9. R. J. Looney, G. N. Abraham, C. L. Anderson, *J. Immunol.* **136**, 1641 (1986).
10. J. M. Allen, unpublished data.
11. Forty-eight hours after transfection with the p135, p90, and p98/X2 cDNAs, COS cells were detached in phosphate-buffered saline (PBS), 1 m*M* EDTA, and incubated with ligand for 30 min at 4°C in PBS, 5% nonfat milk, and 0.2% sodium azide. Bound and free fractions were separated by centrifugation through a 3:2 mixture of dibutyl:dioctyl phthalates. Data were corrected for contribution of nonspecific binding by subtraction of counts per minute (cpm) bound in the presence of 2.5×10^{-4}*M* total IgG.
12. P. Guyre, P. M. Morganelli, R. Miller, *J. Clin. Invest.* **72**, 393 (1983); K. C. Petroni, L. Shen, P. Guyre, *J. Immunol.* **140**, 3467 (1988).
13. One microgram each of p90 and p98/X2 was ^{32}P-labeled at the Bst EII site between domains 2 and 3 and hybridized in 80% formamide, 0.4*M* NaCl and 10 m*M* 2-(*N*-morpholino)propanesulfonic acid (MOPS), *p*H 7.5, with 20 μg of total RNA at 50°C for 3 hours. After S1 nuclease digestion, samples were run on a 1.5% agarose gel and autoradiographed.
14. A. F. Williams and A. N. Barclay, *Annu. Rev. Immunol.* **6**, 381 (1988).
15. M. O. Dayhoff, W. C. Barker, L. T. Hunt, *Methods Enzymol.* **91**, 524 (1983).
16. Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
17. F. Sanger, S. Nicklen, A. Coulson, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5463 (1977).
18. We thank D. Simmons, M. Bevilacqua, A. Peterson, and N. Hogg for discussions and communication of unpublished information, C. Anderson for human IgG_3, and C. Anderson, P. Guyre and M. Fanger for MAbs 32.2, 22.2, and IV.3. J.M.A. is the recipient of a Wellcome Trust fellowship. Supported by a grant from Hoechst AG.

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The Involvement of Platelet Activating Factor in Ovulation

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Follicle rupture during ovulation is associated with inflammation-like changes. Because platelet activating factor (PAF) participates in the inflammatory process, the effect of a PAF-specific antagonist, BN52021, on the ovulatory response was tested in rats. BN52021, administered locally, inhibited follicle rupture in rats stimulated to ovulate with human chorionic gonadotropin (hCG). In addition to suppressing rupture of the follicles, this antagonist suppressed the hCG-stimulated increase in ovarian collagenolysis and vascular permeability. The inhibition of ovulation of BN52021 could be reversed by simultaneous administration of PAF. Furthermore, PAF partially reversed the blockage of ovulation by inhibitors of eicosanoid synthesis. Collectively, these results suggest the involvement of PAF in ovulation. Its role seems to be closely related to the metabolism of arachidonic acid. Thus, modulation of PAF action may serve as an additional target for regulation of reproduction via its action on ovulation.

THE RUPTURE OF MATURE OVARIAN follicles during ovulation involves changes that are also common to inflammation (*1*). Thus, induction of ovulation by gonadotropins, which stimulate increases in follicular tissue–type plasminogen activator (tPA) (*2*) and collagenase (*3, 4*) activities, can be blocked by inhibitors of the cyclooxygenase (*5*) and lipoxygenase (*6*) pathways or arachidonic acid (AA) metabolism. Recently, platelet aggregation and adherence to endothelial cells was observed in periovulatory ovine follicles (*7*). Likewise, margination of platelets in acutely inflamed tissues (*8*) and their high content of mediators, including vasoactive amines and peptides, proteases, chemotactic factors, and growth factors (*9*) were described. These findings prompted us to examine the possible involvement of the platelet activating factor [PAF; 1-*O*-octadecyl (or hexadecyl)-2-acetyl-glycero-3-phosphorylcholine] (*10*) in follicular rupture during ovulation.

Immature, 25- or 26-day-old female Wistar-derived rats were primed with 15 IU of pregnant mare's serum gonadotropin (PMSG) to produce multiple preovulatory follicles. Ovulation was induced with a subcutaneous injection of 4 IU of human chorionic gonadotropin (hCG) 48 to 54 hours after PMSG administration. Approximately 18 to 20 hours after administration of hCG, the rats were killed by cervical dislocation. The ampullae of the oviducts were excised, and the ovulated ova were released and counted under a dissecting microscope. When ovulation was inhibited, the presence of large Graafian follicles was confirmed by examination of the ovaries. The data of unilateral injections into the ovarian bursa were expressed as percent inhibition of the number of ovulated eggs in the oviduct of the treated versus the untreated side.

The involvement of PAF in ovulation was tested by injection of a specific PAF antagonist BN52021 (*11*) [median inhibitory concentration (IC_{50}), 10^{-7}*M*], isolated from the Chinese tree *Ginkgo biloba L.* (*10, 12*). Unilateral injection of the antagonist into the ovarian bursa resulted in a dose-dependent inhibition of follicle rupture from the treated ovary (Table 1). The drug effectively blocked ovulation when administered concomitantly with hCG (0 time), or up to 9 hours after hCG treatment. The inhibition of follicle rupture was prevented when BN52021 (0.75 mg) and PAF (Sigma) were injected into the ovarian bursa simultaneously with hCG (Table 1). Administration of PAF (0.5 to 20 μg per bursa) to rats that were not stimulated by hCG and had their endogenous surge of luteinizing hormone (LH) blocked by Nembutal failed to induce ovulation.

In view of the action of inhibitors of eicosanoid synthesis on follicle rupture (*5,*

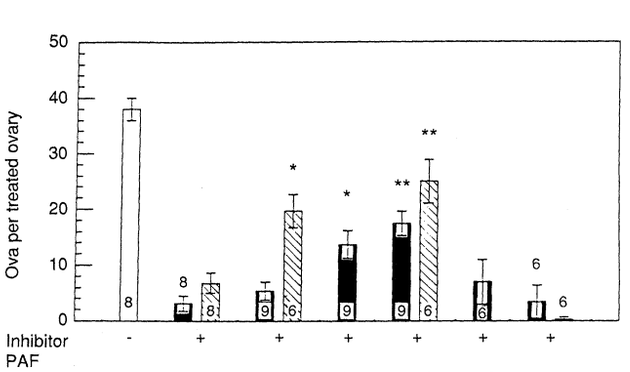

Fig. 1. Induction of ovulation by PAF in rats blocked by inhibitors of eicosanoid synthesis. Immature 25- or 26-day-old rats were injected with 15 IU of PMSG. After 48 to 50 hours, ovulation was stimulated by hCG (4 IU); 3 hours later indo (0.5 mg per bursa, solid bars) or NDGA (0.5 mg per bursa, hatched bars) and PAF were administered simultaneously in 50% dimethyl sulfoxide in saline (v/v) into one ovarian bursa. Ovulation was examined the next morning. Only the number of ova (mean ± SEM) released from the treated ovary are given; the untreated ovaries were not affected significantly by PAF. The number of rats is indicated on the bars. $*P < 0.01$; $**P < 0.001$ versus the rats treated with the same inhibitor only. Analysis of variance revealed a significant ($P < 0.001$) effect of PAF doses on inhibition of ovulation by indo or NDGA. Blank bar, control.

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6), we tested the possibility that administration of PAF could affect the inhibition by indomethacin (indo) (Ikapharm, Ramat Gan, Israel) or nordihydroguaiaretic acid (NDGA; Sigma) (0.5 mg per bursa). PAF (0.1 to 0.5 μg per bursa) partially reversed the inhibitory action of eicosanoid synthesis inhibitors on follicular rupture (Fig. 1). Analysis of variance revealed a significant ($P < 0.001$) effect of PAF doses on inhibition of ovulation by indo or NDGA. Higher doses of PAF (1.0 to 2.0 μg per bursa) were detrimental for ovulation, probably because of toxic systemic effects of the drug, previously described in other systems (*13*).

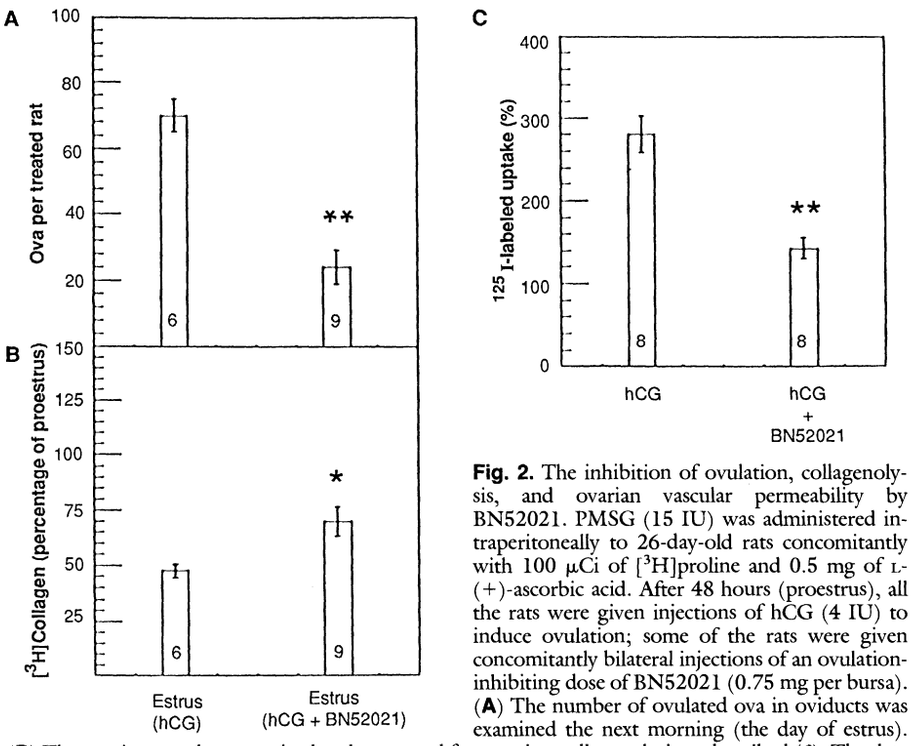

Fig. 2. The inhibition of ovulation, collagenolysis, and ovarian vascular permeability by BN52021. PMSG (15 IU) was administered intraperitoneally to 26-day-old rats concomitantly with 100 μCi of [^{3}H]proline and 0.5 mg of L-(+)-ascorbic acid. After 48 hours (proestrus), all the rats were given injections of hCG (4 IU) to induce ovulation; some of the rats were given concomitantly bilateral injections of an ovulation-inhibiting dose of BN52021 (0.75 mg per bursa). (**A**) The number of ovulated ova in oviducts was examined the next morning (the day of estrus). (**B**) The ovaries were homogenized and processed for assaying collagenolysis as described (*3*). The data are presented as the amount of remaining labeled collagen as a percentage of the labeled collagen measured on the preceding morning (proestrus), which is defined as 100%. (**C**) The uptake of ^{125}I-labeled BSA was estimated by administration of 1 μCi intravenously at the time of hCG administration or 6 hours afterward. The hCG was administered 48 hours after PMSG. Some of the rats were also given bilateral injections of 0.75 mg of BN52021 per bursa. Thirty minutes after injection of the label, blood was collected via a cardiac puncture; the animals were killed, the ovaries were excised, and ^{125}I-labeled BSA in blood and ovaries was counted as described (*14*). The uptake of the label 6 hours after hCG in ovaries is expressed as the percentage of the uptake in rats not treated with hCG, which is defined as 100%. Other details are as in Fig. 1. *$P < 0.01$; **$P < 0.001$ versus the appropriate control group.

Table 1. Inhibition of ovulation by BN52021. The difference between the treated and untreated side was analyzed by the Student *t* test. Values for number of ova and percent inhibition are means ± SEM; *n* = number of rats; NS, not significant.

Treatment	*n*	Number of ova per ovary		Percent inhibition	*P* value
		Untreated	Treated		
BN52021 (mg/bursa)					
0	11	26.6 ± 1.6	21.4 ± 2.4	19.7 ± 9.1	NS
0.1	6	28.0 ± 1.6	24.8 ± 2.1	13.6 ± 8.9	NS
0.3	15	32.7 ± 1.9	20.1 ± 2.0	38.5 ± 6.3	<0.001
0.7	17	26.9 ± 3.2	14.6 ± 2.3	45.5 ± 9.2	<0.01
1.0	11	32.1 ± 2.8	13.5 ± 2.3	57.8 ± 7.2	<0.001
BN52021* (hours after hCG)					
0	9	31.3 ± 3.4	20.1 ± 3.1	35.9 ± 10.0	<0.05
3	11	34.3 ± 3.5	26.2 ± 3.3	23.6 ± 9.4	NS
6	12	30.2 ± 3.6	18.7 ± 2.1	38.0 ± 7.1	<0.05
9	10	34.2 ± 2.5	17.4 ± 3.7	49.1 ± 11.0	<0.01
BN52021 + PAF					
Vehicle	5	39.5 ± 4.6	33.0 ± 4.5	16.5 ± 11.4	NS
BN52021 + PAF†	8	33.6 ± 3.7	35.0 ± 3.4	4.5 ± 10.1	NS

*0.7 mg per bursa. †0.7 mg per bursa and 0.5 μg per bursa, respectively.

Our earlier studies demonstrated that LH and hCG stimulation of ovulation is accompanied by an increase in ovarian collagenolysis (*3*) and in vascular permeability (*14*). These actions of LH and hCG were attenuated by inhibitors of eicosanoid synthesis (*3, 15*). Likewise, BN52021, administered bilaterally into the ovarian bursae, blocked hCG-induced ovulation (Fig. 2A), the increase in ovarian collagenolysis (Fig. 2B), and the uptake of labeled bovine serum albumin (BSA) (Fig. 2C). It seems, therefore, that the concerted action of eicosanoids and PAF is involved in the mediation of LH- and hCG-triggered preovulatory changes in ovarian collagenolysis and vascular permeability.

PAF can induce margination and activation of thrombocytes and leukocytes in vivo (*8*). Several matrix-degrading proteases were identified in human granulocytes (*16*), of which neutrophils constitute over 90%. Neutrophils increase epithelial permeability (*17*). In view of our recent demonstration of a preovulatory increase in ovarian and follicular neutrophil content in the rat (*18*), it seems plausible to suggest that PAF action on ovulation is mediated through blood cells.

The precise mechanisms of PAF involvement in ovulation, as well as the primary cellular origin and targets of its action, remain to be determined. Recently PAF was shown to have a role in ovoimplantation (*19*). Thus, PAF elicited responses similar to the "early pregnancy factor" (*19*), and two unrelated PAF antagonists inhibited ovoimplantation in the rat (*20*) and the mouse (*21*). The present demonstration of PAF involvement in follicle rupture further underlines the similarity between inflammation, implantation, and the ovulatory response (*1*). Moreover, the PAF-related mechanisms may offer additional sites for pharmacologic regulation of fertility through their action on ovulation.

REFERENCES AND NOTES

1. L. L. Espey, in *The Vertebrate Ovary*, R. E. Jones, Ed. (Plenum, New York, 1978), pp 503–532; *Biol. Reprod.* **14**, 233 (1980); H. Lipner, in *The Physiology of Reproduction*, E. Knobil and J. D. Neill, Eds. (Raven, New York, 1988), vol. 1, pp. 447–488; A. Tsafriri, R. Reich, A. O. Abisogun, in *Marshall's Physiology of Reproduction*, G. E. Lamming, Ed. (Churchill Livingstone, London, in press).
2. R. Reich, R. Miskin, A. Tsafriri, *Endocrinology* **116**, 516 (1985); *ibid.* **119**, 1588 (1986).

3. R. Reich, A. Tsafriri, G. L. Mechanic, *ibid.* **116**, 522 (1985).
4. T. I. Morales, J. F. Woessner, J. M. Marsh, W. J. LeMaire, *Biochim. Biophys. Acta* **756**, 119 (1983).
5. D. T. Armstrong and D. L. Grinwich, *Prostaglandins* **1**, 21 (1972); A. Tsafriri, H. R. Lindner, U. Zor, S. A. Lamprecht, *ibid.* **2**, 1 (1972); W. J. LeMaire, N. S. T. Yang, H. R. Behrman, J. M. Marsh, *ibid.* **3**, 367 (1973).
6. R. Reich, F. Kohen, Z. Naor, A. Tsafriri, *ibid.* **26**, 1011 (1983); R. Reich, F. Kohen, R. Slager, A. Tsafriri, *ibid.* **30**, 581 (1985).
7. W. J. Murdoch, *ibid.* **32**, 597 (1986).
8. A. C. Issekutz, M. Ripley, J. R. Jackson, *Lab. Invest.* **49**, 716 (1983).
9. M. H. Ginsberg, in *Advances in Inflammation Research*, G. Weissman, Ed. (Raven, New York, 1981), vol. 2, pp. 53–71.
10. P. Braquet, L. Touqui, T. Y. Shen, B. B. Vargaftig, *Pharmacol. Rev.* **39**, 97 (1987).
11. The chemical formula for BN52021 is 3-*t*-butylhexahydro-4,7*b*,11-trihydroxy-8-methyl-9*H*-1,7α-epoxymethano-1*H*,6α*H*-cyclopenta[*c*]furo[2,3-*b*]-furo[3′,2′:3,4]cyclopenta[1,2-*d*]furan-5,9,12-(4*H*)trione.
12. P. Braquet *et al.*, *Blood Vessels* **16**, 559 (1985).
13. M. A. Martins *et al.*, *Eur. J. Pharmacol.* **136**, 353 (1987).
14. A. O. Abisogun, D. Daphna-Iken, R. Reich, D. Kranzfelder, A. Tsafriri, *Biol. Reprod.* **38**, 756 (1988).
15. A. O. Abisogun, R. Reich, R. Miskin, A. Tsafriri, in *Development and Function of the Reproductive Organs II*, M. Parvinen, I. Huhtaniemi, L. J. Pelliniemi, Eds. (Serono Symposia Review, no. 14, Rome, 1988), pp. 285–293.
16. H. Tschesche, J. Fedrowitz, U. Kohuert, J. Michaelic, H. W. Macartney, *F. Histochem. Cytochem.* **24**, 125 (1986).
17. L. C. Milks, G. P. Conyers, E. B. Cramer, *J. Cell Biol.* **103**, 2719 (1986).
18. A. O. Abisogun, D. Daphna-Iken, A. Tsafriri, *Endocrinology* **122**, 1083 (abstr.) (1988).
19. C. O. O'Neill, *J. Reprod. Fertil.* **73**, 567 (1985); C. Oroczo, T. Perkins, F. M. Clarke, *ibid.* **78**, 549 (1986).
20. N. R. Spinks and C. O. O'Neill, *Lancet* **i**, 106 (1987).
21. G. Acker, F. Hecquet, A. Ettienne, P. Braquet, J. M. Mencia-Huerta, *Prostaglandins* **35**, 233 (1988).

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Atrial Natriuretic Peptide Inhibits a Cation Channel in Renal Inner Medullary Collecting Duct Cells

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The patch-clamp technique was used to examine the effects of atrial natriuretic peptide (ANP) and its second messenger guanosine 3′,5′-monophosphate (cGMP) on an amiloride-sensitive cation channel in the apical membrane of renal inner medullary collecting duct cells. Both ANP ($10^{-11}M$) and dibutyryl guanosine 3′,5′-monophosphate ($10^{-4}M$) inhibited the channel in cell-attached patches, and cGMP ($10^{-5}M$) inhibited the channel in inside-out patches. The inner medullary collecting duct is the first tissue in which ANP, via its second messenger cGMP, has been shown to regulate single ion channels. The results suggest that the natriuretic action of ANP is related in part to cGMP-mediated inhibition of electrogenic Na^+ absorption by the inner medullary collecting duct.

ATRIAL NATRIURETIC PEPTIDE (ANP), a circulating hormone released from the atria of mammalian hearts in response to volume expansion, acts on the kidneys, adrenal glands, and vasculature to regulate fluid and electrolyte homeostasis (*1, 2*). The natriuretic and diuretic effects of ANP are mediated in part by inhibiting Na^+ and water absorption by renal inner medullary collecting ducts (IMCD) (*1–5*). Although this action of ANP has been linked to the intracellular second messenger cGMP (*1–4*), relatively little is known about the cellular mechanisms whereby ANP and cGMP reduce Na^+ absorption by the IMCD.

Electrogenic sodium absorption by the IMCD is a two-step process: passive diffusion across the apical cell membrane through an amiloride-sensitive conductive pathway and active extrusion across the basolateral membrane by a Na^+- and K^+-dependent adenosine triphosphatase (*6, 7*). In a recent patch-clamp study, we reported that electrogenic Na^+ uptake across the apical membrane of IMCD cells in primary culture and in the native epithelium is mediated by a cation channel (*6*). The channel was selective for monovalent cations (P_{Na^+}:P_{Cl^-} = 13:1), inhibited by micromolar concentrations of amiloride, and had a single channel conductance of 28 pS; however, the channel was not regulated by voltage. The present study on IMCD cells was conducted to determine whether ANP reduces Na^+ absorption by inhibiting the cation channel in the apical membrane.

Patch-clamp studies were conducted on rat IMCD cells grown in primary culture as described (*6*). The patch current was measured with a current-to-voltage converter (Yale Mark V design), low-pass filtered at 300 Hz, and digitized at 1 kHz with an IBM AT computer. Data were recorded for at least three 20-s periods (with a 30-s delay between each period) before and after each experimental condition to determine the probability of a single channel being open (P_o). P_o was estimated from the total time spent in the open state divided by the total time of the record (*6*). All experiments were conducted in a paired fashion. Data are reported as the mean ± SEM.

We first examined the effects of ANP on the cation channel in cell-attached patches of the apical membrane. Channels were spontaneously active in 10% of our experiments. We usually observed two or three channels in each patch; occasionally we saw as many as six active channels (Fig. 1A). Addition of

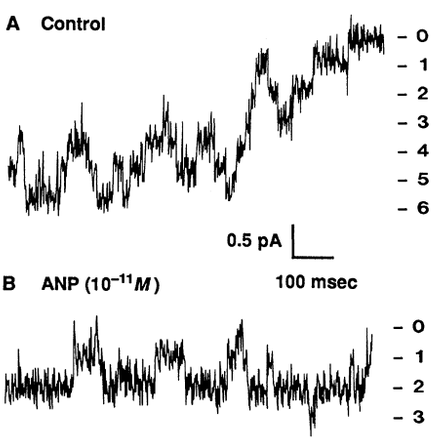

Fig. 1. Current records from a cell-attached patch of the apical membrane. (**A**) Control. Six channels were present in the patch; the single channel P_o was 0.70. (**B**) Three minutes after addition of ANP ($10^{-11}M$) to the bath [rat synthetic ANP, amino acids 1 to 28 (Sigma), the chief circulating form of ANP in rats (*32*)]. Simultaneous openings of more than three channels was not observed in the patch in the presence of ANP. The P_o decreased from 0.70 to 0.54. Vehicle had no effect on channel activity (n = 10). The command potential, Vc, in both records was +20 mV; the voltage across the membrane was the sum of Vc and the resting membrane voltage (~−60 mV). Voltage refers to the cytoplasmic side of the membrane referenced to the interior of the patch pipette. For patches with multichannel events, individual channels were found to be identical and independent by methods described by Palmer and Frindt (*33*). In addition, the number of channels and P_o were determined as described (*6, 33*). The numbers to the right of each current record indicate the number of open channels. Solutions: pipette (cell-attached and inside-out patches) and bath (cell-attached patches): NaCl, 140 m*M*; KCl, 5 m*M*; $CaCl_2$, 1 m*M*; $MgCl_2$, 1 m*M*; and Hepes, 10 m*M*; *p*H 7.4; bath (inside-out patches): NaCl, 5 m*M*; KCl, 140 m*M*; $CaCl_2$, 1 m*M*; $MgCl_2$, 1 m*M*; and Hepes, 10 m*M*; *p*H 7.4.

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