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NEUTRAL LIPASE OF RAT HEART: AN INDUCIBLE ENZYME ?

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SUMMARY. Post-nuclear supernatant (PNS) prepared from homogenates of heparin-pretreated adult rat hearts contains an acid and a neutral lipase activity. Both lipases preferentially hydrolyze endogenous PNS triglycerides (TG). PNS derived from newborn rat hearts, which is depleted of TG, lacks the neutral lipase activity. After dietary trierucate (TE)-induced cardiac lipidosis, the neutral lipase activity in PNS is markedly enhanced. TG-accumulation can also be induced upon in vitro perfusion of rat hearts with Intralipid® and rat serum. Intralipid® -induced lipidosis is accompanied by an increased neutral lipase activity, which can be abolished when protein synthesis is inhibited by cycloheximide. Depletion of cardiac TG, during long-term perfusion, leads to a decrease in PNS neutral lipase activity. When PNS was prepared from hearts 5 h after cycloheximide pretreatment of rats the neutral lipase activities were reduced with a half-life of 6 h. Our data suggest that TG-mediated induction of neutral lipase synthesis is responsible for the increased rate of lipolysis observed during myocardial lipidosis.

Neutral lipase(s) have been demonstrated in rat heart homogenates by Yamamoto and Drummond[1] and by Biale et al.[2]. The enzyme(s), different from lipoprotein lipase (LPL), hydrolyzed long-chain triacylglycerols at least one order of magnitude slower than monoacylglycerols. Therefore their role in triacylglycerol hydrolysis in situ is unknown. Recently, Severson[3] and Rösen et al.[4] also found a neutral acyl-glycerol lipase in rat heart homogenates as well as an acid lipase with pH optima of 7.5 and 4.5, respectively. The determination of the relative contribution of these enzymes to endogenous lipolysis in heart has been attempted recently in our laboratory. In that study[5] in vitro heart perfusions were carried out with methylamine (a lysosomal inhibitor) or diethyl p-nitrophenylphosphate (an inhibitor of the neutral lipase(s)) in order to determine the mutual contributions of acid and neutral lipases to endogenous lipolysis. It was found that an increase of the lipid depot in heart, by previous feeding with trierucate, particularly increased methylamine-resistent lipolysis. Therefore lipidosis probably coincided with an extralysosomal activity increase of endogenous lipolysis.

A strong correlation between endogenous TG and the activity of the lipolytic system has been noted earlier[4,6]. Furthermore, in our laboratory

Abbreviations used: FFA, free fatty acids; LPL, lipoprotein lipase; PNS, post-nuclear supernatant; TE, trierucate; TG, endogenous triglycerides; TO, triolein.

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Jansen et al.[7] demonstrated that feeding rats with high doses of fat (rapeseed oil or olive oil), markedly increased both heparin-releasable (lipoprotein lipase) and non-releasable (possibly the neutral enzyme involved in endogenous lipolysis) lipases in heart. It is the purpose of the present study to investigate the relation between endogenous cardiac TG and neutral lipase activity further.

METHODS AND MATERIALS

Animals. Male Wistar rats (220±20 g body weight) were used throughout the study. They were fed control or TE-rich diets as published elsewhere[6]. Some rats were injected with 5 mg cycloheximide in 0.9% (w/v) NaCl (i.p.) 5 h before sacrifice. Control rats received only saline. Hearts were also obtained from newborn rats of unknown sex directly after birth to prevent feeding of the animals.

Preparation of rat heart PNS. Under ether-anesthesia the hearts were excised quickly, chilled and perfused retrogradely at a rate of 300 beats/min as described before[6,8]. After a 10 min stabilization period in the presence of 11.1 mM glucose, vascular LPL was removed by perfusion with heparin (5 U/ml) for 15 min[10]. After heparin-washout the hearts were minced in a 1 mM phosphate buffer (pH 7.4), washed three times and homogenized as in ref. 5. Hearts from newborn rats were homogenized without heparin pretreatment (groups of 7 hearts were pooled to obtain a suitable amount of tissue). From the homogenates a PNS was prepared exactly as in ref. 5.

Intralipid® perfusion. Lipidosis was induced in rat hearts during perfusion for 1, 2, 4 and 6 h with 1000 ml recirculating buffer containing 8 g soybean TG, 0.5 g phosphatidylcholine and 0.9 g glycerol (introduced with 40 ml 20% Intralipid® from Vitrum, Stockholm) and 1% (v/v) rat serum, supplemented with an amino acid mixture of the same composition of Ham's F 10 nutrient mixture[9]. As control for these Intralipid®-perfusions the same experimental protocol was followed except that the triolein-lecithin vesicles in the Intralipid® were removed by centrifugation (120 min x 72,000 g_{av}). After slicing the fatty top layer, 40 ml of the infranatant was used instead (delipidated "Intralipid"). All buffers were filtered through a 8 µm polycarbonate membrane filter before reaching the aortic canula. After Intralipid®-perfusion the lipid vesicles were washed out during subsequent perfusion with lipid-free buffer until the effluent was clear. Thereupon heparin-perfusion took place for at least 15 min. The effluent collected in the first 2 min was tested for LPL activity. After heparin-washout the PNS was prepared as above. Inhibition of protein synthesis during Intralipid®-perfusion was accomplished by inclusion of cycloheximide (3 µg/ml) in the recirculating perfusion buffer.

Determination of lipolytic activities. Lipolytic activity in PNS was determined with endogenous TG or with 4 µmoles triolein (TO) suspended in 5% (w/v) gum acacia[11]. Incubation was carried out at 37°C in a final volume of 1.0 ml, which contained 15 mg defatted bovine serum albumin, 330 µmoles Tris-acetate buffer of indicated pH and 0.5-2 mg PNS protein. After 45 min the reaction was stopped by the addition of 5 ml acetone. After centrifugation the pellet was reextracted with acetone. The combined acetone fractions, containing the extracted free fatty acids (FFA), were evaporated to dryness at 45°C. FFA in the residue were determined according to Duncombe[12]. Overall FFA recovery using internal standards amounted 90.3±2.5% (n=6). FFA production by lipases in PNS is linear during 120 min and proportional with protein varying from 0.5 to 4 mg/incubation[13]. LPL activity in coronary effluent was determined at 37°C according to Nilsson-Ehle and Schotz[14]. 1 mU of LPL activity represents the release of 1 nmole FFA/min from the TG substrate.

Analytical procedures. TG in PNS was estimated according to Laurell[15] and protein by the biuret method.

Electronmicroscopy. Electronmicrographs were taken from left ventricular sections of hearts previously perfused for 6 h with Intralipid®, after fixation in 3% (w/v) glutaric aldehyde in 0.067 M cacodylate buffer (pH 7.3).

Statistics. Most data are given in mean values ($\bar{X}$) ± standard error of the mean (SEM) with n as the number of observations. Values of P were calculated using Student's t-test. $P<0.05$ was considered to be not significant (NS).

RESULTS

PNS prepared from normal TG-enriched hearts (from which LPL was removed by heparin-perfusion) was incubated at different pH in the absence or presence of 4 mM TO (Fig. 1). Two peaks of lipase activity were observed in all preparations, one at pH 4.5 and one between pH 7 and 7.5. Lipase activity towards endogenous TG in PNS from normal hearts was substantial although the TG content in PNS is low (Table I). Incubation in the presence of added TO (4 mM) resulted in a 30-40% increase in lipase activities at both pH peaks. In PNS prepared from 3 days TE-fed rats, which contained a significantly higher amount of TG (Table I), the lipase activity at pH 4.5 is slightly enhanced and the neutral lipase activity towards endogenous TG is increased ($P<0.001$). The latter is more pronounced when 4 mM TO was present. Control experiments (not shown) in which the concentration of TO was further increased did not yield a further rise in FFA production.

The marked increase in neutral lipase activity towards endogenous TG in PNS from hearts of TE-fed rats suggested a relation between lipase activity and the amount of endogenous TG. Therefore we tested the pH activity curve of lipase activities in PNS prepared from newborn rat hearts. In these hearts LPL activity is virtually absent[16]. Table I indicates that the endogenous TG content was significantly lower than in adult hearts. As illustrated in Fig. 2

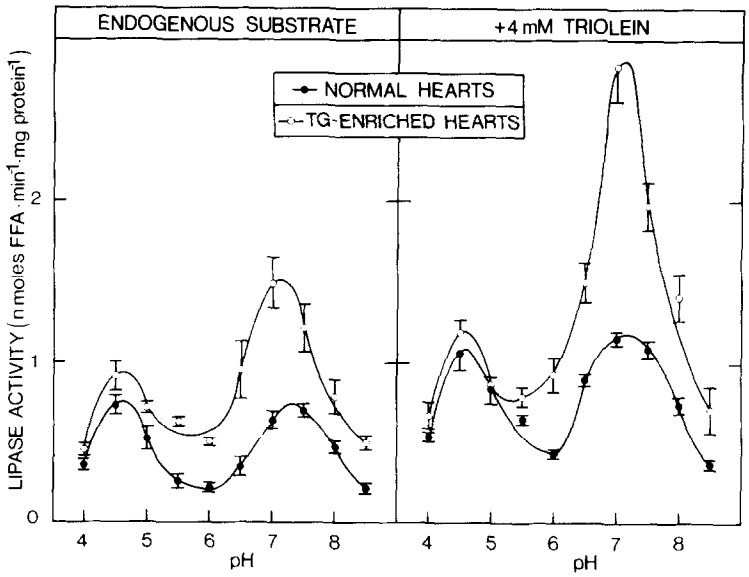

Fig. 1. pH activity curves of lipase activities in PNS from homogenates of normal and TG-enriched rat hearts. $\bar{X}$ ± SEM, n=3-5,

TABLE I

ENDOGENOUS TRIGLYCERIDE (TG) CONTENT IN RAT HEART POST-NUCLEAR SUPERNATANT (PNS)

Condition	TG in PNS (mM)[a]	P vs control
Hearts from normal, adult, rats (control)	0.085 ± 0.008 (17)[b]	
" " 24 h fasted rats	0.087 ± 0.013 (3)	NS
" " 1 day TE-fed rats	0.219 ± 0.033 (4)	<0.001
" " 3 days TE-fed rats	0.648 ± 0.068 (6)	<0.001
" " newborn rats[c]	0.013 ± 0.004 (3)	<0.005

[a]Floating fat in PNS was first removed by centrifugation; [b]$\bar{X}$ ± SEM (n);
[c]7 hearts were pooled for one PNS.

lipase activities in the absence of added TO were very low (comp. Fig. 1). In the presence of 4 mM TO, however, the acid lipase activity reached normal values while the neutral lipase activity was still only 30% of the rate in adult hearts. This, again, points out the dependency of neutral lipase activity on endogenous TG content.

To investigate the relation between the duration of TE feeding and the increase in cardiac neutral lipase activity, PNS was prepared from control and overnight fasted rats, and after one or 3 days of TE feeding. Overnight fasting did not significantly affect either acid or neutral lipase activity (Fig. 3)

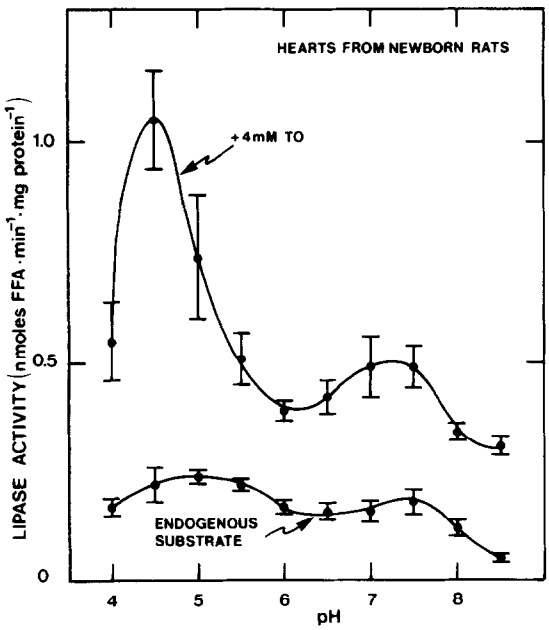

Fig. 2. pH activity curves of lipase activity in PNS from homogenates of newborn rat hearts. $\bar{X}$ ± SEM, n=3.

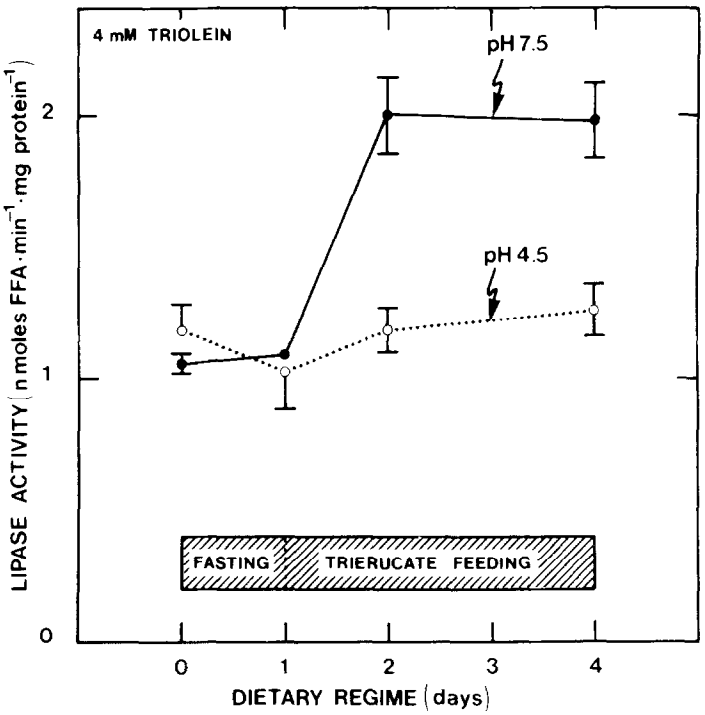

Fig. 3. The effect of fasting and dietary trierucate upon cardiac acid and neutral lipase activities. $\bar{X}$ ± SEM, n=2-5.

while also endogenous TG content remained constant (Table I). Subsequent TE feeding for one night only slightly increased acid lipase activity but led to a marked rise in neutral lipase activity and endogenous TG content (Table I). During continuous TE feeding cardiac TG content rose further but left the high neutral lipase activity unaltered. The relation between cardiac lipidosis and the increase in neutral lipase activity was also proved by in vitro perfusion of hearts from rats fed normal chow with a TG-rich perfusion buffer. Therefore the perfusion buffer was supplemented with Intralipid® and an amino acid mixture, to assure sufficient supply of nutrients during a 6 h perfusion. Myocardial LPL was activated by the addition of 1% (v/v) rat serum to the buffer. Hydrolysis of Intralipid®-TG will provide the heart with an excess of FFA which, in our non-working hearts, will be esterified and stored in fat droplets. Indeed, as demonstrated by electronmicroscopy of the left ventricle after 6 h of Intralipid®-perfusion (Plate I) numerous fat droplets were formed in the myocytoplasm. Moreover, Fig. 4 (left part) demonstrates a gradual increase of TG in PNS during Intralipid®-perfusion while the heparin-releasable LPL activity was enhanced. Commensurate to the increase in tissue TG content, the neutral lipase activity is increased drastically (450%) after a lag phase of 1-2 h. The increase in acid lipase activity is less and amounts to about 70% at the end of a 6 h Intralipid®-perfusion.

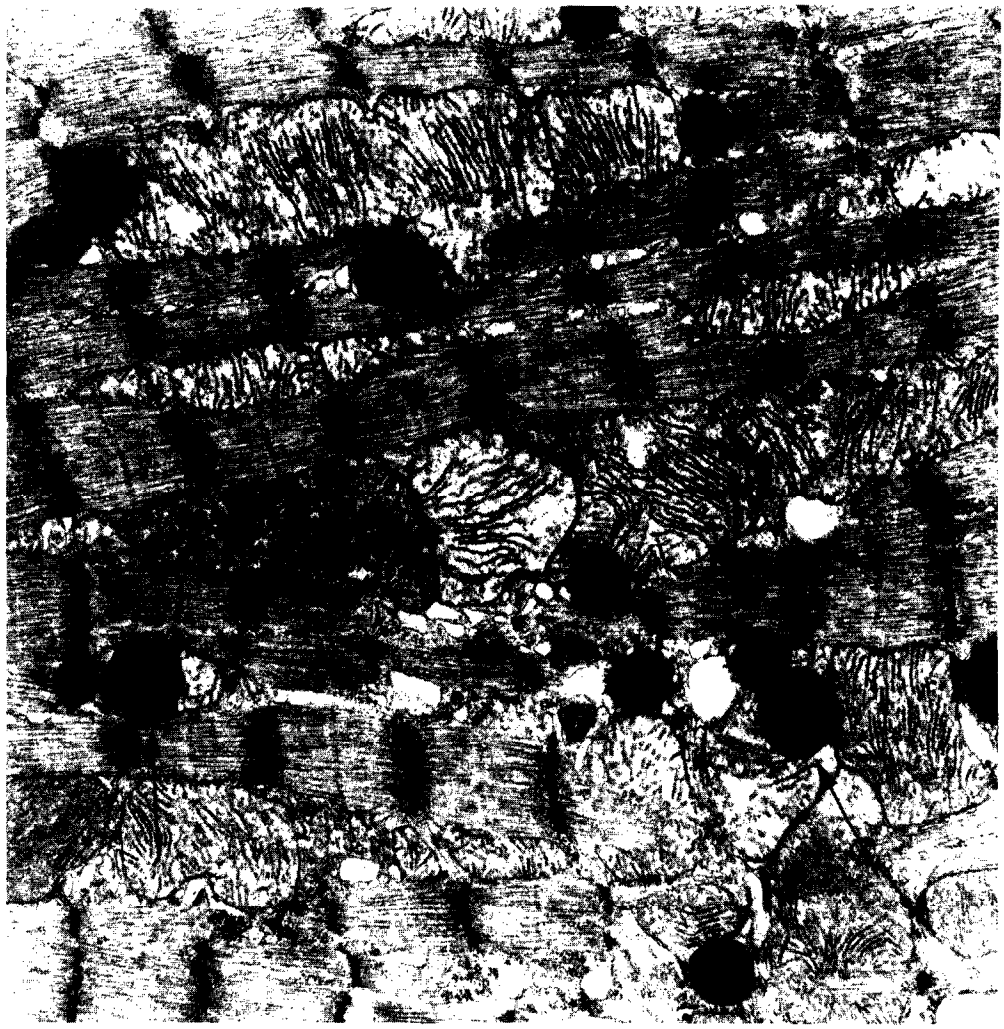

Plate 1. Electronmicrograph of a section of left ventricular heart tissue after a 6 h perfusion with Intralipid®-supplemented perfusion buffer. Note the engorgement of the cytosol with lipid droplets (arrow). (Magnification 15,000 x).

To investigate whether the increase in neutral lipase activity is due to synthesis of new enzyme molecules or to activation and stabilization of enzyme present by endogenous TG, we tested the effect of the protein synthesis inhibitor cycloheximide (3 μg/ml) during perfusion (Fig. 4, middle part). In the presence of cycloheximide heparin-releasable LPL activity slowly decreases, indicating indeed inhibition of protein synthesis. However, the remaining LPL stores were still able to induce a rise in endogenous TG, although less when compared with hearts during Intralipid®-perfusion in the absence of cycloheximide. As indicated in Fig. 4 (middle part), the acid and neutral lipase activity remained fairly constant despite lipidosis. Perfusion with delipidated "Intralipid" (Fig. 4, right part) also increased heparin-releasable LPL activity but endogenous cardiac TG dropped gradually during

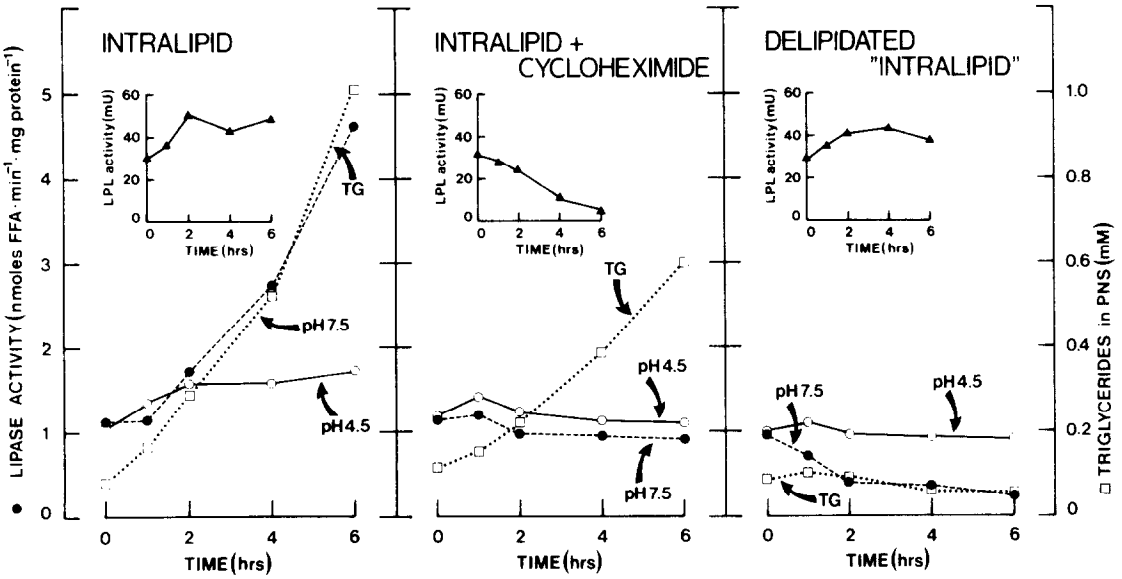

Fig. 4. Alterations of cardiac acid (o), neutral (•) and lipoprotein lipase activities (LPL, ▲), and endogenous TG (□), during perfusion in the presence of Intralipid® (left part), Intralipid® + cycloheximide (middle part) and delipidated "Intralipid" (right part). Lipase activities were determined during incubation in the presence of 4 mM TO. See for details Methods and Materials. $\bar{X}$, n=2.

prolonged perfusion. The acid lipase activity remained constant but the neutral lipase activity dropped to 20% of the initial value. In PNS prepared from heart homogenates of control and TE-fed rats 5 h after cycloheximide treatment (5 mg, i.p.) the neutral lipase activity, determined in the presence of 4 mM TO, dropped from 1.08±0.04 (n=8) to 0.61 (n=2) nmoles FFA.min^{-1}.mg protein^{-1} in control rats and from 2.09±0.15 (n=6) to 1.18 (n=2) nmoles FFA. min^{-1}.mg protein^{-1} in hearts from TE-fed rats. In both groups of the hearts the half-life of the neutral lipase was about 6 h.

DISCUSSION

Previous work from our laboratory has indicated the relation between cardiac TG accumulation and overall tissue lipolytic activity[5-7]. Furthermore the relative importance of the non-lysosomal lipase activity in lipid degradation was assessed[5]. In these studies the activity of tissue lipases was determined by detecting ^{3}H-FFA released from added ^{3}H-TG substrate[5,7]. This method, however, underestimates the actual lipase activity in homogenates since the acid as well as the neutral lipase preferentially hydrolyze endogenous TG (Fig. 1, Table I). Indeed, the difference between neutral lipase activity in the absence or presence of 4 mM TO (about 0.5 nmoles FFA/min/mg protein at pH 7.5) agrees well with the neutral lipase activity measured with added radioactive TO[5].

Comparing the neutral lipase activities in PNS from hearts of normal (adult) rats, newborn rats and from TG-enriched hearts led us to consider the possibility that the enzyme is induced by endogenous TG. This was confirmed by experiments in which Intralipid®-perfusion of normal rat hearts led to myocardial lipidosis and to a cycloheximide-sensitive increase in neutral lipolytic activity. This indicates that, indeed, the synthesis of new enzyme was responsible for the increase in lipolytic capacity.

A stabilizing effect of endogenous TG on the neutral enzyme could be excluded since TE-induced lipidosis could not prevent degradation of the neutral lipase in cycloheximide pretreated rats which took place at the same rate when compared with control hearts. In conclusion we propose that intracellular TG induce synthesis of the neutral enzyme. The high increase of neutral enzyme activity, compared to lysosomal activity, assigns a major contribution of the former enzyme to the increase of the rate of lipolysis during lipid accumulation in rat heart[5,6]. The phenomena observed ressemble in some aspects the development of lipolytic enzyme activity during differentiation of preadipocytes to fat-containing cells[17]. In preadipocytes acid lipase is the main triacylglycerol lipase in contrast to fat loaded cells where the neutral enzyme predominates[17].

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