B Cells of Aging Rats: Impaired Stimulus-Secretion Coupling But Normal Susceptibility to Adverse Effects of a Diabetic State

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A diabetic state impairs B-cell function and survival. We tested whether the negative effects are exacerbated by the aging process. Islets were isolated from old (63.3 ± 2.3 weeks) and young (11.3 ± 0.5 weeks) inbred Wistar rats. Age did not affect DNA and insulin content, yet both glucose-induced (27.8 mmol/L) and arginine-induced induced (10 mmol/L) insulin responses in old islets were significantly reduced. Islets were transplanted under the kidney capsule of recipients that were either nondiabetic or severely diabetic after streptozotocin (STZ) treatment (blood glucose > 20 mmol/L). Following 8 weeks' transplantation to nondiabetic recipients, perfused kidneys with grafts of old islets exhibited the same insulin responses to glucose as grafts of young islets. However, responses to arginine were reduced in grafts of old islets (28 ± 4 μU/min) relative to grafts of young islets. (70 ± 18 μU/min, $P < .05$). Insulin mRNA content was similar in grafts of old islets and grafts of young islets. Following 8 weeks' transplantation to diabetic recipients, 27.8 mmol/L glucose failed to induce insulin secretion in grafts of old islets and grafts of young islets alike, whereas arginine-induced insulin secretion was unaffected in grafts of old islets but reduced in grafts of young islets. Insulin mRNA content was reduced to a similar extent by the diabetic state (to 28% in grafts of old islets and to 27% in grafts of young islets grafts in nondiabetic recipients). We conclude that aging, although leading to impaired stimulus-secretion coupling, does not increase susceptibility to the negative effects of a diabetic state on B-cell function as presently tested.

A DIABETIC STATE impairs B-cell function[1] and survival.[2] Several mechanisms are involved. Overstimulation leading to B-cell exhaustion causes B-cell insensitivity to glucose.[3] Evidence also exists for a direct glucotoxic effect.[4] Furthermore, elevated free fatty acids, commonly associated with diabetes, induce insensitivity to glucose.[5,6]

Other factors modulate B-cell susceptibility to the adverse effects of a diabetic state. Marked species differences in susceptibility have been demonstrated. The genetic background of species differencies has not been elucidated. However, it has been proposed that the regeneration potential of B cells is of crucial importance.[7]

Aging is known to decrease replicative capacity,[7] thereby limiting replacement of damaged B cells. Aging may also impair stimulus-secretion coupling in B cells in animals[8-12] and in man.[13] Both of these effects of aging are apt to decrease the capacity of B cells for insulin secretion and increase the possibility of B-cell exhaustion.

The aim of the present study was to test the hypothesis that B-cell secretory and biosynthetic potentials are more severely decreased by a diabetic state at older versus younger age. B-cell functions of islets from old and young rats were initially characterized after short-term tissue culture. Islets were then transplanted under the kidney capsule of nondiabetic and diabetic syngeneic recipients. We evaluated whether an 8-week period of diabetes had a more profound effect on arginine-induced insulin secretion and on insulin mRNA content in grafts of old versus young islets.

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MATERIALS AND METHODS

Animals

The experiments were approved by the Stockholm Ethics Committee for Animal Experiments. Inbred Wistar-Furth rats (B & K Universal, Sollentuna, Sweden) were used. All islet donors were females, whereas all recipients were males. All rats were allowed free access to water and a standard pelleted diet (B & K) under 12-hour light cycles. Diabetes was induced by streptozotocin (STZ) in male recipient rats at 8 weeks of age. At that time, STZ (kindly supplied by Dr William E. Dulin, Upjohn, Kalamazoo, MI) was injected intravenously at a dose of 65 mg/kg. Two weeks later, blood glucose levels were measured from blood obtained by tail snipping. Rats with blood glucose levels greater than 20.0 mmol/L were selected as recipients.

Isolation of Islets

Islets of Langerhans were isolated by collagenase treatment essentially as previously described.[14] Collagenase was obtained from Boehringer (Mannheim, Germany). The isolation procedure was identical for all pancreases as to the concentration of collagenase and the automated shaking procedure in a water bath at 37°C. Each pancreas yielded 150 to 300 islets. After isolation, islets were cultured overnight in RPMI 1640 supplemented with 11.1 mmol/L glucose, 2.1 mmol/L L-glutamine, and 10% (vol/vol) heat-inactivated fetal bovine serum (Sigma, St Louis, MO).

Insulin Secretion From Isolated Islets

After overnight culture, islets from old and young rats were preincubated at 37°C for 30 minutes in Krebs-Henseleit bicarbonate (KHB) buffer[15] supplemented with 10 mmol/L HEPES, 2 g bovine serum albumin/L (Sigma), and 4.7 mmol/L glucose. After preincubation, triplicate batches of six islets from old or young islet donors were incubated in 200 μL KHB buffer at 37°C in a water bath with shaking in sequence as follows: 30 minutes with 4.7 mmol/L glucose, 30 minutes with 27.8 mmol/L glucose, 20 minutes with 4.7 mmol/L glucose, and 20 minutes with 4.7 mmol/L glucose and 10 mmol/L arginine. Incubation media were frozen and stored at −20°C until insulin assay. At the end of incubations, islets were collected, washed with glucose-free KHB

buffer, and sonicated in 200 μL redistilled water. The aqueous homogenates were subsequently assayed for insulin and DNA content.

Proinsulin Biosynthesis in Isolated Islets

Isolated islets, cultured as described, were preincubated at 37°C for 30 minutes in KHB buffer containing 4.7 mmol/L glucose. After preincubation, triplicate batches of 20 islets from old and young islet donors were incubated at 37°C for 2 hours without shaking in 100 μL KHB buffer containing 27.8 mmol/L glucose and 50 μCi/mL ^{3}H-leucine (Amersham, Bucks, UK). The islets were then washed with glucose-free KHB buffer containing nonradioactive leucine (10 mmol/L) and sonicated in 200 μL redistilled water. The aqueous homogenates were subsequently assayed for total protein biosynthesis, proinsulin biosynthesis, and DNA content. Total protein biosynthesis was estimated by measurement of radioactivity in the trichloroacetic acid–precipitable fraction of the homogenate. Proinsulin and insulin were separated from other proteins in the islet homogenates by an immunoblotting technique.[16]

Preparation of Isolated Islets for RNA Extraction

Groups of 200 islets were cultured overnight in RPMI 1640 as described earlier, and subsequently washed with glucose-free phosphate-buffered saline (pH 7.4). They were homogenized in 250 μL 4-mol/L guanidinium thiocyanate, 25 mmol/L sodium citrate (pH 7.0), and 0.1 mol/L 2-mercaptoethanol, frozen in dry ice–ethanol, and then stored at −80°C until extraction of RNA.

Glucose, Insulin, and DNA Assays

Blood glucose determinations were made by a glucose oxidase method using reagent strips (Boehringer) and a reflectance meter (Boehringer). Immunoreactive insulin level was measured by radioimmunoassay[17] using rat insulin as a standard, ^{125}I-labeled insulin as a tracer, and our own antibodies raised against porcine insulin. For measurements of insulin content, we used the aqueous homogenates of islets obtained at the end of incubations for insulin secretion. Islet insulin content was determined in these aqueous homogenates following overnight extraction at 4°C with acid-ethanol. Islet DNA content was determined by measurements of DNA in the aqueous homogenates using the method described by Hinegardner.[18]

Islet Transplantation

Isolated islets were cultured overnight in RPMI 1640 as described earlier. Approximately 200 islets were then transplanted under the left kidney capsule as previously described.[19,20] The number of transplanted islets was purposely kept low to avoid normalizing blood glucose in the diabetic recipients.

Kidney Perfusion Experiments

Eight weeks after transplantation, the rats were anesthetized by intraperitoneal injection of 50 mg/kg sodium pentobarbital. The graft-bearing kidneys were isolated and perfused as previously described,[19,20] with minor modifications. Kidney perfusions were performed at a constant flow rate of 2.5 mL/min using KHB buffer supplemented with 20 g/L bovine serum albumin (Sigma), 20 g/L dextran T-70 (Pharmacia, Uppsala, Sweden), and 4.7 mmol/L glucose. The KHB buffer medium was kept at 37°C and continuously gassed with a gas mixture of 95% O_2 and 5% CO_2. After an equilibration period of 30 minutes, stimulations with 27.8 mmol/L glucose and 10 mmol/L arginine were performed in sequence (Fig 1). Time zero was defined as the start of perfusion with 27.8 mmol/L glucose. Effluents were collected into prechilled tubes containing aprotinin (Bayer, Leuerkusen, Germany) at a final concentration of greater than 500 KIE/mL, and stored at −20°C until insulin assay. At the end of the perfusions, islet grafts were dissected with parts of the kidney capsule still adhering to the grafts. The grafts were then frozen and stored in liquid nitrogen until extraction of RNA.

Quantification of Preproinsulin mRNA

Total RNA was extracted from isolated islets and islet grafts by acid guanidium thiocyanate-phenol-chloroform extraction.[21] Total RNA content of the extracts was measured by spectrophotometry (OD A260 nm = 40 μg/mL). Levels of preproinsulin mRNA were determined by a nonsaturated solution hybridization assay.[22,23] A synthesized oligonucleotide corresponding to 58 nucleotides of the rat preproinsulin 2 cDNA flanked by *Bam*HI and *Kpn*I restriction sites was inserted into pGEM-3Zf(+) (Promega, Madison, WI). This fragment differs in one position from the rat preproinsulin 1 cDNA, dividing the 58-nucleotide probe into 29 and 28 nucleotides after RNase treatment. The resulting vector, prINS2, was transcribed in vitro to obtain labeled antisense RNA and unlabeled sense RNA (Promega, Madison, WI). For synthesis of labeled antisense RNA, the vector was linearized by *Eco*RI and transcribed with SP6 RNA polymerase in the presence of 3 μmol/L ^{35}S-UTP (>37 × 10^{12} Bq/mmol). Unlabeled sense RNA was obtained by transcription with T7 RNA polymerase after linearization with *Xba*I. The DNA template was removed by RQ1 DNase I (Promega). Transcripts were separated from unincorporated nucleotides on Sephadex G-50 columns (Nick column; Pharmacia). The concentration of unlabeled sense RNA was determined spectrophotometrically. Three serial dilutions of each extract in 20 μL 0.2× SET (1 × SET contains 1.0% sodium dodecyl sulfate, 20 mmol/L Tris hydrochloride, pH 7.5, and 10 mmol/L EDTA) were mixed with 20 μL 2× hybridization solution (20,000 cpm antisense RNA, 1.2 mol/L NaCl, 8 mmol/L EDTA, 1.5 mmol/L dithiothreitol, 50% deionized recrystallized formamide, and 40 mmol/L Tris hydrochloride, pH 7.5). After hybridization at 70°C for 18 hours, 1 mL RNase solution (40 μg/mL RNase A [Boehringer], 100 U/mL RNase T1 [Boehringer], 100 μg/mL salmon sperm DNA, 0.3 mmol/L NaCl, 2 mmol/L EDTA, and 10 mmol/L Tris hydrochloride, pH 7.5) was added and the samples were incubated at 37°C for 1 hour. RNase-resistant radioactivity was precipitated by addition of 100 μL ice-cold 100% trichloroacetic acid, followed by an incubation on ice for 30 minutes. The precipitates were collected by filtration through GF/C filters (Whatman International, Maidstone, UK) and quantified by liquid scintillation counting. Parallel hybridizations with increasing amounts of unlabeled sense RNA allowed construction of standard curves. Preproinsulin mRNA in the extracts was calculated by comparison to the standard curve. Background radioactivity, determined by hybridizations without graft extracts and sense RNA, was less than 1% of the input radioactivity. The assay was linear in the range one to 50 times the background. All quantifications of preproinsulin mRNA in graft extract are based on at least three serial dilutions within the linear range of the assay.

Statistical Analysis

Results are expressed as the mean ± SE. Integrated insulin responses were calculated as the increment above the secretion rates that were recorded immediately before administration of glucose or arginine, and converted to mean secretion rates by division with appropriate time periods. Tests of significance were performed using the two-tailed Student's *t* test for paired and unpaired differences. To ensure normal distribution of values for arginine-induced insulin secretion, these were logarithmically converted before significance testing.

RESULTS

Experiments With Isolated Islets

Characteristics of islet donor rats. Old islet donor rats were aged 66.3 ± 2.3 weeks and young donor rats 11.3 ± 0.5 weeks. Body weight was 250 ± 7 g in old donors and 185 ± 4 g in

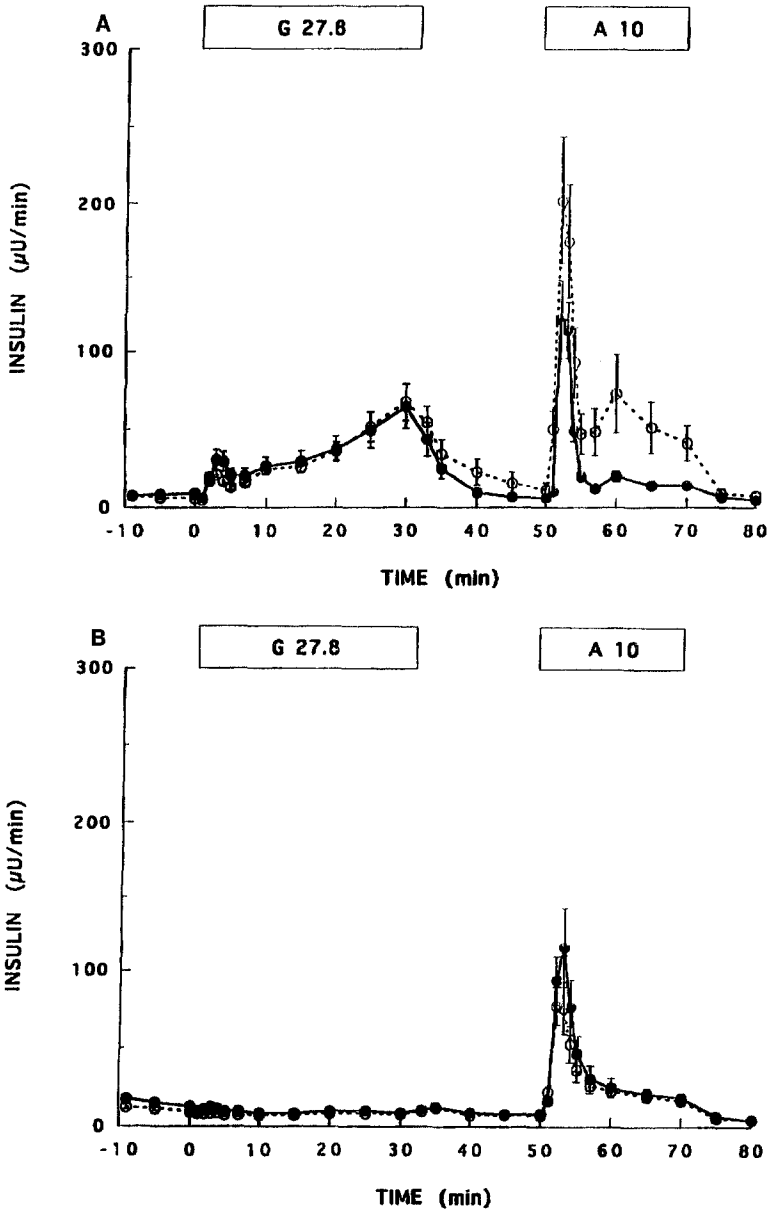

Fig 1. Graft insulin responses to 27.8 mmol/L glucose followed by 10 mmol/L arginine. Kidneys with islet grafts from normal (A) or diabetic (B) recipients were perfused. Donor islets were from either young (○) or old (●) rats. Results are the mean ± SE of 10 to 11 experiments in each series.

young donors. Age did not affect fasting blood glucose levels (4.6 ± 1.0 mmol/L in old *v* 4.8 ± 0.1 mmol/L in young).

Islets and insulin secretion. Neither DNA nor insulin contents differed between islets from old and young rats. Basal insulin secretion in the presence of 4.7 mmol/L glucose was similar between islets from old and young rats (Table 1). However, in comparison to islets from young rats, old islets secreted only 59% as much insulin in response to 27.8 mmol/L glucose and only 36% in response to 10 mmol/L arginine.

Table 1. Insulin Secretion, DNA Content, and Insulin Content in Isolated Islets

Islet Type	Insulin Secretion (nU/min/ng DNA)			DNA Content (ng/islet)	Insulin Content (μU/ng DNA)
	Glucose 4.7 mmol/L	Glucose 27.8 mmol/L (incremental secretion)	Arginine 10 mmol/L (incremental secretion)		
Young (n = 6)	5.8 ± 0.6	60.1 ± 6.9	11.8 ± 0.9	22.6 ± 4.0	19.0 ± 1.1
Old (n = 6)	4.4 ± 0.9	35.2 ± 3.8*	4.2 ± 0.6†	26.8 ± 1.9	17.2 ± 0.8

NOTE. Insulin secretion was tested in batch-type incubations following overnight culture. Arginine was tested in the presence of 4.7 mmol/L glucose. Results are the mean ± SE of the number of observations indicated in parentheses.

*$P < .05$ *v* young.

†$P < .001$ *v* young.

Table 2. Proinsulin Biosynthesis in Islets From Young and Old Rats

Group	DNA Content (ng/islet)	PIB (dpm/ng DNA)	TPB (dpm/ng DNA)	PIB/TPB (%)
Young (n = 6)	27.6 ± 2.3	98.2 ± 18.3	474.9 ± 70.6	20.4 ± 2.3
Old (n = 6)	25.1 ± 1.8	129.4 ± 14.7	561.7 ± 65.9	23.2 ± 1.1

NOTE. PIB and TPB were measured in overnight-cultured islets after a 2-hour incubation with ^{3}H-leucine. Results are the mean ± SE of the number of observations indicated in parentheses.

Abbreviations: PIB, proinsulin biosynthesis; TPB, total protein biosynthesis.

Proinsulin biosynthesis in isolated islets. There were no differences in total protein biosynthesis, proinsulin biosynthesis, or the ratio of proinsulin biosynthesis to total protein biosynthesis between old and young islets (Table 2).

Transplantation Experiments

Characteristics of recipient rats. Levels of blood glucose in diabetic recipients were markedly and consistently elevated throughout the transplantation period (Table 3). The age of the transplanted islets did not influence blood glucose in diabetic recipients or in normal recipients. Body weight was expectedly lower in diabetic than in nondiabetic recipients.

Insulin secretion from islet grafts. Basal and 27.8-mmol/L glucose–induced insulin secretion was similar when comparing grafts of old islets to normal recipients (GO → N) with grafts of young islets to normal recipients ([GY → N] Fig 1 and Table 4). However, arginine-induced insulin secretion from GO → N was significantly lower than secretion from GY → N (Table 4).

In grafts to diabetic recipients (GO → D and GY → D), insulin secretion in response to 27.8 mmol/L glucose was abolished. Arginine-induced insulin secretion was not affected by recipient diabetes in grafts consisting of older islets (GO → D *v* GO → N), but was significantly decreased in grafts consisting of young islets (GY → D *v* GY → N).

Insulin mRNA content of islet grafts. A chronic diabetic environment markedly decreased insulin mRNA content in the islet grafts. However, there was no difference in the decrease of insulin mRNA content between old and young islet grafts. Hence, a diabetic environment decreased insulin mRNA content

Table 3. Characteristics of Recipient Rats

Rat Type	Body Weight (g) at Transplantation	Blood Glucose (mmol/L) Before Transplantation	Blood Glucose (mmol/L) 8 Weeks After Transplantation
Normal (n = 11) grafted with young islets	356 ± 7	5.1 ± 0.2	4.6 ± 0.1
Normal (n = 10) grafted with old islets	338 ± 6	4.9 ± 0.3	5.1 ± 0.2
Diabetic (n = 11) grafted with young islets	247 ± 8	23.0 ± 0.9	21.3 ± 1.0
Diabetic (n = 11) grafted with old islets	253 ± 12	23.5 ± 1.0	23.5 ± 1.3

NOTE. Diabetes was induced in recipients by STZ. Each recipient was transplanted with approximately 200 islets from normal syngeneic donors. Results are the mean ± SE of the number of observations indicated in parentheses.

Table 4. Insulin Release From Islet Grafts

Graft Condition	Insulin Secretion (μU/min) Glucose 4.7 mmol/L	Glucose 27.8 mmol/L (incremental secretion)	Arginine 10 mmol/L (incremental secretion)
GY → N (n = 11)	6.5 ± 1.2	27.5 ± 4.5	70.1 ± 18.3
GO → N (n = 10)	8.7 ± 0.6	26.1 ± 7.2	28.1 ± 4.2*
GY → D (n = 11)	11.2 ± 1.5	−1.4 ± 0.5	30.0 ± 5.3†
GO → D (n = 11)	15.2 ± 1.6	−3.3 ± 0.6	36.3 ± 7.2

NOTE. Following an 8-week transplantation period, graft-containing kidneys were perfused in succession with 4.7 mmol/L glucose, 27.8 mmol/L glucose, and 10 mmol/L arginine. Arginine was tested in the presence of 4.7 mmol/L glucose. Results are the mean ± SE of the number of observations indicated in parentheses.

*$P < .05$ *v* GY → N.

†$P < .05$ *v* GY → N.

72% in old grafts and 73% in young grafts relative to grafts to normal recipients (Table 5).

DISCUSSION

In our transplantation experiments, 8 weeks of a diabetic environment completely abolished glucose-induced insulin responses in grafts from old and young islets. At first glance, these results seem to compromise the possibility to test for an interaction between a diabetic state and aging on insulin secretion. However, it is known that desensitization of glucose-induced insulin secretion is a rapidly induced phenomenon that does not itself indicate a decrease of B-cell capacity.[1] Arginine-induced insulin secretion at a defined glucose level has, on the other hand, been validated as a good indicator of B-cell capacity both in animals[24] and in man.[25] Consequently, the latter parameter was deemed appropriate and sufficient to assess major effects of aging on insulin secretion under diabetic conditions.

Interpretation of aging effects in the present study was facilitated by diabetic conditions being the same for old and young islets, as assessed from blood glucose levels in recipients before and after the 8-week period of transplantation. Also, transplanted islets from young and old donors had similar size as assessed from DNA measurements. (Islet size could be important for islet oxygenization,[26] as well as for the speed and completeness of vascularization of grafted islets.)

The major finding of our study is that aging fails to increase B-cell susceptibility to adverse effects of a diabetic state. Hence, arginine-induced insulin secretion was decreased by the

Table 5. Total RNA and Insulin mRNA Content in Islet Grafts

Group	Total RNA (μg/graft)	Insulin mRNA (fmol/graft)
GY → N (n = 11)	6.16 ± 0.38	80.9 ± 5.5
GO → N (n = 9)	7.12 ± 0.58	66.4 ± 5.1
GY → D (n = 11)	7.27 ± 0.57	21.9 ± 4.9*
GO → D (n = 11)	8.89 ± 0.71	18.3 ± 2.9*

NOTE. Following kidney perfusions, islet grafts were removed, frozen, and stored in liquid nitrogen until extraction and further processing. Results are the mean ± SE of the number of observations indicated in parentheses.

*$P < .01$, N *v* D.

diabetic state in grafts from young rats, but not in grafts from old rats. The reasons for the possibly greater impact of a diabetic state on this parameter in young grafts are not known and require further investigation.

Although aging did not exacerbate the negative influences of a diabetic state, it was clear that islets from older rats were in some respects less responsive to secretory stimuli than islets from young rats. Hence, before transplantation, isolated islets from older rats demonstrated smaller insulin responses to glucose and to arginine than isolated islets from younger rats. A decrease with age of glucose-induced insulin secretion from isolated islets agrees with many[8-12] but not all[27] previous studies. Insulin responses to arginine have not been previously compared between old and young rats.

Arginine-induced secretion was decreased in GO → N versus GY → N. This effect paralleled that seen in isolated islets. However, in response to glucose, GO → N released similar amounts of insulin as GY → N. The latter finding is discordant with results in isolated islets. It was previously suggested that the collagenase treatment used to isolate islets would perturb glucose-induced insulin secretion more with advancing age of the pancreas.[28] Although conditions of islet isolation were similar for old and young pancreases, we cannot rule out a greater vulnerability of older islets to collagenase treatment. Such a putative effect could be thought to subside during subsequent in vivo conditions, and this could explain the divergent results obtained in isolated islets and grafts.

In summary, the present study provides evidence that aging in the rat does not increase B-cell susceptibility to the adverse effects of a diabetic state. Furthermore, we demonstrate that decreased arginine-induced insulin secretion is a consistent feature of aging.

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