acyl derivatives to penetrate across the inner mitochondrial membrane^{16,17} and to be transported to the sites of oxidation in the mitochondria. A reduction in tissue levels of carnitine has been demonstrated in ischemic¹⁸⁻²¹ and FFA supplemented heart¹⁴ and is supposed to exaggerate ischemic damage of myocardium, presumably by accelerating the accumulation of fatty acyl derivatives. From this point of view, protective effects of exogenous carnitine on the metabolic derangement in ischemic heart have been reported in several studies.^{19,21-23} However, antiarrhythmic effects of exogenous carnitine have been reported only in the incidence of protection against ventricular fibrillation in the dogs with coronary artery occlusion.^{21,22}

The purpose of this study was to evaluate the effects of L-carnitine on the ventricular arrhythmias in the dogs with acute myocardial ischemia and a supplement of excess FFA.

MATERIALS AND METHODS

Twenty-one mongrel dogs weighing 8 to 12 kg were anesthetized with intravenous sodium pentobarbital (30 mg/kg). Ventilation was maintained by means of a Harvard animal respirator with room air. The dogs had been fasted for 16 to 20 hours. A left thoracotomy was performed through the 4th intercostal space. The pericardium was opened and the heart was slightly suspended. A short area of the left anterior descending branch of the coronary artery was dissected free from surrounding tissue and the dissected artery was ligated by a silk thread together with 23 gage needle located beside it. By drawing off the needle, incomplete occlusion of coronary artery was produced. After 80 minutes of coronary ligation, a bolus of 200 u/kg of heparin and 5 ml/kg of Intralipid[®] containing soy-bean oil were injected intravenously. After additional 60 minutes, beating hearts were removed from the animals and transmural tissue (1-1.5g) representing ischemic area (supplied by the ligated artery) and nonischemic area (supplied by circumflex artery) were rapidly excised. Briefly the respective tissues were frozen with Wollenberger

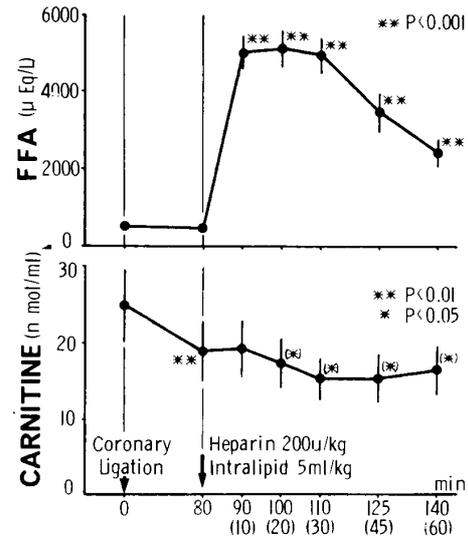


Fig. 1. Effects of coronary ligation and administration of heparin and Intralipid on the plasma levels of FFA and carnitine. About carnitine, asterisks without parenthesis represent the significance of change with respect to the level before coronary ligation. Asterisks inside the parenthesis represent the significance of change with respect to the level after 80 minutes of coronary ligation.

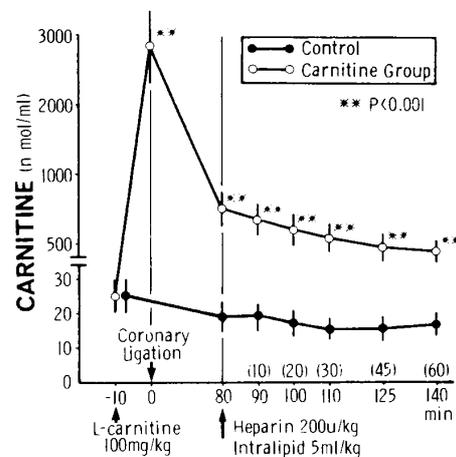


Fig. 2. Plasma levels of carnitine in dogs pretreated with 100 mg/kg of L-carnitine (carnitine group). Asterisks represent the significance of change with respect to the control levels.

clamp cooled to the temperature of liquid nitrogen. The frozen and pressed samples were cracked into fragments on a block of dry ice and stored at -70°C . Extraction and analysis of carnitine, short and long chain acyl carnitine, ATP and FFA were made on these frozen tissues within three days. Plasma levels of carnitine and FFA were determined before and after 80 minutes

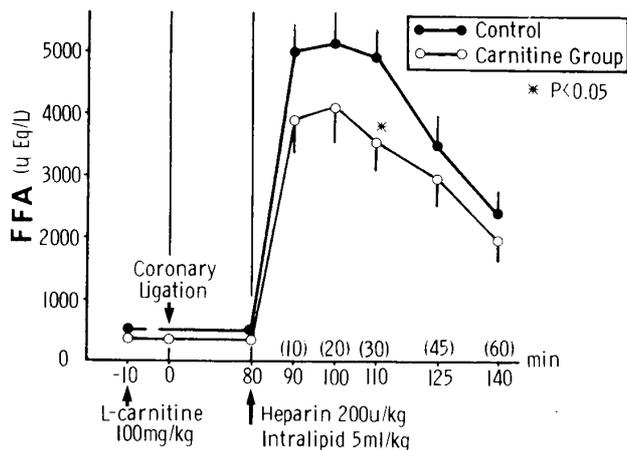


Fig.3. Effect of L-carnitine on the plasma levels of FFA. An asterisk represents the significance of change with respect to the control level.

of coronary ligation and at 10, 20, 30, 45 and 60 minutes after the injection of heparin and Intralipid.

Electrocardiograms were recorded continuously during the experiment by a Holter electrocardiographic recorder and analysed by DYNAGRAM Model DCG-6000 (Instrument for Cardiac Research Inc., New York).

In ten dogs, 100 mg/kg of L-carnitine (provided by Otsuka Pharmaceutical Factory Inc., Japan) was administered intravenously over five minutes. After additional five minutes, the coronary artery was ligated. Eleven dogs without L-carnitine were used as the controls.

Free carnitine was determined enzymatically using carnitine acetyl transferase by the method of Marquis and Fritz²⁴. Short and long chain acyl carnitine were assayed as free carnitine after alkaline hydrolysis by the method of Pearson et al.²⁵ ATP was determined by enzyme assay using hexokinase and glucose-6-phosphate dehydrogenase²⁶ and FFA was determined by the method of Itaya and Ui²⁷.

Values were represented as mean \pm SE. Statistical analysis was made by paired or non-paired Student's t test, as appropriate.

RESULTS

Plasma levels of FFA and carnitine

Plasma levels of FFA and carnitine in the control dogs are shown in Fig. 1. Plasma FFA remained unchanged after 80 minutes of coronary ligation. Following the injection of heparin and Intralipid, plasma FFA rapidly increased ($p <$

TABLE I SCORING SYSTEM FOR VENTRICULAR ARRHYTHMIAS

0	No VPBs
1	Isolated unifocal VPBs < 5/min
2	Isolated unifocal VPBs > 5/min
3	Multifocal VPBs
4	Couplets or salvos
5	Early VPBs (R on T)
6	Ventricular tachycardia
7	Ventricular fibrillation

Grading system for VPBs by Lown and Wolf²⁸ was modified.

VPB = ventricular premature beat

0.001), remained at high levels for 30 minutes and gradually decreased thereafter. Plasma carnitine levels decreased after 80 minutes of coronary ligation ($p < 0.01$), and decreased much more after the injection of heparin and Intralipid.

In the dogs pretreated with 100 mg/kg of L-carnitine, plasma levels of carnitine maintained far higher levels throughout the experiment as compared with that in the controls ($p < 0.001$) (Fig. 2). Pretreatment of L-carnitine suppressed the increase in plasma FFA levels induced by heparin and Intralipid after 30 minutes ($p < 0.05$) (Fig. 3).

Ventricular arrhythmias induced by acute myocardial ischemia and supplement of excess FFA

Ventricular arrhythmias were quantified by an arbitrary scoring system that was produced by modifying the grading system for ventricular premature beats by Lown and Wolf²⁸ (Table I). Maximum score during every 10 minutes was expressed in individual cases (Table II) and changes of the mean score were shown in Fig. 4.

In untreated dogs, ventricular arrhythmias occurred in all cases following coronary artery occlusion. They usually begin to appear within a few minutes after coronary ligation, reached at a maximum score and subsided thereafter up to 80 minutes, although three different types of the occurrence were observed in individual cases (Fig. 5). In 10 cases, ventricular arrhythmias occurred only in an early period after coronary ligation and disappeared after 40 minutes. In a case, they increased up to 60 minutes after coronary ligation and subsided after 80 minutes. In 9 cases, they continued up to 80 minutes after coronary ligation. Excess FFA induced by

TABLE II VENTRICULAR ARRHYTHMIAS INDUCED BY CORONARY ARTERY OCCLUSION AND SUPPLEMENT OF EXCESS FFA

Case No.	Weight (kg)	Coronary occlusion								Heparin + Intralipid								min
		↓ 10	20	30	40	50	60	70	80	90	100	110	120	130	140			
<i>Control</i>																		
1	11	4	4	4	5	5	6	5	0	0	1	1	1	4	4			
2	11	6	6	2	0	0	2	1	1	1	3	4	4	3	6			
4	10	4	2	3	2	2	1	2	4	4	2	4	2	4	4			
6	10	4	7															
8	11	0	0	0	1	4	2	2	4	4	4	4	1	1	2			
10	8	0	0	1	0	0	0	0	1	1	0	0	0	0	0			
12	10	0	2	3	0	2	1	0	0	0	1	4	0	4	1			
14	9	0	0	3	4	3	3	4	4	3	4	6	4	6	4			
16	9	0	0	0	0	0	1	1	0	3	1	4	4	1	4			
18	10	0	1	1	0	0	0	0	1	4	1	4	3	3	3			
20	8	4	6	4	4	3	3	3	6	6	6	4	6	4	3			
<i>L-carnitine treated</i>																		
3	9	0	1	3	4	4	4	4	4	3	4	3	4	4	3			
5	9	0	4	1	2	1	3	4	2	1	3	3	6	1	3			
7	10	0	0	0	0	0	0	0	0	1	0	0	0	0	1			
9	11	0	0	1	1	1	1	1	1	0	0	0	0	0	0			
11	9	0	4	1	1	0	0	1	1	0	1	0	3	3	3			
13	12	0	0	1	1	1	1	1	1	4	1	0	0	0	1			
15	8	1	0	0	1	1	1	3	3	3	3	4	4	4	4			
17	9	0	0	0	1	0	1	3	3	1	1	4	3	4	4			
19	8	4	4	4	3	3	4	1	6	3	1	3	3	3	4			
21	10	0	0	1	0	0	1	1	0	1	0	1	1	1	1			

Maximum score for every 10 minutes was expressed according to the scoring system for ventricular arrhythmias as shown in Table 1.

heparin and Intralipid increased again the grade of ventricular arrhythmias.

Pretreatment of L-carnitine reduced the grade of arrhythmias in early phase of myocardial ischemia (10 to 20 minutes) and the arrhythmias induced by excess FFA. These effects of L-carnitine were more clear in the cases in which every score in a period from 40 to 80 minutes of coronary occlusion was below 2 (Fig. 6).

Tissue levels of carnitine derivatives, FFA and ATP

Changes in the tissue levels of various metabolites following coronary artery occlusion and supplement of excess FFA are shown in Table III. After 140 minutes of coronary occlusion, free and total carnitine decreased, whereas long chain acyl carnitine increased in ischemic myocardium.

ATP decreased and FFA increased in ischemic myocardium (compared with nonischemic myocardium by paired t test). Pretreatment of L-carnitine 100 mg/kg tended to suppress the decrease in free carnitine from 525 ± 62 to 651 ± 65 nmol/g and ATP from 1.63 ± 0.48 to 2.31 ± 0.53 μ mol/g in ischemic myocardium. On the other hand, it also tended to suppress the increase in long chain acyl carnitine from 500 ± 91 to 454 ± 60 nmol/g, although those changes were statistically not significant (by non-paired t test).

DISCUSSION

Two types of ventricular arrhythmias were observed in this study. One was induced by coronary artery occlusion and the other was provok-

TABLE III EFFECTS OF L-CARNITINE ON TISSUE LEVELS OF VARIOUS METABOLITES

		Carnitine				ATP	FFA
		Free	Short chain acyl	Long chain acyl	Total	(μ mol/g)	(μ Eq/g)
		(n mol/g)	(n mol/g)	(n mol/g)	(n mol/g)		
<i>Nonischemic area</i>							
No treatment	(10)	878 \pm 146	415 \pm 31	261 \pm 23	1544 \pm 169	5.26 \pm 0.13	3.89 \pm 0.28
L-carnitine treated	(10)	968 \pm 97	368 \pm 30	213 \pm 26	1551 \pm 116	5.34 \pm 0.17	3.49 \pm 0.30
<i>Ischemic area</i>							
No treatment	(10)	525 \pm 62*	361 \pm 34**	500 \pm 91**	1386 \pm 149*	1.63 \pm 0.48***	4.41 \pm 0.34*
L-carnitine treated	(10)	651 \pm 65***	318 \pm 39	454 \pm 60**	1523 \pm 104**	2.31 \pm 0.53***	4.27 \pm 0.38**

Values are expressed per gram wet tissue weight and represented as mean \pm SE. The number of animals is given in parenthesis. Asterisks represent the significance of change between nonischemic and ischemic areas. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$

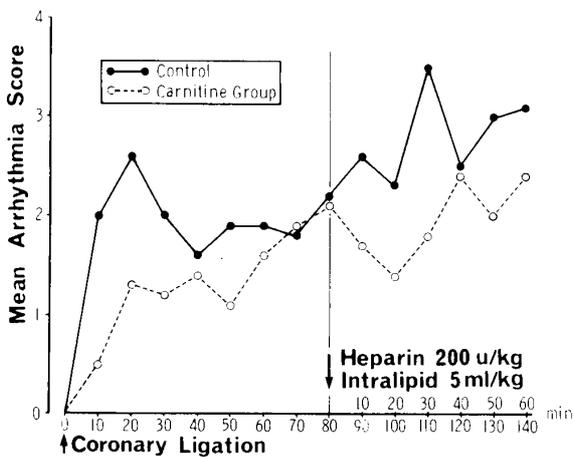


Fig.4. Effect of L-carnitine on the ventricular arrhythmias induced by coronary artery occlusion and supplement of FFA. Effect of L-carnitine on the mean score of ventricular arrhythmias was evaluated in all cases. Scores in 10 minutes after coronary ligation and in 30 minutes after the supplement of excess FFA were significantly reduced by L-carnitine.

ed by the supplement of excess FFA.

Early ventricular arrhythmias following coronary artery occlusion

The early ventricular arrhythmias which occur within minutes after coronary artery occlusion has been electrophysiologically explained to result from reentry in ischemic ventricular muscle²⁹ However, important local metabolic events have been also demonstrated to occur within seconds and minutes of coronary occlusion, namely the increase in extracellular potassium, the in-

crease in tissue lactate, the increase in tissue partial pressure of carbon dioxide followed by marked acidosis and the increase in tissue cyclic adenosine monophosphate (AMP).³⁰ At the same time, general metabolic changes also occur, namely an increased blood lactate, increased blood FFA concentrations, increased circulating catecholamines and an increased circulating cyclic AMP.³⁰ And these metabolic factors are thought to play an important role in the genesis of ischemic arrhythmias, probably by causing abnormalities in the action potential of ischemic myocardial cells.³⁰

Recently a reduction in tissue levels of carnitine has been demonstrated in ischemic myocardium¹⁸⁻²¹ and the accumulated intermediate metabolites in FFA oxidation has been proposed as a cause of the cellular damage in ischemic myocardium.¹³

In our previous study,³¹ after 15 minutes of coronary occlusion, tissue levels of free carnitine decreased by 40 percent in ischemic myocardium, whereas long chain acyl carnitine and long chain acyl coenzyme A (CoA) increased by 133 percent and 48 percent respectively. And pretreatment of L-carnitine significantly prevented the depletion in tissue levels of free carnitine and ATP, and the accumulation of long chain acyl carnitine and long chain acyl CoA. In this study, however, no significant changes by L-carnitine were observed in the tissue levels of metabolites, although L-carnitine tended to suppress the decrease in free carnitine and ATP, and the increase in long chain acyl carnitine. The supposed reason is that coronary occlusion of 140 minutes

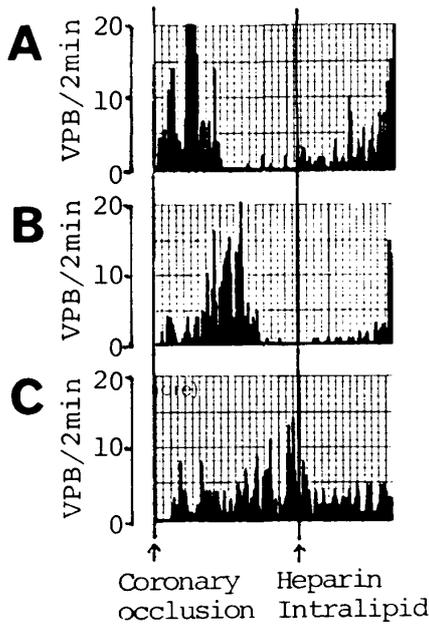


Fig.5. Examples of ventricular arrhythmias induced by coronary artery occlusion and supplement of excess FFA. Incidence of ventricular premature beats (VPBs) was expressed on a histogram by the number for every one minute (a part of the trend chart automatically analysed by DYNAGRAM Model DCG-6000).

A: VPBs occurred only in a early phase after coronary ligation and supplement of excess FFA increased the incidence of VPBs (Case 2).

B: The incidence of VPBs gradually increased up to 60 minutes after coronary ligation and subsided thereafter. Supplement of excess FFA slightly increased the incidence (Case 1).

C: The occurrence of VPBs continued up to 80 minutes after coronary ligation. The incidence of VPBs was not increased by excess FFA (Case 3).

duration was too long for L-carnitine to effect the significant improvement in the tissue levels of metabolites.

Since high levels of long chain acyl CoA inhibits adenine nucleotide translocase³² that is the regulator of the egression of ATP across the inner mitochondrial membrane, accumulation of long chain acyl CoA is supposed to exaggerate the depleted ATP production by reduced oxygen supply in ischemic myocardium. And the ratio of long chain acyl CoA to free carnitine was suggested to be important in regulating the activity of several enzymes during early phases of ischemia.¹⁹ However, in these periods, the increase in long chain acyl carnitine was nearly three times of the increase in long chain acyl CoA.^{31,33,34} In

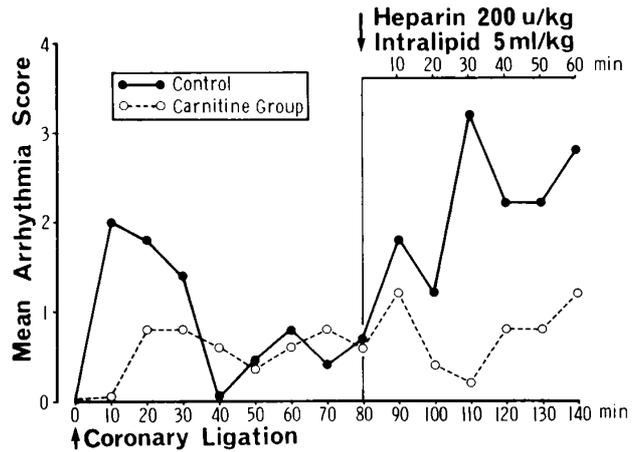


Fig.6. Effect of L-carnitine on excess FFA induced arrhythmias. Effect of L-carnitine on excess FFA induced arrhythmias was evaluated in the cases in which every score in a period from 40 to 80 minutes of coronary occlusion was below 2. Pretreatment of L-carnitine prevented the aggravation of the grade of ventricular arrhythmias induced by excess FFA. Control = Case 2, 10, 12, 16, 18. Carnitine group = Case 7, 9, 11, 13, 21.

addition, negative correlation was observed not only between ATP and the ratio of long chain acyl CoA to free carnitine, but also between ATP and the ratio of long chain acyl carnitine to free carnitine.³¹ And the effect of L-carnitine was more prominent in the reduction in long chain acyl carnitine than that in long chain acyl CoA.³¹ It has been also reported that long chain acyl carnitine is a more powerful inhibitor of the activity of bovine heart Na^+, K^+ -ATPase than long chain acyl CoA,³⁵ and it is also an inhibitor of the Ca^{++} -ATPase activity of cardiac sarcoplasmic reticulum isolated from dog hearts.³⁶ Serious ventricular arrhythmias in early phase following coronary occlusion were prevented by the pretreatment of L-carnitine. And in this study, ventricular fibrillation occurred only in a case of the controls. Taken together with the results of our previous studies^{21,31} 8 of 52 untreated dogs (15.4%) manifested ventricular fibrillation, whereas only one of 26 dogs treated with L-carnitine (3.4%) had fibrillation 5 to 15 minutes after coronary occlusion. From these observations, accumulation of long chain acyl carnitine appears to be one of the factors that cause ventricular arrhythmias in ischemic heart.

Ventricular arrhythmias induced by excess FFA
When excess FFA was supplemented in cases

in which ventricular arrhythmias subsided 40 to 80 minutes after coronary occlusion, the grade of ventricular arrhythmias increased again. Pretreatment of L-carnitine reduced the grade of these arrhythmias and tended to reduce the increased tissue long chain acyl carnitine levels.

Arrhythmogenic effect of high plasma FFA in acute myocardial infarction has been reported in man¹ and animals^{2,3} and several explanations have been proposed as the mechanisms⁴⁻¹¹. However those results could not be repeated by other investigators^{37,38}. Although the difference has been explained that arrhythmogenic effect was due not to the absolute level of FFA but rather to the FFA/albumin molar ratio,³ the results in this study suggest that the accumulated long chain acyl carnitine may also play an important role in the genesis of the ventricular arrhythmias induced by excess FFA, as well as coronary artery occlusion. In other words, whether excess FFA are able to provoke arrhythmias or not may be due not only to FFA/albumin molar ratio, but also to the tissue carnitine concentrations that is essential for the oxidation of long chain fatty acyl derivatives.

From this point of view, administration of L-carnitine is supposed to be beneficial to prevent serious arrhythmias in ischemic heart disease, presumably by restoring the impaired FFA oxidation.

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