Characterization of Pulmonary and Myocardial 
Beta-Adrenoceptors with 
S-1’-[Fluorine-18]Fluorocarazolol

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S-1’-[18F]fluorocarazolol was administered to healthy volunteers to assess its potential for noninvasive measurement of regional pulmonary and myocardial beta-adrenoceptor densities. Methods: High-specific activity fluorocarazolol was intravenously injected on two separate occasions within a 1-wk interval. The initial injection was without pretreatment, but before the second injection, the volunteers either inhaled salbutamol (2 x 200 µg aerosol) or they ingested pindolol (3 x 5 mg during a 12-hr interval). Twenty-eight PET time frames of 31 planes were acquired over a period of 60 min after each injection. Blood samples were drawn and analyzed for the presence of fluorocarazolol and radioactive metabolites. Results: Uptake of fluorocarazolol in the target tissues was nearly unaffected by salbutamol or pindolol. Pulmonary and myocardial tissue-to-plasma concentration ratios of fluorocarazolol reached plateau values of 11.6 ± 0.6 (lungs) and 18.1 ± 1.0 (heart) at 45-50 min postinjection. These values were reduced to 2.0 ± 0.4 and 2.0 ± 0.6 after treatment with pindolol. Conclusion: These data indicate that:

1. Pulmonary and myocardial uptake of radioactivity after intra-venuous administration of S-1’-[18F]fluorocarazolol represents radioligand binding to beta-adrenoceptors.
2. Pulmonary binding occurs mainly in alveoli rather than in airway smooth muscle under these conditions.
3. Binding kinetics do not preclude quantification of receptors with compartment models.

Key Words: beta-adrenoceptor density; fluorine-18-fluorocarazolol; PET

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PET with appropriate radioligands offers the possibility of studying receptors noninvasively in man. Carazolol, a lipophilic, nonsubtype-selective beta-adrenoceptor antagonist, can be labeled with the positron emitters 11C (1,2) or 18F (3). The suitability of carazolol for PET studies of beta-adrenoceptors has been demonstrated in mice, rats and minipigs (1—4). Uptake in the target organs (heart, lungs) is substantial and it can be blocked preventing tissue uptake of S-1’-[18F]fluorocarazolol (5), which indicates that fluorocarazolol and propranolol compete for binding to the same receptor sites.

In vivo competition studies showed that fluorocarazolol binds to the beta-1 and beta-2-subtypes. Beta-1 subtype-selective antagonists (CGP 20712A and ICI 89,406) inhibited 18F uptake in rat heart (predominantly beta-1-adrenoceptors) more potently than in rat lungs (predominantly beta-2-adrenoceptors (5)). In contrast, beta-2 subtype-selective drugs (ICI 118,551 and propranolol) were more potent in the lungs than in the heart (2,5). Radioactive metabolites appeared in rat plasma, but bound radioactivity in heart and lung represented mainly parent compound even at 60 min postinjection (6). In vivo saturation studies indicated receptor densities of 6.0 and 21 pmole/g tissue in rat heart and lung, respectively (7).

Thus, animal experiments indicate that S-(fluorocarazolol may be a useful ligand for PET evaluation of beta-adrenoceptors in patients suffering from asthma, chronic obstructive pulmonary disease, cystic fibrosis, hypertension or heart failure, conditions which are associated with altered receptor densities or an altered coupling of the receptors to distal parts of the transduction chain (8—13). Such studies can also be performed with another radioligand, S-[11C]-CGP 12177 (14—20). However, the synthesis of this compound is difficult and in our hands not sufficiently reliable for routine clinical studies. S-1’-[18F]fluorocarazolol is more easily prepared and it may prove a useful alternative to S-[11C]-CGP 12177. Here, we report the results of the first PET studies in healthy volunteers using S-1’-[18F]fluorocarazolol.

MATERIALS AND METHODS

Subjects

Healthy volunteers were recruited according to the following criteria: age 18—40 yr, prebronchodilator forced expiratory volume in 1 sec (FEV1) >=80% of predicted, nonsmoker or ex-smoker (smoking terminated for more than 1 yr). Excluded were people with a positive history for wheezing and tightness of the chest, upper respiratory tract infections in a period shorter than 4 wk before the study, presence of pulmonary diseases including asthma, presence of airway hyperresponsiveness, atopy (at least one positive reaction to known allergens in intracutaneous skin tests), use of beta-mimetics or theophylline, high blood pressure or heart failure, pregnancy or suspected pregnancy.

All volunteers underwent the following screening: anamnesis, physical examination, routine blood biochemistry to assess kidney and liver function, electrocardiogram, skin tests for allergic reactions and pulmonary function tests (spirometry and determination of airway hyper-responsiveness). Airway responsiveness to methacholine was determined using the 2-min tidal breathing method of Cockcroft et al. (21,22). The study was approved by the Medical Ethics Committee of the University Hospital. Each subject gave written, informed consent.

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### TABLE 1

<table>
<thead>
<tr>
<th>Date</th>
<th>Volunteer no.</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Weight (kg)</th>
<th>Pretreatment</th>
<th>Injected mass (nmole)</th>
<th>Receptor occupancy lung (%)</th>
<th>Receptor occupancy heart (%)</th>
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</thead>
<tbody>
<tr>
<td>30-11-94</td>
<td>1</td>
<td>23</td>
<td>M</td>
<td>67</td>
<td>None</td>
<td>0.64</td>
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<td>07-12-94</td>
<td></td>
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<td></td>
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</tr>
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<td>08-03-95</td>
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<td>21</td>
<td>M</td>
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<td>None</td>
<td>0.36</td>
<td>1.24</td>
<td>0.52</td>
</tr>
<tr>
<td>15-03-95</td>
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<td>24</td>
<td>M</td>
<td>76</td>
<td>Salbutamol</td>
<td>2.84</td>
<td>1.51</td>
<td>0.77</td>
</tr>
<tr>
<td>12-04-95</td>
<td>4</td>
<td>36</td>
<td>M</td>
<td>67</td>
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<td>0.99</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19-04-95</td>
<td>5</td>
<td>27</td>
<td>F</td>
<td>56</td>
<td>None</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24-05-95</td>
<td>6</td>
<td>28</td>
<td>M</td>
<td>76</td>
<td>Pindolol</td>
<td>2.84</td>
<td>1.51</td>
<td>0.77</td>
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<td></td>
<td>Salbutamol</td>
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<td></td>
<td></td>
</tr>
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<td>02-06-95</td>
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<td></td>
<td>Salbutamol</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>20-09-95</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
<td>None</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>29-11-95</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td>None</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± s.d.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.3 ± 0.8</td>
<td>1.6 ± 0.8</td>
<td>0.8 ± 0.3</td>
</tr>
</tbody>
</table>

Mean ± s.d. values are shown for each parameter. The number of subjects is shown in parentheses.

### Radioligand

S-Desisopropylcarazolol (enantiomeric excess >98%) was prepared as reported previously (6). S-1'-[18F]fluorocarazolol was synthesized by reacting the precursor with [18F]fluoroacetone (3,6) and purified by HPLC. The specific activity was 50 ± 24 TBq/mnmole (1400 ± 680 Ci/mnmole) and the radiochemical purity was >99.8%. The ligand was dissolved in 0.5 ml ethanol/propylene glycol/0.9% NaCl (1/2/2 v/v/v). Before injection this solution was filtered (0.22 μm) and 7.5 ml 0.9% NaCl was added via the filter. The solution was sterile and pyrogenic. S-1'-fluorocarazolol. HCl passed the test on “acute toxicity” (European Pharmacopeia; Dutch Pharmacopeia Ed. IX) at a 10,000-fold higher dose than was administered to humans.

### Study Protocol

At the beginning of the study, a cannula was placed in a vein of one of the lower forearms. Another cannula was placed in the radial artery of the contralateral arm, after patency of the ulnar artery had been proven by the Allen-test. The arterial cannula was inserted under local anesthesia with lidocaine. The venous cannula was used for injection and the arterial line for blood sampling.

The volunteer was then placed in the PET camera (FWHM = 6 mm). A rectilinear scan was made for proper positioning (heart and lungs in the field of view). Next, a transmission scan was produced, using the internal 68Ge/68Ga sources, to correct for attenuation. S-1'-[18F]fluorocarazolol (on average 56 MBq = 1.5 mCi) was injected over a period of 1 min, using a Medrad OP-iOO remote-controlled pump. Lines were carefully flushed with saline to ensure complete delivery of the radioligand.

Data acquisition was started at the onset of injection; 8 frames of 15 sec were followed by 4 frames of 30 sec, 4 frames of 1 min, 4 frames of 2 min, 6 frames of 4 min and 2 frames of 10 min. Total duration of the study was 60 min. Arterial blood samples (2 ml)
were drawn at 0.5-min intervals during the initial 5 min and at 10-min intervals from 10 to 60 min postinjection. Radioactivity in plasma and in a cell pellet (5 min 3000 g) was determined in all samples using a gamma counter that was cross-calibrated with the PET camera. Additional samples (3 ml) drawn at 1, 2, 5, 10, 20, 40 and 60 min were used for metabolite analysis (see below). The volunteer left the camera when data acquisition had ended and the cannulas were removed.

After an interval of at least 1 wk, the volunteer returned for the second part of the study in which the influence of a beta-2 agonist or a beta-adrenoceptor antagonist on tissue uptake of S-1'-[18F]fluorocarazolol was assessed. Two different treatments were compared:

1. Some volunteers inhaled salbutamol (Ventolin®, 2 × 200 µg aerosol) 30—40 min prior to injection of the radioligand.
2. Other volunteers took pindolol (Viskeen®, orally, 5 mg on the evening before the experiment, 5 mg on the morning before the experiment and 5 mg 30—40 min before injection of the radioligand).

Cannulas were placed in a vein of each of the lower forearms. No arterial catheter was used in the second part of the study to keep inconvenience to a minimum. One cannula was used for injection and the other for blood sampling. Tracer injection, data acquisition and sampling were performed as on day one.

**Metabolite Analysis**

Plasma was analyzed for the presence of S-1'-[18F]fluorocarazolol and radioactive metabolites by methods published previously (23). Untreated plasma samples were directly applied to a Chromsphere Biomatrix (150 × 4.6 mm i.d.) column with M3 guard column. The mobile phase was 10 mM K2HPO4:acetonitrile (90:10 v/v, pH 7.5) and the flow rate 1.5 ml.min⁻¹. Twenty-four fractions of the eluate were collected over a period of 12 min. Radioactivity in the fractions was determined using the gamma counter. An independent estimate of the fraction of unmetabolized ligand was obtained by determining protein-bound radioactivity in human plasma by ultrafiltration (MPS-1 reusable micropartition system with YMT-30 membrane, Amicon, Beverly MA). It has been shown previously that about 73% of native S-1'-[18F]fluorocarazolol is bound to plasma proteins, but its radioactive metabolites have negligible protein binding (23).

**Data Evaluation**

Regions of interest (ROIs) were drawn on both lungs, using the transmission scan and avoiding hilar structures. A ROI for the left

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**TABLE 2**

Parameters of the 'Slow Kinetic Phase' of Washout in Target Tissues

<table>
<thead>
<tr>
<th>Volunteer no.</th>
<th>Heart</th>
<th>Lungs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Y-intercept (ECAT cts × 10⁶)</td>
<td>Rate constant (min⁻¹)</td>
</tr>
<tr>
<td>1</td>
<td>26.6</td>
<td>0.0106</td>
</tr>
<tr>
<td>2</td>
<td>24.1</td>
<td>0.0066</td>
</tr>
<tr>
<td>3</td>
<td>27.9</td>
<td>0.0081</td>
</tr>
<tr>
<td>4</td>
<td>24.8</td>
<td>0.0089</td>
</tr>
<tr>
<td>5</td>
<td>34.1</td>
<td>0.0067</td>
</tr>
<tr>
<td>6</td>
<td>24.8</td>
<td>0.0080</td>
</tr>
<tr>
<td>Control mean ± s.d.</td>
<td>0.0062 ± 0.0015</td>
<td>26.7 ± 2.2</td>
</tr>
<tr>
<td>2</td>
<td>23.4</td>
<td>0.0094</td>
</tr>
<tr>
<td>3</td>
<td>22.6</td>
<td>0.0075</td>
</tr>
<tr>
<td>Salbutamol mean ± s.d.</td>
<td>0.0084 ± 0.0010</td>
<td>25.1 ± 0.8</td>
</tr>
<tr>
<td>4</td>
<td>16.2</td>
<td>0.0145</td>
</tr>
<tr>
<td>5</td>
<td>13.0</td>
<td>0.0150</td>
</tr>
<tr>
<td>Salbutamol mean ± s.d.</td>
<td>0.0148 ± 0.0003*</td>
<td>13.0 ± 3.8*</td>
</tr>
</tbody>
</table>

*Significantly different from the corresponding value for the heart.
†Significantly different from the control value.
Details regarding the participants and the study protocol are presented in Table 1. All volunteers (5 men, 1 woman; age range 21–36 yr; median age 27 yr) had normal spirometric values: average FEV₁ 103% of predicted, range 92%–114%, average vital capacity 105 (92–123) % of predicted. None of the subjects showed bronchial hyperresponsiveness to methacholine: the provocation concentration for a 20% decrease of FEV₁ (PC₂₀) was >20 mg/ml in all cases.

Injection of S-¹-[¹⁸F]fluorocarazolol by the remote-controlled pump did not cause any change in blood pressure, heart rate or electrocardiogram of the volunteers. Transverse sections of the thorax of a single subject before and after ingestion of pindolol are shown in Figure 1. The volunteer is on his back; the direction of observation is from his feet towards his head. In the control study (without pindolol), the heart and lungs were clearly visible; at planes originating from the lower part of the thorax the right liver lobe came into the field of view. After ingestion of pindolol, heart and lungs were no longer visible, but the hepatic uptake of S-¹-[¹⁸F]fluorocarazolol was unaffected. Inhalation of salbutamol before administration of the radioligand did not alter the PET images in any way. PET images acquired after salbutamol treatment are therefore not shown in Figure 1.

Kinetics of S-¹-[¹⁸F]fluorocarazolol Uptake in Heart and Lung

After injection of the radioligand, tissue levels of radioactivity rose to a maximum followed by a rapid decline to a relatively stable plateau (Fig. 2). This 'slow kinetic phase' represented a higher level of radioactivity per volume in the heart than in the lungs (Fig. 2). A biexponential function was fitted to the tissue washout curves; parameters calculated for the slow component of this function (i.e., the slow kinetic phase) are shown in Table 2. Salbutamol did not significantly influence the slow kinetic phase in either heart or lungs, but pretreatment of subjects with pindolol induced a more rapid washout of radioactivity from the target organs (Fig. 2; Table 2). Pindolol reduced cardiac and pulmonary radioactivity to 39% and 56% of the control at 60 min postinjection, whereas salbutamol had no significant effect (average reduction <5% from 30–60 min postinjection, Fig. 2).

Clearance of Radioactivity from Plasma

 Injected radioactivity was initially rapidly cleared from plasma (to 6.3% ± 0.3% of the peak level within 10 min), but the subsequent clearance was slow (to 3.1% ± 0.4% after 60 min, see Fig. 3). Inhaled salbutamol did not affect the plasma clearance of the radioligand, but ingested pindolol retarded it. Circulating levels of radioactivity from 10 to 60 min postinjection were significantly (1.7–1.8 fold) higher in pindolol-treated subjects than in control subjects (Fig. 3).

Appearance of Labeled Metabolites in Plasma

Radioactive metabolites appeared rapidly in human plasma after injection of S-¹-[¹⁸F]fluorocarazolol. The fraction of total plasma radioactivity representing unmodified radioligand is plotted as mean ± s.d.
Tissue-to-Plasma Concentration Ratios

If tissue radioactivity is considered to represent bound radioligand only (see Discussion) and the contribution of radioactive metabolites is subtracted from total plasma radioactivity, [tissue]/[plasma] concentration ratios of the radioligand can be calculated. In heart and lung, these ratios slowly rise to a plateau value that is reached after 45-50 min (Fig. 5).

DISCUSSION

Advantages and Disadvantages of Using S-1'-[Fluorine-18]-Fluorocarazolol Rather Than S-[Carbon-11]-CGP 12177

We could produce S-1'-[18F]fluorocarazolol with lower maximum yield than S-[11C]-CGP 12177 but with much higher specific activity (Table 3). The yield could still be optimized and it was already sufficient for human studies. The high specific activity of S-1'-[18F]fluorocarazolol allowed PET scanning to be performed with low receptor occupancy, i.e., lack of pharmacological effects (see below). In contrast, planned studies with S-[11C]CGP 12177 often had to be canceled for medical-ethical reasons as no low-mass injection was possible.

Both CGP-12177 and fluorocarazolol are potent, nonsubtype-selective beta-adrenoceptor antagonists with subnanomolar affinities to beta-adrenoceptors (Table 3). CGP 12177 is hydrophilic whereas fluorocarazolol is a more lipophilic ligand. The lipophilicity of fluorocarazolol as compared to CGP-12177 resulted in a higher nonspecific binding and a more extensive first-pass metabolism in humans (Table 3). However, CGP 12177 did not appreciably cross the blood-brain barrier whereas fluorocarazolol could be used for PET studies of beta-adrenoceptors in the central nervous system (data reported elsewhere).

Receptor Occupancy in Target Tissues

Tissue concentrations of fluorocarazolol were calculated from PET images using the calibration factor of the camera and the specific activity of the injected radioligand. During the slow kinetic phase, maximal concentrations ranged from 0.03 to 0.085 pmole/ml in the heart and from 0.015 to 0.06 pmole/ml in the lungs (raw data; tissue volume including air).

Washout of 18F from the target tissues after injection of S-1'-[18F]fluorocarazolol showed the typical biexponential kinetics that have also been described for S-[11C]CGP 12177, an established beta-adrenoceptor ligand (19). The rapid component of this kinetics represented the vascular phase and nonspecific binding, whereas the slow component mainly represented radioligand binding to beta-adrenoceptors (19). Estimations of beta-adrenoceptor density in the heart of healthy volunteers using S-[11C]-CGP 12177 ranged from 7.0 ± 1.4 [uncorrected mean ± s.d. (24)] to 10.0 ± 1.7 pmole/ml [corrected for spillover from blood and partial volume effect (25)]. Pulmonary beta-adrenoceptor density in healthy subjects, measured by the same technique, were 2.0 ± 0.2 pmole/ml tissue (including air) (18). Maximal receptor occupancies in the present study, calculated from tissue concentrations of S-1'-[18F]fluorocarazolol and in vivo data for tissue Bmax (18,24,25) ranged from 0.4 to 1.2% in the heart and from 0.75% to 3% in the lungs (see Table 1). At the end of the experiment (50–60 min post injection), values were ca 0.50% lower. It is therefore not surprising that injection of S-1'-[18F]fluorocarazolol had no measurable effect on heart rate or blood pressure, although carazolol has no intrinsic sympathomimetic activity.

Mechanisms Underlying Tissue Uptake of Radioactivity

Orally administered pindolol had a strong effect on S-1'-[18F]fluorocarazolol uptake in the heart and lung but not in the liver of healthy volunteers (Figs. 1, 2, 5; Table 2). The pindolol effect suggests that myocardial and pulmonary radioactivity represents radioligand binding to beta-adrenoceptors, whereas liver uptake is largely determined by other mechanisms (e.g., membrane transport and metabolism). Inhalation of salbutamol had no measurable effect on pulmonary radioactivity at intervals >20 min although salbutamol seemed to induce a more

TABLE 3

Properties of the Beta-Adrenoceptor Ligands

<table>
<thead>
<tr>
<th>S-[11C]-CGP 12177</th>
<th>S-1'-[18F]-fluorocarazolol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum yield (GBq)</td>
<td>1.85</td>
</tr>
<tr>
<td>Specfic activity (TBq/m mole)</td>
<td>0.37–18.5</td>
</tr>
<tr>
<td>Physical half-life (min)</td>
<td>20.4</td>
</tr>
<tr>
<td>Lipophilicity (log P&lt;sub&gt;octanol/buffer pH 7.4&lt;/sub&gt;)</td>
<td>−0.5 (29)</td>
</tr>
<tr>
<td>Affinity β&lt;sub&gt;1&lt;/sub&gt;-subtype (nM)</td>
<td>0.33 (30)</td>
</tr>
<tr>
<td>Affinity β&lt;sub&gt;2&lt;/sub&gt;-subtype (nM)</td>
<td>0.90 (30)</td>
</tr>
<tr>
<td>Metabolism in humans (during 60 min scan)</td>
<td>Negligible according to (31, 32) (see Fig. 4)</td>
</tr>
</tbody>
</table>

FIGURE 5. Tissue-to-plasma concentration ratios of S-1'-[18F]fluorocarazolol in human tissues. Tissue radioactivity was assumed to be 100% unmodified radioligand; plasma radioactivity was corrected for the presence of radioactive metabolites. Data are plotted as mean ± s.d.
Effect of Pindolol on Radioligand Clearance

In the presence of pindolol, levels of radioactivity in plasma from 10 to 60 min after injection of S-1-[18F]fluorocarazolol were almost twice as high as in the control situation (Fig. 3). Moreover, the ratio of the concentrations of parent/metabolites was significantly increased (Fig. 4). The effect of pindolol may be caused by two different (but not mutually exclusive) mechanisms:

1. Pindolol blocked beta-adrenoceptors and it caused a more rapid washout of the radioligand from the target organs, resulting in higher concentrations in plasma (see Fig. 2).

2. Pindolol competed with S-1-[18F]fluorocarazolol for the same metabolic pathway in the lung and thus caused less rapid degradation and excretion of the radioligand.

CONCLUSION

S-1-[18F]fluorocarazolol seemed a useful radiopharmaceutical for PET studies of beta-adrenoceptors in human heart and lungs. The myocardium and the peripheral lung were clearly visualized; uptake in these tissues was strongly inhibited after ingestion of pindolol. Tissue-to-plasma concentration ratios of the radioligand increased to a plateau value which was reached at 45–50 min postinjection. Kinetics of 18F uptake and release in the target organs were compatible with determination of receptor densities with compartment models. After submission of this article, another article was published in which it was shown that myocardial beta-adrenoceptor density in experimental animals can be accurately determined by a dual-injection protocol (28).

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REFERENCES


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