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Diagnosis of cardiac thrombosis in patients with atrial fibrillation in the absence of macroscopically visible thrombi

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Summary. Cardiac thrombosis due to atrial fibrillation (AF) has been recognized as the most common cause of cerebral embolism. However, sometimes no macroscopic thrombus is found at autopsy in the heart of a victim of this type of cerebral embolism. We investigated morphological changes in the left atrial endocardium of 31 patients (including 21 cases with AF) who had died of cerebral embolism. "Rough endocardium" (RE) seen macroscopically provided evidence for the existence of atrial thrombosis. The RE that appeared in AF cases was due to a granular and wrinkled appearance of the endocardium associated with oedematous and fibrous thickening. Fibrin-thread deposits were also always distinguishable. Mural thrombi and oedema with neutrophil infiltration in the subendocardium could be seen under the microscope. Small areas of endothelial denudation and thrombotic aggregations were commonly observed by scanning electron microscopy (SEM). These SEM lesions were significantly more frequent in cases with AF than in controls ($P<0.001$). The diagnostic success rate for atrial thrombosis among cases with AF increased from 33.3% to 81% when thrombi proven by histological investigation of the areas with RE were added. Left atrial RE may be an anatomically relevant finding for the existence of atrial thrombosis with AF, when the thrombosis cannot be detected upon gross observation at autopsy.

Key words: Atrial fibrillation – Cerebral embolism – Rough endocardium – Cardiac thrombosis – Autopsy diagnosis

Introduction

Atrial fibrillation (AF) is one of the most important causes of cerebral embolism especially amongst the elderly (Åberg 1969; Wolf et al. 1978, 1987; Kannel et al. 1982). It is not rare to find an absence of cardiac thrombi in patients at autopsy who have been diagnosed clinically as having cerebral embolism from a cardiac thrombus, due to AF. These cases cause difficulty for pathologists and we therefore investigated morphologically relevant changes in the atrial endocardium. These permit a diagnosis of the previous existence of cardiac thrombi.

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Materials and methods

We investigated 31 autopsies of cases of major cerebral embolism (Table 1) and 7 autopsy controls. Both the clinical and pathological criteria of cerebral embolism given by the Harvard Cooperative Stroke Registry (Mohr et al. 1978) and the National Institute of Neurological and Communicative Disorders and Stroke (1981) were used in this study. However, the diagnosis was made only when the patient met both of the following sets of criteria: (1) for pathological diagnosis, demonstration of an infarcted area (frequently haemorrhagic infarction) in the brain, and evidence of an embolus in the cerebral artery, or emboli to other organs, or absence of severe stenosis or occlusion in arteries supplying an infarcted region, and (2) for clinical diagnosis, sudden onset of symptoms, and establishment of a likely source for embolus in the heart including atrial fibrillation, without other causes of stroke.

Sources of emboli other than the heart, such as atherosclerotic thrombi in carotid artery, were excluded.

The duration of the clinical course from the main attack to the death of each embolic patient in this study was within 30 days (mean8 $\pm$ 7 days). The patients ranged from 43 to 97 years in age (average 69 $\pm$ 12 years old). AF was detected both before and after the attack in 21 of the 31 patients (Table 1).

Seven autopsy controls who died of non-stroke diseases had no history of AF or any cardiac disease. Their average ages was nearly the same as that of the embolic patients (67 $\pm$ 9 years old).

The left atrial, auricular, ventricular and valvular endocardium were carefully examined macroscopically in both groups to seek grossly identifiable abnormal findings of the endocardium.

Five or six serial tissue blocks (each 50 $\times$ 5 mm in size) were obtained from the left atrial endocardium of each case (including controls) and fixed in 10% phosphate-buffered formalin (pH 7.2). When macroscopic changes, including the cloudy and somewhat thickened endocardium with a rough and wrinkled appearance, identified as "rough endocardium" described below, were found, these lesions were included in the tissue blocks. Serial sections were

Table 1. Endocardial changes at autopsy in the cases who died of cerebral embolism with or without atrial fibrillation

Case no.	Age	Sex	Valvular or subendocardial changes	RE	Gross thrombi in LA	Microscopic findings of left atrial endocardium			
						Ed.	Hem.	Inf.	Thrombus
AF cases									
1	52	F		+	0	+	0	0	+
2	56	M	Mi-V	0	+	+	0	+	+
3	58	M		+	+	0	0	+	+
4	59	M	Mi-V	+	+	+	0	+	+
5	70	M		+	0	0	0	0	0
6	71	F	Mi & Ao-V	+	0	+	0	+	+
7	72	M	Ao-V	+	+	+	0	+	+
8	74	M		+	0	0	0	0	+
9	74	M		+	+	+	+	+	+
10[a]	74	F		+	0	+	0	0	+
11[a]	74	M		+	0	0	0	0	+
12	76	F	VENT	0	0	0	0	0	0
13[a]	76	M	VENT	+	0	+	0	+	+
14	78	F		+	0	+	0	+	+
15[a]	78	F	Mi-V	+	0	+	0	+	+
16	80	F		+	+	+	+	+	+
17[a]	81	F		+	0	+	+	+	+
18	83	F		+	+	+	+	+	+
19[a]	84	M		+	0	+	0	+	+
20[b]	63	M		0	0	0	0	0	0
21[b]	76	M		0	0	0	0	0	0
Non-AF cases									
1	43	M	Mi-V	0	0	0	0	0	0
2	46	M	Mi-V	0	0	0	0	FC	0
3	52	M		0	0	+	0	FC	0
4	52	M	VENT	0	0	0	0	0	0
5	54	M	Ao-V	0	0	0	0	0	0
6	69	M	VENT	0	0	0	0	0	0
7	76	F	Mi-V	0	0	0	0	0	0
8[b]	74	F		0	0	0	0	0	0
9[b]	75	M		0	0	0	0	0	0
10[b]	97	M		0	0	0	0	0	0

AF, Atrial fibrillation; LA, left atrium; RE, rough endocardium; M, male; F, female; Mi or Ao-V, thromboendocarditis of mitral or aortic valve; VENT, left ventricular thrombosis associated with subendocardial infarct; Ed., oedema; Inf., leucocyte infiltration; FC, foam cells; Hem., fresh and old haemorrhage; +, positive; 0, negative;
[a] Case for SEM study
[b] Artery-to-artery embolism; embolus origin of no. 3 in non-AF group could not be determined

cut at 5 μm to stain with haematoxylin and eosin, phosphotungstic acid hematoxylin (PTAH) and the Azan Mallory method.

Scanning electron microscopic (SEM) observations were performed on the left atria with rough and wrinkled appearances of 6 embolic cases with AF (nos. 10, 11, 13, 15, 17, 19 in Table 1) and on the smooth normal atria of 7 controls.

Tissue blocks 2.5 × 1 cm were excised and washed in saline with heparin sodium (5000 units/dl) and fixed in 10% buffered formalin within 2 h of death. The fixed tissue were cut equally into five small specimens with razor blades. After conventional preparation, the endocardial surface were observed under a SEM (Hitachi S-650). All endocaridal surfaces of the specimens were photographed under the SEM. The numbers of diseased regions involved in thrombus formation and leucocyte adhesion to the endocardia were calculated in photographs of areas of (250 × 250) μm^2. Twenty areas randomly chosen from each tissue block were observed, making a sum of 100 areas in each case. The size of the lesions was not taken into consideration for the calculation. To obtain valid data, SEM photographs were observed double-blind (with regard to whether an AF patient or a control patient was being observed). The difference in frequency of the lesions between the two groups was evaluated statistically by comparision of the means of the total numbers of lesion spots (modified two-sample *t*-test by Cochrans and Cox).

Results

Apart from macroscopic thrombosis, seen in 7 cases, the left atrial endocardium showed abnormal gross findings in 17 of 21 cases of AF (81%): oedematous and fibrous thickening with cloudy appearances, fine and coarse granular surfaces, large and small wrinkles, and scattered aggregation of fibrin-like villous material (Fig. 1) were all seen. We called these findings "rough endocardium (RE)". Endocardial bleeding and various degrees of haemosiderin deposition were also found in 5 cases.

When no AF had been recorded in the clinical course, the atrial endocardium did not show RE. However, 2 non-AF cases showed fine granular surface and small wrinkles, but exihibited no findings such as oedematous

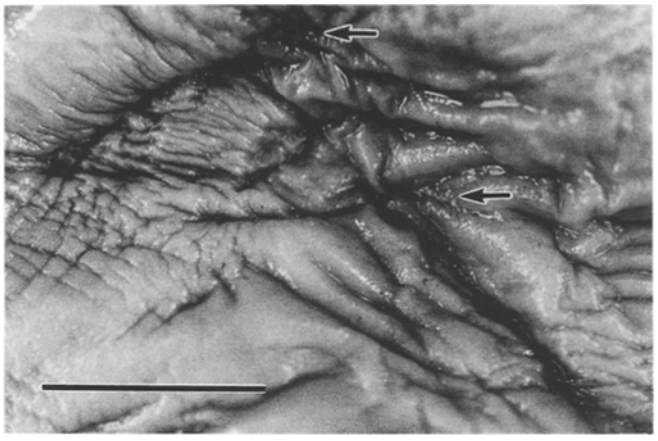

Fig. 1. Rough endocardium (RE) in the left atrium showed fine and coarse granular and wrinkled surface, scattered small haemorrhages, haemosiderin deposits (*arrows*) and fibrin deposition. Case 17; *bar* = 10 mm

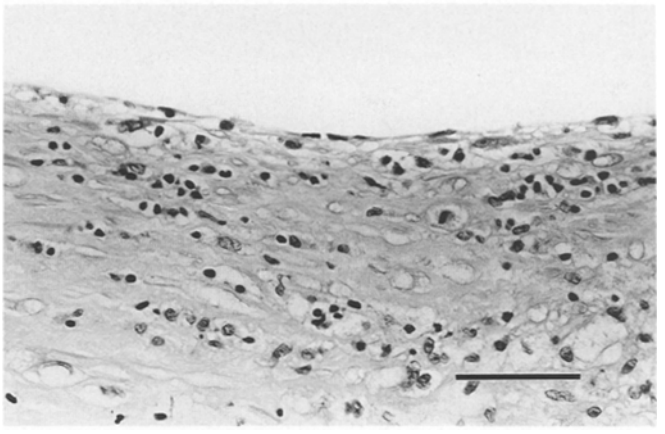

Fig. 3. Oedematous swelling with infiltration of neutrophils and lymphocytes is seen in the subendocardium of RE in the AF group. Haematoxylin and eosin stain; *bar* = 100 μm

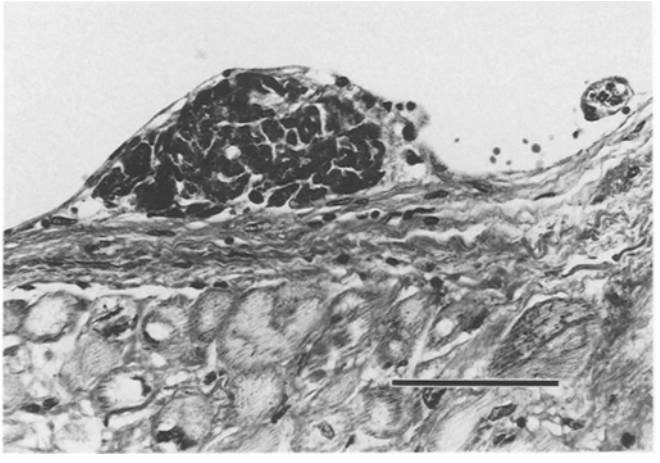

Fig. 2. An organizing microthrombus on the surface of RE near the left auricle in the atrial fibrillation (AF) group. Haematoxylin and eosin; *bar* = 100 μm

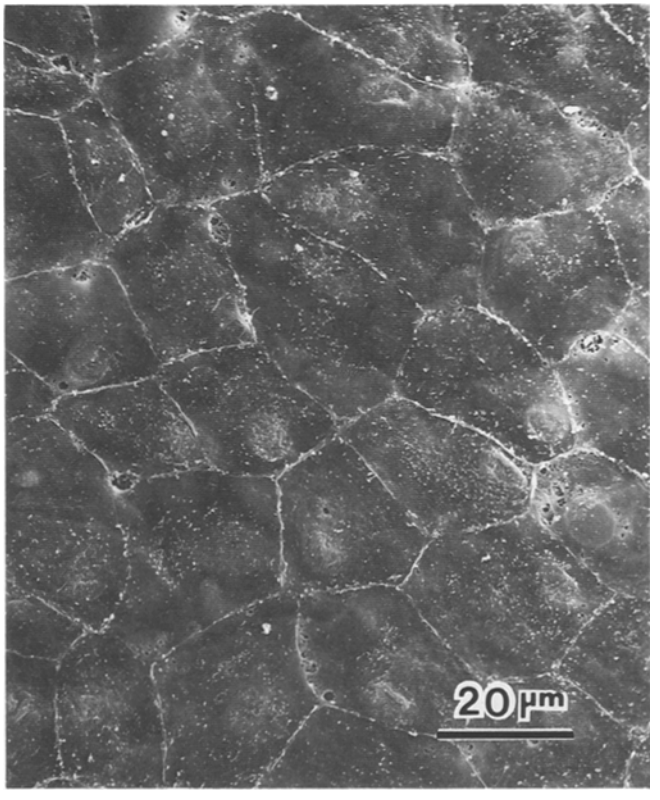

Fig. 4. The left atrial endocardium in the controls shows a flat and continuous paving-stone-like arrangement of endothelial cells. SEM

and cloudy thickening, coarse granular surface large wrinkles. The endocardium of these 2 cases, therefore, could not be categorized as RE.

None of the 7 autopsy controls exhibited RE.

The correlation between RE and AF among embolic cases was statistically significant ($P<0.001$) in a chi-square analysis.

In the AF group, microscopic investigations on RE revealed mural thrombi which were either fresh or organized (Fig. 2) in 16 of the 17 cases (94%) (Table 1). Apart from thrombi, oedematous swelling of the fibrously thickened subendocardial tissue, slight infiltration of neutrophils, lymphocytes and plasma cells (Fig. 3), haemorrhage and haemosiderin deposition, and blood plasma infiltration were all encountered. Four cases in the AF group had no obvious RE in the left atrium, but 1 case showed organized microthrombi near the auricle without frank infiltration of inflammatory cells or plasma in the subendocardium.

In the non-AF group, the 2 previously described cases which showed fine granular surfaces and small wrinkles had foam cell infiltration in the atrial endocardium (Table 1). One of them showed a slightly oedematous change, in addition to foam cells in the endocardium.

If thromboendocarditis or ventricular thrombosis due to subendocardial infarction existed, the atrial endocardium was not always involved in the specific change.

The correlation between AF and atrial thrombosis, and the relation between RE and atrial thrombosis were both statistically significant ($P<0.001$). Control cases were free of the histological changes mentioned above.

On SEM endothelial cells in the left atria of the controls, with neither AF nor RE, showed uniform polygon-

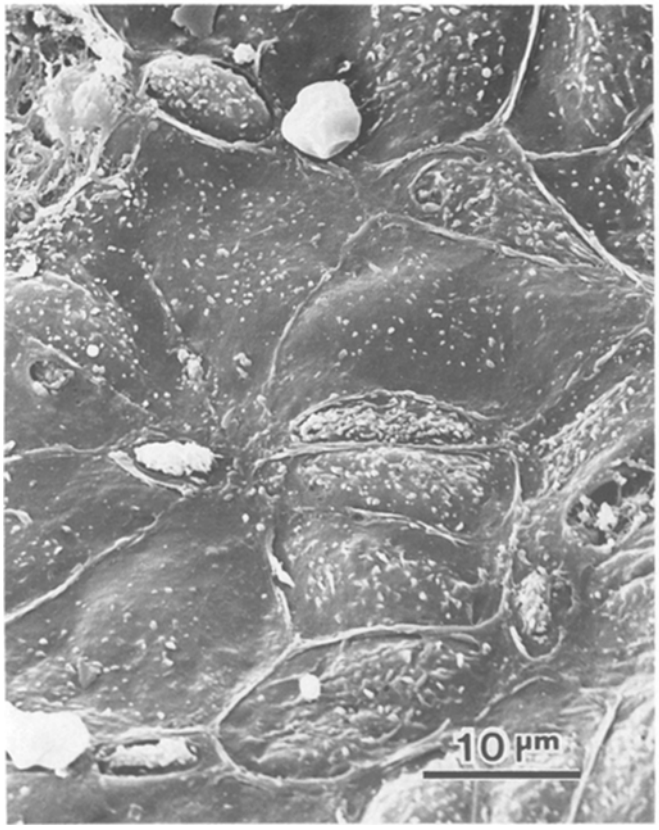

Fig. 5. Radial and irregular arrangements of the endothelial cells centering on a small denuded area of RE in the AF group. The arrangement indicates regeneration of endothelial cells which have abundant surface villi. SEM

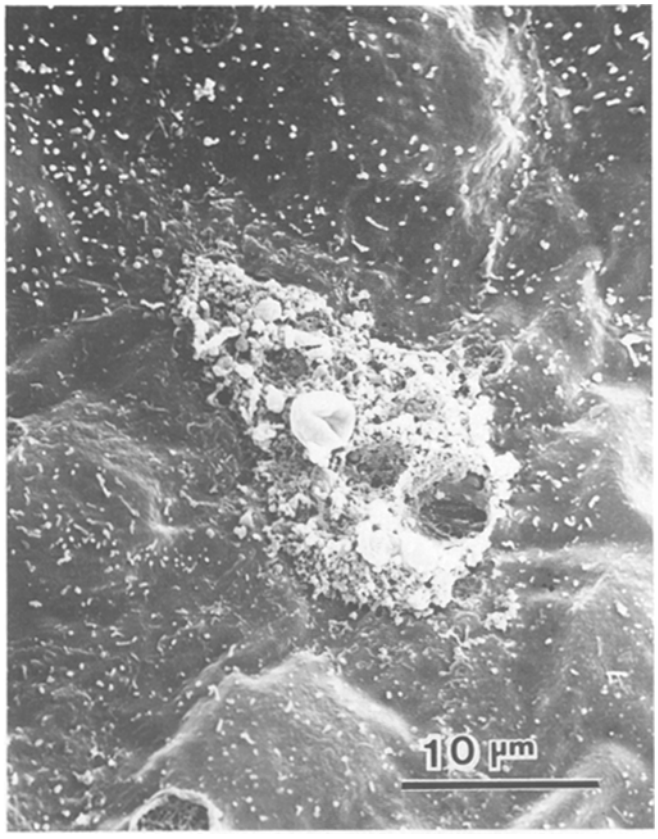

Fig. 6. One of scattered small foci of endothelial injuries caused by AF. A small endothelial desquamation in the area of RE is covered by a microthrombus in the AF group. SEM

al and flat shapes in regular arrangement like paving stones (Fig. 4). There was neither endothelial desquamation nor thrombus formation on the endocardium, but some endothelial cells possessing microvilli and attached by a few mononuclear cells or neutrophils were present.

Endothelial cells in RE are, in general, polygonal but very irregular both in size and shape, and bulge slightly towards the lumen. They are arranged radially and centred on small areas of endothelial denudation, which suggests that endothelial regeneration progresses after injury (Fig. 5).

The surface of endothelial cells in RE often shows many elongated microvilli of various lengths and has small aggregates of platelets or fresh small thrombi attached (Fig. 6), even in cases in which no macroscopic thrombi are observed. Monocytes and neutrophils which attach occasionally to the surface of less injured endothelium extended their cytoplasmic processes into the intercellular spaces between endothelial cells.

We calculated the total number of changes including monocyte and neutrophil adhesion, thrombus formation and endothelial desquamation, in each case. The mean number of lesions in the RE of 6 cases of AF was 524 ± 114, while that of the control group was 68 ± 27: a significant difference ($P < 0.001$) (Table 2).

The average number of blood cells adhering to each given area was significantly larger in the AF group. There was also a characteristic difference in the nature

Table 2. Frequency of endocardial changes in the atrium with or without AF: the total number of all lesions in SEM photographs of 100 randomly chosen areas of $(250 \times 250)\ \mu m^2$

Cases (No. in Table 1)	Total numbers of lesions in $100 \times [(250 \times 250)\ \mu m^2]$	Grades of lesions	
		WBCs adhesion	Thr.
RE & AF[a]			
1 (No. 10)	382	3+	2+
2 (No. 11)	470	3+	2+
3 (No. 13)	423	3+	3+
4 (No. 15)	715	3+	3+
5 (No. 17)	539	3+	3+
6 (No. 19)	616	3+	3+
Controls[b]			
1	80	1+	0
2	78	1+	0
3	62	1+	0
4	18	±	0
5	90	1+	0
6	104	1+	0
7	42	1+	0

RE, Rough endocardium; AF, atrial fibrillation; WBCs, white blood cells; Thr., microthrombi
Evaluation of lesion scoring: ±, 1–20; 1+, 20–100; 2+, 100–200; 3+, 200–400 lesions; 0, undetectable
Statistical difference (total numbers of lesions) between group[a] and group[b]: $P < 0.001$

of endothelial lesions in the two groups, that is thrombus formation. It was observed only in the AF group (Table 2).

The frequency of detectable thrombi in the left atrium of AF cases was only 33.3% (7 in 21) on gross observation, but it increased to 81% (17 in 21) when microscopic thrombosis was identified (Table 1).

Emboli in the cerebral arteries supplying infarcted area were revealed macroscopically and/or microscopically in 17 of 21 AF cases and in 8 of the 10 non-AF cases.

Discussion

It has been generally accepted that the major source of the cerebral emboli of AF patients is atrial and/or auricular thrombi (Cerebral Embolism Task Force 1986 and 1989; Caplan 1986; Peterson 1990), but some cases of cerebral embolism, diagnosed clinically as being of cardiac origin (because of repeated episodes of AF) had no cardiac thrombosis at autopsy macroscopically. In our series 14 of 21 cases (66.7%) with AF had no macroscopic thrombosis in the heart (Table 1). According to the pathological study by Davies and Pomerance (1972), thrombosis in the left atrium was found in only 46 of 74 patients with long-term AF with or without cerebral embolism. The discovery rate of gross cardiac thrombosis in the disease is regrettably low.

In order to make a definite diagnosis whether cerebral emboli come from the heart in cases of cerebral embolism without gross cardiac thrombosis, we looked for characteristic and relevant macroscopic findings in the heart.

We consider RE of the atrium to be a relevant finding for atrial thromboses which are no longer apparent. When we found RE, all of the cases had AF and 16 of 17 cases had cardiac thrombosis. Conversely, cases with neither AF nor RE had no cardiac thrombosis. Of 14 cases without RE, 13 had no cardiac thrombosis. Thus it is not necessary to hesitate to make a clear diagnosis of "cerebral embolism of cardiac origin" if RE is found in the left atrium.

RE is typically thickened, coarsely granular and largely wrinkled endocardium with villous projections. Haemorrhage and fibrin-thread deposits are sometimes also present. The lesion is composed histopathologically of oedematously and fibrously thickened subendocardium assoicated with denudation of endothelial cells, haemorrhage, leucocytic infiltration dominantly of neutrophils and microthrombi adhered to denuded subendothelium or covered by endothelium in the organizing stage.

Light microscopic investigation of RE without recognition of gross cardiac thrombosis in the AF group revealed mural microthrombi in almost all of them. However, three-quarters of cases without RE in the AF group failed to show microthrombi on the atrial endocardium.

Fibrotic thickening of the endocardium may be the scar of chronic repeated injury of endocardium. There appears to be a close causal relationship between AF and RE ($P<0.001$) and the detection rate of thrombus in the RE of AF patients reached 94.1% (16 in 17) when microscopic investigations were carried out.

Though the pathogenesis of RE caused by AF is unclear, there are some possible explanations. Random and frequent contraction of the left atrial wall in AF can induce turbulent blood flow, giving rise to shear forces on both atrial endocardium and blood corpuscles. Endothelium is injured by turbulent shear force and the blood will also tend to stagnate in the atrium (Skinner et al. 1963).

When RE is recognized in cases of AF, it strongly suggests that the cases have exhibited prior atrial thrombosis.

Thickening of the endocardium is seen also in endocarditis parietalis fibroplastica (Löffler 1936) or endomyocardial fibrosis (Davies 1948). An infiltrate of eosinophils and round cells with mural thrombosis is observed in the subendocardium of the active phase, and followed by fibrous endocardial thickening in the organized stage of this disorder. The apices and the inflow portions of bilateral ventricles are preferentially affected; however, the atria are relatively spared. Endocarditis parietalis is thus distinguishable from the RE associated with AF.

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