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Down's syndrome
(Na^+/K^+)-ATPase
ouabain binding
platelets
serotonin
sodium transport

Sodium Transport, Ouabain Binding, and (Na^+/K^+)-ATPase Activity in Down's Syndrome Platelets

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Summary

The mechanism of low serotonin content in Down's syndrome (DS) platelets was studied by comparing the activity of (Na^+/K^+)-ATPase, ouabain binding, and rate of outward movement of sodium in platelets from DS and control subjects.

Ouabain inhibited (Na^+/K^+)-ATPase activity in sodium iodide-extracted platelet membranes from 10 normal subjects had an average value of 2.87 ± 0.20 μmol P_i/hr/10^9 platelets whereas the activity from DS platelet membranes was 2.13 ± 0.19 μmol P_i/hr/10^9 platelets ($P < 0.02$). Total ATPase activity in normal platelet membranes was 3.58 ± 0.33 μmol P_i/hr/10^9 platelets whereas that from DS membranes was 2.49 ± 0.35 μmol P_i/hr/10^9 platelets ($P < 0.02$). (Na^+/K^+)-ATPase activity is decreased in DS platelets.

Ouabain binds specifically to (Na^+/K^+)-ATPase sites on membranes. The amount of ouabain bound by DS platelets is less than that by control platelets. The average amount of ouabain bound was 1.08 ± 0.04 × 10^{-15} mol/10^9 platelets for controls and 0.86 ± 0.02 × 10^{-15} mol/10^9 platelets for DS ($P < 0.001$). There appear to be fewer (Na^+/K^+)-ATPase sites on DS platelets compared to normal.

The outward movement of Na^+ in platelets is catalyzed by the enzyme (Na^+/K^+)-ATPase. The average ouabain-sensitive outward movement of sodium in normal platelets was 9.03 ± 0.46 μmol/hr/10^9 platelets. For DS subjects the average outward movement was 50% of normal, 4.41 ± 0.14 μmol/hr/10^9 platelets. The rate of outward Na^+ transport is significantly less ($P < 0.001$) in DS compared to normal platelets.

Decreased serotonin content of DS platelets may be secondary to decreased (Na^+/K^+)-ATPase activity, which results in decreased rate of outward Na^+ transport and increased Na^+ content. As serotonin is cotransported with Na^+, the decreased rate of serotonin uptake may be secondary to increased Na^+ content of DS platelets.

Speculation

If decreased (Na^+/K^+)-ATPase activity, decreased rate of Na^+ outward transport, increased Na^+ content, and decreased rate of serotonin uptake were also present in synaptosomes of DS patients, decrease in amine content might occur and result in altered neurotransmission.

It has been known for several years that the content of serotonin (5HT) in platelets from Down's syndrome (DS) patients is decreased (12). A number of studies have been undertaken to elicit the mechanism of this observed decrease of serotonin in DS platelets. Jerôme and Kamoun (9) and Bayer and McCoy (2) demonstrated that in DS platelets, the V_{max} for serotonin uptake is reduced and the K_m for serotonin is increased. Boullin and O'Brien (4) showed that the ATP content of DS platelets was decreased and suggested that this decrease may be responsible for the low 5HT content as 5HT is stored complexed with ATP in the platelet. Bayer and McCoy (2) studied AMP, ADP, and ATP synthesis in DS and normal platelets. They showed that ATP synthesis is increased in DS platelets and that the ATP/5HT ratio in DS platelets is actually greater than normal. It was suggested that availability of ATP should not be a causative factor in the decreased content of serotonin in DS platelets (2). Lott *et al.* (11) showed that the outward rate of movement of serotonin was normal in DS platelets and suggested that the decreased serotonin content could be explained by a normal rate of outward but a decreased rate of inward movement of 5HT. McCoy *et al.* (14) showed that the content of sodium in DS platelets was increased and that the total ATPase activity was decreased in DS platelets. The proposal was advanced that decreased ATPase activity resulted in a decreased rate of outward movement of sodium, and consequently an increase in platelet sodium content and decreased rate of inward movement of serotonin.

We report in this communication further studies which demonstrate that in DS platelets, (Na^+/K^+)-ATPase activity is decreased, the binding of ouabain decreased, and the outward movement of Na^+ decreased compared to normal platelets.

MATERIALS AND SUBJECTS

Blood was obtained from male and female DS subjects aged 10–25 years who lived at home and were enrolled at the Winnifred Stewart School for Retarded Children or at the Western Industrial Research and Training Centre. Written informed consent from parent or guardian was received to obtain necessary blood samples. Control samples were from laboratory personnel and student volunteers in the age range 14–30 years, with the majority in the age range 18–22 years. Informed consent was obtained from parents of those subjects who were less than age 18 years. Blood was taken by venipuncture with a plastic syringe with EDTA as anticoagulant. All subsequent work was done in polypropylene and polystyrene tubes. Radioactive chemicals were obtained from New England Nuclear Corporation. [^{3}H]Ouabain with a specific activity of 11.7 and 12.0 Ci/mM was received as a benzene-ethanol solution which was evaporated and the ouabain dissolved in glass-distilled H_2O. ^{22}Na was received as a carrier-free solution of NaCl. [^{32}P]ATP was obtained in an aqueous solution which varied in specific activity from 10–35 Ci/mmol.

Other chemicals used were obtained from Baker, Fisher, and Sigma chemical companies.

METHODS

PLATELET ISOLATION

Fifty milliliters of blood was collected in 5-ml anticoagulant (0.1 M EDTA; 0.15 NaCl, pH 6.6). The blood was sedimented at 200 × *g* for 20 min, the red blood cells discarded, the plasma spun at 200 × *g* for 15 min, the pellet discarded, and the platelets in the platelet-rich plasma counted, using a Coulter Counter model B

particle counter (aperture current^{-1}, 1/4; amplitude^{-1}, 1/2; with thresholds at 7 and 70). The platelet-rich plasma was then pelleted at 1000 × *g* for 20 min, the pellet washed in 0.9% NaCl with 1 mM EDTA at pH 7.4, resuspended, centrifuged again at 1000 × *g* for10 min, then resuspended in a buffer suitable to the assay to be performed.

(Na^+/K^+)-STIMULATED, OUABAIN-INHIBITED ATPase ASSAY (25)

The platelet pellet was resuspended in a buffer containing 5 mM EDTA in 1.0 mM Tris, pH 7.4, and disrupted by sonication. To the sonicate was added ice-cold NaI which extracts Ca^{++}- and Mg^{++}-ATPase but leaves (Na^+/K^+)-ATPase in the platelet membrane. After extraction on ice for 30 min, the membrane fragments were centrifuged and resuspended in 1.0 ml 5 mM EDTA, pH 7.4. The (Na^+/K^+)-ATPase assay was run with Na^+/K^+ or ouabain to determine ouabain-inhibited enzyme activity. The activity was determined by measuring the [^{32}P]P_i liberated from terminally labeled [^{32}P]ATP during a 10-min incubation period. (Na^+/K^+)-stimulated, ouabain-inhibited ATPase was calculated by subtracting from total ATPase, ouabain-resistant ATPase. The net activity equalled ouabain-sensitive (Na^+/K^+)-ATPase.

OUABAIN BINDING TO PLATELET (25)

The platelet pellet, isolated as in the previous experiment, was resuspended in 4.0 ml incubation buffer. A 1.0-ml portion was taken for recentrifugation and resuspended in a sodium-free buffer to demonstrate the requirement for sodium in ouabain binding; 1.0 ml was taken for zero time and two 1.0-ml samples for incubation. To each tube was added [^{3}H]ouabain (2.5 μCi) and tubes were incubated for 1 hr at 37°. The reaction was terminated by washing platelets with ice-cold solution containing 4.0 mM ouabain in 0.15 M NaCl.

The platelet was dissolved in 2 N NaOH and bound radioactivity determined using a liquid scintillation counter. The results are expressed as total disintegrations per min of ouabain bound to platelets in the presence of sodium minus ouabain bound in the absence of sodium to determine Na^+-sensitive ouabain binding. From this the moles of ouabain bound were calculated.

OUTWARD ^{22}Na TRANSPORT IN HUMAN PLATELETS (25)

The platelet pellet was resuspended in an incubation buffer at 1.5–3.5 × 10^9 platelets/ml. To the platelet suspension was added 8.0 μCi ^{22}Na and this was incubated at room temperature for 2 hr (see Fig. 1) to load platelets. The ^{22}Na concentration was similar in normal and DS platelets at this time. The platelets were divided in two, centrifuged, and resuspended in reaction buffer with or without 0.25 mM ouabain. The platelets were incubated at 37° and sampled at specific intervals over a 10-min period (see Fig. 2). The reaction was terminated by placing a 100-μl aliquot into a tube in an ice water bath, adding 2.0 ml ice-cold saline, and centrifuging at 4°. The supernatant was discarded, the tube wiped clean, and the ^{22}Na remaining in the pellet determined using a Beckman Gamma 300 counter. The amount of sodium transported, labeled and unlabeled/hr/10^9 platelets, was calculated. The total $^{22}Na^+$ efflux minus ouabain-resistant efflux was equal to the ouabain-sensitive $^{22}Na^+$ efflux. The percentage of $^{22}Na^+$ ions in the total Na^+ pool was calculated on the basis of equilibrium and thus the Na^+ efflux (labeled and unlabeled) could be calculated. Although it is possible that reuptake of $^{22}Na^+$ during measured efflux occurs, in view of experiments conducted with regard to loading time (Fig. 1), it is felt that the reuptake of $^{22}Na^+$ would not contribute significantly to the results.

RESULTS

Na^+/K^+)-ATPase ACTIVITY IN NORMAL AND DS PLATELETS

Many assays were run under a variety of conditions in an attempt to demonstrate the presence of (Na^+/K^+)-stimulated, ouabain-inhibited ATPase activity in homogenates of human platelets. It was not possible in these extensive studies to consistently demonstrate significant (Na^+/K^+)-ATPase activity. For this reason, the NaI extraction method of Nakao *et al.* (15) was employed. This method extracts Mg^{++}- and Ca^{++}-ATPase but leaves (Na^+/K^+)-ATPase intact in the platelet membrane.

The ouabain-inhibited (Na^+/K^+)-ATPase activity of platelet membranes from 10 normal subjects had an average value of 2.87 ± 0.20 μmol $P_i/10^9$ platelets while the average (Na^+/K^+)-ATPase activity for 10 DS subjects was 2.13 ± 0.19 μmol P_i/hr/10^9 platelets (see Table 1). This difference in enzyme activity was significant ($P < 0.02$). The total ATPase activity of normal platelet membranes averaged 3.58 ± 0.33 μmol P_i/hr/10^9 platelets while the activity from DS platelet membranes was 2.49 ± 0.35 μmol P_i/hr/10^9 platelets. This difference in enzyme activity was also significant ($P < 0.02$). DS platelets (Na^+/K^+)-ATPase represented 69% of the total ATPase activity compared to normal platelets which had 74% of total ATPase activity in this form. Although both (Na^+/K^+)-ATPase and total ATPase activity in DS platelets are decreased, the ratio of Na^+/K^+ to total ATPase is similar to that of normal platelets.

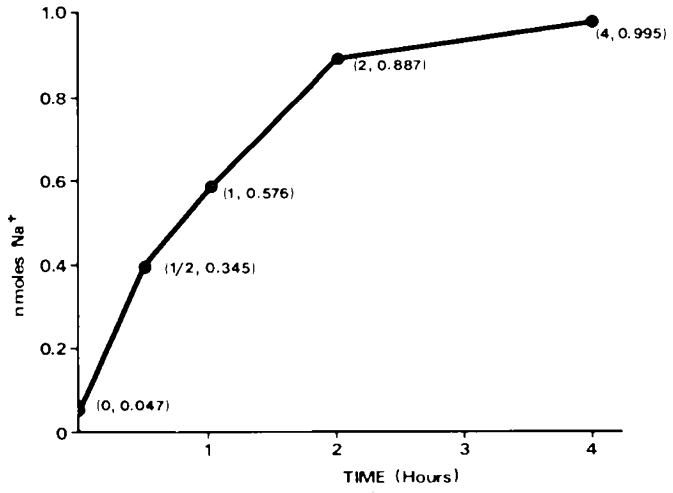

Fig. 1. Isolated platelets were resuspended in incubation buffer containing $^{22}Na^+$. Platelets were incubated and samples taken for radioactive content at the times indicated. The figures are the mean of two experiments.

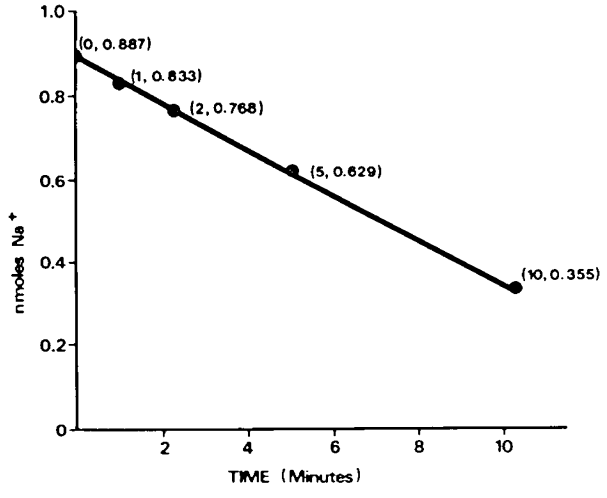

Fig. 2. Platelets were resuspended in incubation buffer containing $^{22}Na^+$ for 2 hr. The platelets were then divided and incubated either in NaCl/KCl reaction buffer or reaction buffer containing ouabain. Aliquots were taken at times indicated, radioactivity in the platelets determined. From this data, the rate of $^{22}Na^+$ efflux was calculated.

OUABAIN BINDING TO PLATELETS

The quantity of ouabain bound to both normal and DS platelets was small, but was less in DS than normal platelets (Table 2). The results were expressed as disintegrations per min per 10^9 platelets and as moles of ouabain bound per 10^9 platelets. The average amount of ouabain bound was equivalent to 15,098 dpm/10^9 platelets for normal and 11,838 dpm/10^9 platelets for DS subjects. When disintegrations per min were converted to molar concentration $\times 10^{-15}$, the average amount of ouabain bound was 1.08 ± 0.04 $\times 10^{-15}$ mol/10^9 platelets for normal and 0.86 ± 0.02 $\times 10^{-15}$ mol/10^9 platelets for DS subjects. This decreased binding of ouabain by DS platelets was significant ($P < 0.001$).

Table 1. *(Na^+/K^+)-stimulated, ouabain-sensitive ATPase activity in NaI-treated platelet membranes*[1]

	μmol P_i released/hr/10^9 platelets					
	(Na^+/K^+)-ATPase (after NaI)		Total ATPase (after NaI)		% (Na^+/K^+)-ATPase/total ATPase	
	Normal	DS	Normal	DS	Normal	DS
	3.54	3.09	4.97	3.85	71.2	80.1
	3.50	2.38	3.69	2.57	95.0	92.2
	2.52	2.85	2.92	3.46	86.4	81.8
	2.67	2.37	3.94	2.86	69.2	83.9
	3.42	1.53	5.40	1.70	63.3	90.2
	2.43	1.94	2.73	2.20	89.0	87.5
	2.14	1.35	2.48	1.62	86.2	83.2
	3.84	1.40	4.30	1.69	89.4	83.1
	2.29	1.85	2.80	1.89	81.8	98.0
	2.30	2.51	2.52	3.04	91.5	82.1
Av.	2.87	2.13	3.58	2.49	82.3	86.2
SEM	±0.20	±0.19				
Significant difference (P)		<0.02	<0.02			
DS/normal		74.2	69.6			

[1] Calculations to convert counts per min to micromoles of P_i released: A) total ATPase $= \frac{\text{cpm } [^{32}P]P_i \text{ released/hr/}10^9 \text{ platelets}}{\text{total } [^{32}P]\text{ATP added (cpm)}} \times 100\% = \%$ of input [^{32}P]ATP cleaved/hr/10^9 − [(% cleaved) (nmol input ATP (labeled and labeled)] = μmol of P_i released/10^9 platelets; B) ouabain-resistant ATPase, calculated as above; C) ouabain-sensitive (Na^+/K^+)-ATPase = total ATPase − ouabain-resistant ATPase.

Table 2. *Ouabain bound by whole platelets*[1]

	Normal		Down's syndrome	
	dpm bound/10^9 platelets	$\times 10^{-15}$ mol/10^9 platelets	dpm bound/10^9 platelets	$\times 10^{-15}$ mol/10^9 platelets
	15,670	1.18	9,676	0.77
	14,793	1.13	13,266	0.88
	13,014	0.99	10,912	0.83
	12,881	0.98	10,824	0.82
	13,570	1.03	11,200	0.85
	19,206	1.30	14,978	1.01
	15,991	0.99	13,732	0.88
	15,664	1.02	10,117	0.79
Av.	15,098	1.08	11,838	0.86
SEM	±732	±0.04	±673	±0.02
DS/normal		79.3		
Significant difference (P)		<0.001		

[1] Calculations were made as follows: A) dpm ouabain bound/10^9 platelets ÷ 2.2 $\times 10^6$ dpm/μCi = nCi bound ouabain/10^9 platelets; B) nCi/10^9 platelets ÷ specific activity (Ci/mM) = pM ouabain bound/10^9 platelets; C) pM × volume used = mol × 10^{-15}/10^9 platelets.

^{22}Na OUTWARD TRANSPORT IN NORMAL AND DS PLATELETS

The purpose of the study was to compare the rate of outward movement of $^{22}Na^+$ in normal and DS platelets. The average ouabain-sensitive outward movement of $^{22}Na^+$ in normal platelets from eight subjects was 103,742 dpm $^{22}Na^+$/hr/10^9 platelets (0.342 pmol) (Table 3). The outward movement of combined labeled and unlabeled Na^+ in normal platelets averaged 9.03 nmol Na^+/hr/10^9 platelets. For eight DS subjects the average ouabain-sensitive outward movement of ^{22}Na was 50,780 dpm (0.168 pmol) $^{22}Na^+$/hr/10^9 platelets and for combined labeled and unlabeled Na^+ the average outward movement was 4.41 nmol Na^+/hr/10^9 platelets. Calculated on the basis of $^{22}Na^+$ efflux of combined labeled and unlabeled Na^+ efflux, the rate of outward Na^+ transport is significantly less ($P < 0.001$) in DS compared to normal platelets.

DISCUSSION

A number of theories have been proposed to account for low serotonin content of DS platelets. Kamoun and Jerôme (10) have suggested that a decreased absorption of tryptophan by the decreased number of chromaffin cells in the distal colon of DS patients may result in decreased platelet serotonin content. Boullin and O'Brien (4) proposed that decreased ATP content in DS platelets would limit the amount of serotonin that could be complexed for storage in the platelet. O'Brien and Groshek (16) proposed that increased shunting of tryptophan through the kynurenine pathway in DS may increase available tryptophan for serotonin synthesis. A study of vitamin B_6 metabolism in DS done by measuring tryptophan metabolites during deoxypyridoxine administration suggested that DS patients are depleted of vitamin B_6 more readily than control subjects (12).

Serotonin is actively transported and concentrated in platelets and this uptake can be inhibited by a number of agents such as imipramine (23) and ouabain (5). The uptake of serotonin is energy dependent and Sneddon has shown it to be linked to the inward movement of sodium into the platelet (21, 22). The outward movement has been proposed to be a passive efflux (17) but other investigators have found the rate of efflux to be correlated to the metabolism of the cell (20), and concluded that serotonin release is an active process (23). A review of published work of other investigators and our own work has led us to propose that the decreased content of serotonin in DS platelets is due to a decreased rate of uptake but a normal rate of efflux of serotonin.

The present work was undertaken to determine if abnormalities in the rate of Na^+ movement could be associated with decreased V_{max} for serotonin entry into DS platelets. Sneddon's work (21) on rat platelets showed that when the platelet concentration of Na^+ was increased by ouabain inhibition of (Na^+/K^+)-ATPase, the inward rate of serotonin transport was decreased. Our previous work (14) showed that the mean sodium content of DS platelets was significantly increased: 6.79 ± 0.40 μg/10^9 platelets compared to 3.32 ± 0.21 μg/10^9 platelets in controls. One explanation for the increased Na^+ content in DS platelets is the present demonstration that the rate of outward movement of Na^+ is approximately 50% that of ^{22}Na is associated with a doubling of Na^+ content in DS platelets.

In the literature, we were unable to find data on the rate of outward movement of Na^+ in normal human platelets. This paper provides these normal values and shows that the rate averaged 9.03 nmol Na^+/hr/10^9 platelets for combined labeled and unlabeled sodium in normal platelets. The rate was significantly slower, 4.41 nmol Na^+/10^9 in DS platelets.

The outward movement of Na^+ is linked to the inward movement of K^+. The hydrolysis of the terminal phosphate of ATP by (Na^+/K^+)-ATPase is accompanied by the outward movement of

Table 3. *Na^+ ions transported out by platelets (ouabain-sensitive efflux only)*[1]

	Normal			Down's syndrome		
	dpm $^{22}Na^+$/hr/10^9 platelets	pmol $^{22}Na^+$/hr/10^9 platelets	nmol Na^+/hr/10^9 platelets (labeled and unlabeled)	dpm $^{22}Na^+$/hr/10^9 platelets	pmol $^{22}Na^+$/hr/10^9 platelets	nmoles Na^+/hr/10^9 (labeled and unlabeled)
	93,120	0.307	8.15	47,790	0.158	4.15
	109,490	0.362	9.53	46,060	0.152	4.00
	124,960	0.413	10.87	48,620	0.161	4.23
	111,960	0.370	9.71	51,340	0.169	4.46
	120,480	0.398	10.47	51,520	0.170	4.48
	89,690	0.297	7.80	48,430	0.160	4.21
	82,210	0.272	7.15	51,340	0.169	4.46
	98,030	0.324	8.52	61,120	0.203	5.31
Av.	103,742	0.342	9.03	50,780	0.168	4.41
SEM	±5,407	±0.017	±0.46	±4,322	±0.005	±0.14
DS/normal			48.6			
Significant difference (P)			<0.001			

[1] Calculations were made as follows: A) dpm $^{22}Na^+$ transported out/hr/10^9 ÷ 2.2 × 10^6 dpm/μCi = nCi$^{22}Na^+$ transported/hr/10^9 platelets; B) nCi/hr/10^9 platelets ÷ specific activity of $^{22}Na^+$ = pmol $^{22}Na^+$ transported/hr/10^9 platelets; C) pmol/hr/10^9 platelets × ratio of unlabeled to labeled Na^+ = nmoles Na^+ transported/hr/10^9 platelets.

3 molecules of Na^+ and the inward movement of 2 molecules of K^+ (7). The present work shows that there is a significant decrease in (Na^+/K^+)-ATPase activity in DS platelets and also of total ATPase activity. It cannot be stated that the decreased rate of outward movement of $^{22}Na^+$ in DS platelets is due to decreased activity of (Na^+/K^+)-ATPase but it is probable there is a close relationship between these two observations. Barthel (1) has shown that the time courses of serotonin and $^{42}K^+$ uptake are parallel. The proposal is made that serotonin uptake requires the asymmetric distribution of sodium and potassium between blood platelets and the medium. This asymmetry is maintained by (Na^+/K^+)-ATPase.

The decrease in quantity of [^{3}H]ouabain bound by DS platelets paralleled the decrease in (Na^+/K^+)-ATPase. It is thus probable that the decreased enzyme activity may be due to a decreased number of enzyme sites on DS platelet membranes. Decreased [^{3}H]ouabain binding occurred both in DS intact platelets and NaI-extracted membranes. The binding of ouabain is thought to be specific to (Na^+/K^+)-ATPase (6). There is no evidence yet that there is a direct relationship between these observations and trisomy for chromosome 21.

Based on the present and preceding work, a model is proposed to account for the low serotonin content of DS platelets. (Na^+/K^+)-ATPase activity is decreased, probably due to a decreased number of enzyme sites in DS platelet membrane. The decreased enzyme activity results in a decreased rate of outward movement of Na^+ which results in an increased content of Na^+ in DS platelets, a decreased K^+ content in DS platelets (13). As serotonin is cotransported with Na^+, the inward movement of this amine is also decreased. Because the outward movement of serotonin is normal in DS platelets (11), the net effect is a low serotonin content in DS platelets.

The uptake and storage of amines in platelets has many characteristics of the amine uptake process in synaptosomes. The platelet has been proposed as a peripheral model for amine uptake in the central nervous system (3, 18). If decreased (Na^+/K^+)-ATPase activity were present in DS neutral and glial cells, similar findings in relation to amine uptake may be present and modify normal neuronal transmission processes in Down's syndrome.

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