Tumor Location with 1-Aminocyclopentane [^{11}C] Carboxylic Acid: Preliminary Clinical Trials with Single-Photon Detection

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High specific activity [^{11}C] Carboxyl-labeled 1-aminocyclopentane-carboxylic acid ([^{11}C] ACPC) was tested as a tumor-scanning agent in thirty-eight patients. This artificial amino acid clears the blood to a level of less than 12% within 45 min; thus, imaging is possible within the useful life of C-11. [^{11}C] ACPC can be produced in amounts adequate for clinical scanning. Doses between 12 and 45 mCi were given by i.v. injection, and scans obtained only in the single-photon mode gave clinical information on the sites of tumors. There was no evidence of any toxic effects from [^{11}C] ACPC, and the radiation doses as extrapolated from animal data are approximately 0.01 rad per mCi for the whole body and less than 0.06 rad per mCi for the pancreas. In all but five of the 38 patients [^{11}C] ACPC scans were compared with those obtained with Ga-67 citrate. There were 19 positive [^{11}C] ACPC scans and 24 positive Ga-67 scans. The results indicate that [^{11}C] ACPC is likely to be of diagnostic value for cancer patients if used in conjunction with positron tomography instrumentation.

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We are continuing to seek better methods for the early detection of malignant neoplasms and for assessing their location, size, and extent (*1*). Carbon-11 has excellent potential for use in labeling organic and biochemical substances (*2*), since its positron-decay mode with resultant annihilation photons offers unusual possibilities for imaging with much-improved spatial resolution through computer-assisted tomography (*3,4*). These obvious advantages of C-11 are limited by its half-life of 20.4 min, which restricts its use to rather rapid biologic processes and requires the availability of methods for fast chemical synthesis and adequate radioprotective measures for the radiochemist. On the other hand, its short half-life permits the administration of millicurie doses of C-11-labeled agents, which may result in excellent imaging statistics and make possible repeat studies after intervals as short as 2 hr.

From a review of the literature, it appeared that C-11-labeled 1-amino-cyclopentane carboxylic acid ([^{11}C] ACPC) might be a potential tumor-scanning agent because the stable form of this drug, "cycloleucine," was studied as a cancer chemotherapeutic agent in the early 1960s (*5*). This artificial cyclic amino acid, labeled with C-14 in the carboxyl group, was shown by Berlinguet et al. (*6*) and Sterling et al. (*7*) to localize in several types of animal malignancies. Further studies in our institution confirmed and extended this work. We accordingly developed methods for rapid synthesis, purification, and pharmaceutical testing of carboxy-labeled [^{11}C] ACPC (*8*). Clinical trials were then deemed justified, and phases 1 and 2 studies were undertaken with ap-

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proval by our Committee on Human Studies, and after [^{11}C] ACPC had been approved by the U.S. Food and Drug Administration for use as a new investigational drug.

MATERIALS AND METHODS

[^{11}C] ACPC preparation. The amino acid was labeled on the carboxyl atom in a hot cell at the local 86-in. 22-MeV proton cyclotron* complex using the Strecker amino acid synthesis (*8*) reaction at high temperature and pressure. The purified product was transported from the ORNL cyclotron complex to the ORAU laboratory for radiopharmaceutical processing in a clean room equipped with a laminar-flow hood. After pH adjustment to 7.0 ± 0.2 with 1 N HCl, the solution was filtered through a sterile 0.22-μ microfilter into a sterile precapped vial. Final radioassay was done using a sodium iodide crystal that had been calibrated with standards obtained from the U.S. National Bureau of Standards. To test for pyrogens, a 15-min Pyrotest technique† was used (*9*) because the short half-life of C-11 precludes testing with rabbits in the conventional USP manner.

Clinical studies. Subjects for this study were referred to us on an outpatient basis from a local chest disease hospital, the University research center and hospital and from private practicing physicians. Scans were performed in 38 patients with active malignant neoplastic disease, neoplastic disease in "remission," or suspected malignancy.

In the first ten patients the radiopharmaceutical was injected through the tubing of a running i.v. infusion (5% dextrose in 0.45% NaCl). Since no reactions were observed, the subsequent patients received their doses by direct i.v. injection. Volumes were always less than 12 ml and total doses ranged from 12 to 45 mCi. The weight of the stable ACPC did not exceed 0.1 mg per kg. ACPC at this level is innocuous from a toxic standpoint (*5*), and unpublished animal work indicates that over a wide range the amount of stable ACPC administered does not alter the tissue distribution of C-14 and C-11-labeled ACPC. Torso scans were started about 5 min after injection using a 5-in. dual-head total-body rectilinear scanner equipped with high-energy collimators. Anterior, posterior, and combined views were obtained. Instrumentation for taking full advantage of the positron emission of C-11 is not yet in use in our laboratory, and the present work is based exclusively on images made with single-photon detection and without correction for decay during scanning. A window setting of 440 to 620 keV was used to cover the 510-keV positron annihilation photons. After the first torso scan was made, which usually required about 20 min, there was often enough activity left for a total-body scan or an area of interest scan.

In all except five cases Ga-67 citrate scans were obtained within a few days of the [^{11}C] ACPC scan, for the purpose of comparing the relative value of the two radiopharmaceuticals in cancer detection and, if possible, to determine whether the two were equally deposited in non-neoplastic inflammatory areas. Gallium-67-citrate scans were done two days after injection of 70 μCi per kg of body weight.

Hematologic and clinical chemical observations. Before the injection of [^{11}C] ACPC, at least twice during the first 2 hr after the dose, and once on the next day, the first ten patients had the following tests performed: CBC, differential leukocyte count, platelet count, reticulocyte count, bleeding time, prothrombin time, partial thromboplastin time, LDH, SGOT, bilirubin, plasma hemoglobin, and urinalysis. In the subsequent 15 patients these tests were done only before injection of the tracer and 24 hr postinjection. The tests were then discontinued.

Blood samples for radioassay were obtained before injection and at 5, 15, 30, 60, and 120 min after injection. Total-body counts and radioassay of urine following the scanning procedure were used to determine the mode of excretion and to obtain dosimetry data.

RESULTS

Observations for toxicity. There was no immediate or delayed local or general reaction to the [^{11}C] ACPC, and no significant changes in any of the laboratory tests. Although late laboratory tests (after 24 hr) were not obtained, general observation of the patients revealed no findings suggestive of late complications.

Blood clearance and urinary excretion. Blood radioactivity was measured, and the percentage of the administered dose remaining in the blood was calculated. There was rapid and uniform clearance of activity: by 5 min only 25% remained in the blood, and by 45 min not more than 12%. Urinary C-11 excretion was measured in 25 of the patients using the first urine specimen 2 hr after administration and showed an average activity excretion of 1.1% of the dose for this period. Whole-body counts before and after voiding confirmed the almost complete retention, and the absence of any appreciable loss of C-11 activity by decarboxylation.

Radiation dosimetry. Table 2 gives dosimetry estimates based on the tissue distribution observed in rats 30 min after administration of [^{11}C] ACPC (*10*). Calculations were performed by the ORAU Radiopharmaceutical Internal Dosimetry Center. Although the human estimates were extrapolated from

TABLE 1. COMPARISON OF SCANNING RESULTS OBTAINED WITH [^{11}C] ACPC AND Ga-67 CITRATE

Diagnosis	No. of patients	No. of positive scans		No. of lesions detected		Comments
		[^{11}C] ACPC	Ga-67 Citrate	[^{11}C] ACPC	Ga-67 Citrate	
Cancer of lung	12	10	12	17	25	
Multiple myeloma	1	0	1	0	2	
Ameloblastoma	1	1	1	3	2	
Cancer of breast with metastases	3	3	2	3	2	Uptake of [^{11}C] CPC and Ga-67 citrate at site of previous mastectomies.
Cancer of breast without active lesions	2	0	0	0	0	Scanning was done following mastectomy.
Lymphoma	4	3	4	4	12	
Hodgkin's disease, active	1	0	1	0	1	
Hodgkin's disease in remission	3	0	0	0	0	
Metastatic adenocarcinoma, P.S.U.	1	1	1	2	2	Questionable lesions in mediastinum and abdomen.
Colon cancer postoperative without evidence for metastases	1	0	0	0	0	Confusing LUQ and pelvic activity. No clinical evidence for active neoplastic process.
Cancer of pancreas	1	0	0	0	0	Lesion present.
Melanoma	1	0	0	0	0	Clinically free of disease.
Suspected malignancy	2	1	2	1	4	One bone and one pulmonary lesion.
Total	33	19	24	30	50	

the rat, the fact that the excretion is low in humans, as it is in rats, tends to justify this extrapolation. Our experience has shown that later scans are never more informative than initial ones, and thus the relatively large doses we have sometimes used are probably not necessary. We believe that under our conditions of imaging, an adequate dose is 0.25–0.3 mCi per kg (20 mCi).

Time relationships. Most of the scans were started at 5 min after injection and torso scans were obtained in about 20 min. In 23 patients there was enough radioactivity remaining to start another scan at 30–45 min after beginning the first. In general, there was little or no difference in the amount or clarity of information provided by the first and second views, either with regard to tumors or normal tissues, except that the cardiac and vascular images soon become somewhat less pronounced. In the few instances in which images of the kidneys were seen, they tended to be weaker in the second view.

Normal distribution as shown by imaging. No normal persons were studied with [^{11}C] ACPC, but based on areas apparently uninvolved by disease a comparison of all the scans suggests some patterns that probably represent the normal distribution. The liver was consistently the organ of greatest uptake and was clearly visualized in all 38 patients, although in two of them it appeared fainter than in the others. The spleen was clearly seen in 17 patients and was questionably visible in eight more. (Two patients had had splenectomy.) In a few instances in which there

TABLE 2. ESTIMATED RADIATION DOSE TO REFERENCE MAN FROM I.V. ADMINISTRATION OF [^{11}C] ACPC

Organ	Radiation dose* (rads/mCi)	
	Male	Female
Total body	0.011	0.011
Pancreas	0.058	0.057
Liver	0.021	0.023
Spleen	0.022	0.021
Kidney	0.023	0.023
Lung	0.019	0.019
Muscle	0.014	0.014
Marrow	0.017	0.016
Testis	0.017	—
Ovary	—	0.020

* Based on 30-min tissue distribution of C-14-labeled ACPC in rat following i.v. administration (0.04 mg/kg), assuming immediate uptake by organs followed by complete decay in situ.

were early and late scans, the spleen was seen somewhat better on the early one. The heart (presumably myocardium) was believed seen in 20 patients, but was never very prominent. In five patients the early scans showed distinct localization in the proximal portions of the veins of injection (not at sites of injection) extending into the larger draining vessels. It was conjectured that this could be temporary deposition in the walls of the vessels. The region of the salivary glands was excluded from the scan in many instances, but when the lower part of the head was visualized, the nasopharynx was rather dense, and the salivary glands were frequently but not invariably seen. Breasts never showed prominent uptake, but were seen in three of 15 female patients. The mediastinum was difficult to evaluate as separate from the heart, and in the patients with lung cancer frequently contained tumor; in about one-fourth of the patients it was thought to contain some normal accentuated activity in the absence of tumor.

Of the 38 patients, ten had a rounded zone of decreased activity, 4–10 cm in diam, in the left upper quadrant of the abdomen. In some instances this activity was as low as room background. We considered that these areas might represent air in the stomach, but we were puzzled by the degree of reduction of activity in view of the presence of posterior tissues and the high energy of the radiation. In one patient such a zone was seen in the upper part of the lower quadrant. As expected (*8*), no definite activity was visualized in the pancreas in contrast to the rat.

In seven patients the kidneys were seen, especially in the earliest scans (10–30 min), but never very prominently. There was definite visualization of the bladder in 18 patients out of 34 in whom the area was adequately scanned. Faint visualization of the genitalia was seen in one-third of the males and two-thirds of the females, when the areas were included in the scans. Distinctly low, homogeneous background activity characterized the lower abdominal region in 29 of 38 cases. Of the other nine, at least three had known or strongly suspected tumor in this region.

In several patients there was a faint band of activity, arching with its convexity cephalad, extending from the regions of the axillae to the sternoclavicular region. The significance of this band was not apparent (Fig. 4). No activity was seen in bone or marrow in any instance.

Areas of post-operative change and inflammation. We had little opportunity to evaluate areas of inflammation. In two instances concentration of [^{11}C] ACPC was slightly increased at the site of a previous mastectomy; in one there was faint "lung" uptake at the site of a right upper lobectomy done 10 yr previously. One patient had modest uptake in a hip joint with nonspecific arthritis. Another had severe osteoarthritis of the knee, prominent on a Tc-99m phosphate scan but negative on the [^{11}C] ACPC scan. In one patient with proven tuberculosis there was faintly positive uptake at the site of the lesion. In another with known tuberculosis and probable lung cancer, both types of lesion were believed visualized. In one patient who had had radiation therapy to submandibular lymph nodes and the salivary glands, the Ga-67 citrate scan was strongly positive, as has been reported in other cases of radiation sialitis (*11*), but the [^{11}C] ACPC showed no concentration. Another patient had a positive Ga-67 scan shortly

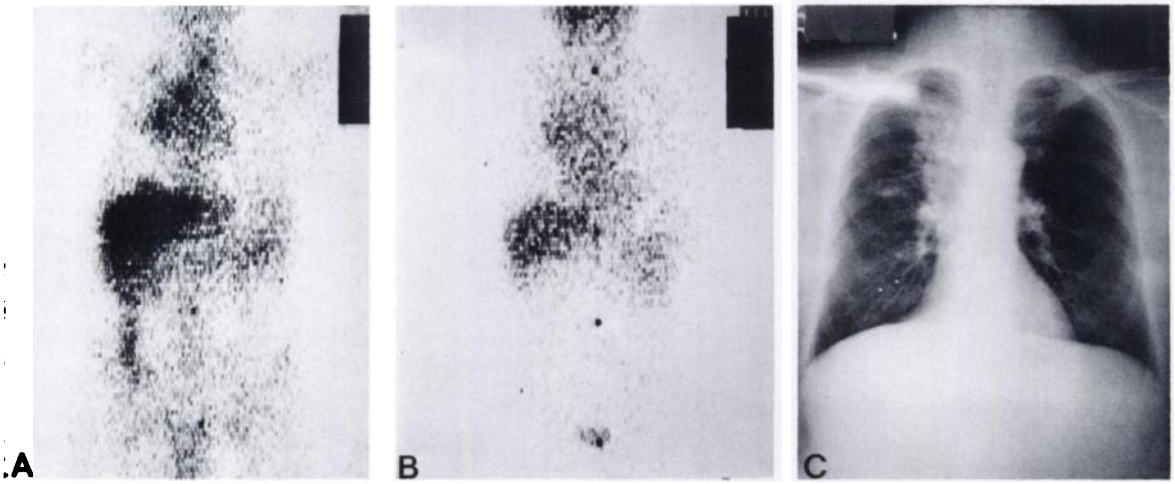

FIG. 1. Anterior ^{67}Ga citrate (A) and [^{11}C] ACPC (B) scans and chest x-ray (C) in patient with carcinoma involving mediastinum and upper lobe of right lung. Gallium shows lesions with greater clarity. [^{11}C] ACPC in addition shows heart. Both scans suggest splenomegaly. Note low activity in lower abdomen with [^{11}C] ACPC except for urinary bladder. (Both scans enhanced by computer technique.)

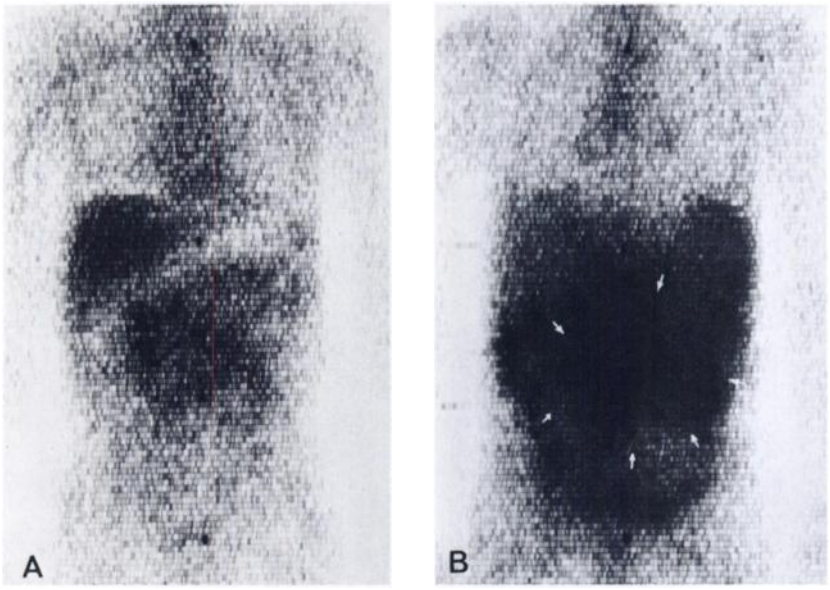

FIG. 2. Anterior scans on 55-year-old patient with non-Hodgkin lymphoma showing large abdominal mass. The [^{11}C] ACPC scan (A) shows vein of injection in patient's right arm, cardiac shadow just above the diaphragm, pale zone in left upper quadrant, and large mid-abdominal mass. Ga-67 scan (B) shows more uptake in tumor but size of lesions is obscured by activity in colon. Both scans (especially the Ga-67) show widening of mid-mediastinum unconfirmed by radiographic tomography.

after surgical removal of a group of lymph nodes; there was no concentration of [^{11}C] ACPC at the operative site.

Localization in tumors. Table 1 summarizes the results in patients scanned with [^{11}C] ACPC and Ga-67 citrate, and Figs. 1, 2, 3, and 4 show examples of [^{11}C] ACPC scans. A comparison with Ga-67 citrate was made, because the latter is a good all-purpose tumor-scanning agent and because a large group of our patients had lung cancer, in which this agent has been proven valuable (*12,13*). In general, the [^{11}C] ACPC showed a definite tendency to deposit in known malignant lesions, but, the clarity of visualization was generally poorer with the [^{11}C] ACPC. The Ga-67 showed a total of 50 lesions, whereas the [^{11}C] ACPC showed only 30; nevertheless, the number of patients with totally normal [^{11}C] ACPC scans and abnormal Ga-67 scans was only four. On the other hand, there were three lesions called positive on the [^{11}C] ACPC scan (not yet confirmed histologically) and not seen on the Ga-67 scans. One was in cancer of the lung, one in cancer of the breast, and one in the ameloblastoma. An additional case of extensive carcinoma of the lower abdomen was seen well with the [^{11}C] ACPC, but uncertain with the Ga-67 because of bowel interference.

Five additional patients were scanned with [^{11}C] ACPC but not with Ga-67. Three with cancer of the breast, post therapy had normal scans; one was presumably free of disease, and two had abnormal bone scans not paralleled by the [^{11}C] ACPC scans. A patient with carcinoma of the prostate post operatively had a normal bone scan and [^{11}C] ACPC scan. A single patient with inoperable cancer of the pancreas showed a liver metastasis as a cold spot with [^{11}C] ACPC.

DISCUSSION

This study has shown that [^{11}C] ACPC can be administered to patients without any early or late reactions and without changes in standard hematologic or clinical-chemical test values. Adequate doses for imaging can be given without exceeding acceptable radiation levels for diagnostic procedures.

Localization in several types of malignant neoplasms was clearly demonstrated and occurred very rapidly. In 13 patients who were scanned twice within 65 min, the tumor image was never better in the second scan, and in three instances was fainter,

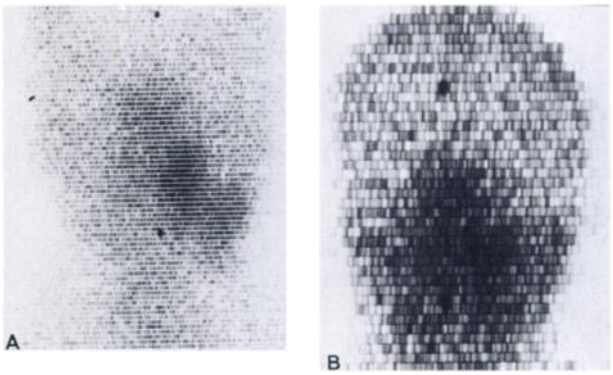

FIG. 3. Anterior view of head of patient with treated Hodgkin's disease showing, in the Ga-67 scan (A), evidence of radiation sialitis on the left but symmetrical salivary-gland and nasopharyngeal uptake of [^{11}C] ACPC (B).

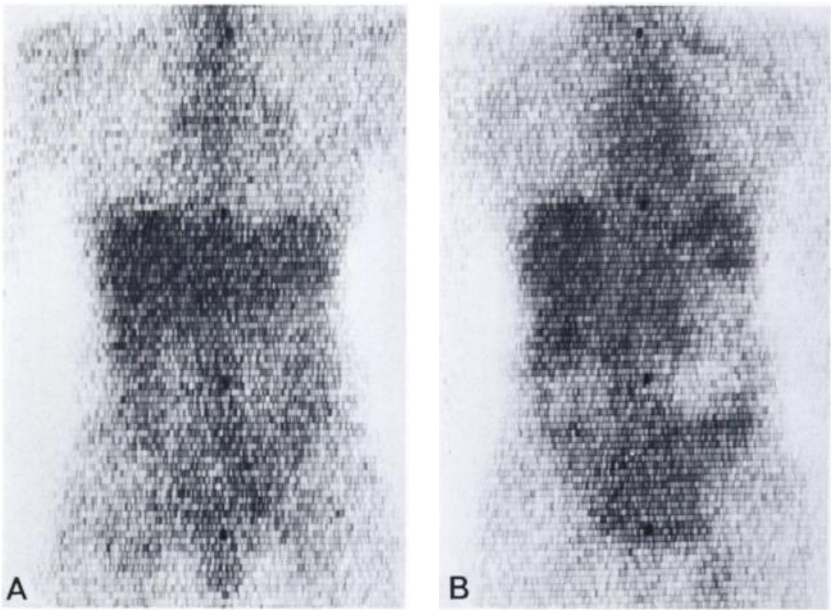

FIG. 4. Ga-67 citrate (A) and [^{11}C] ACPC scan (B) in patient proven to have metastatic adenocarcinoma, primary site unknown. Lower abdomen is believed to contain extensive tumor, although it was not easily palpable. The abnormality there is more definite in [^{11}C] ACPC scan, in part because normal colon activity in Ga-67 view would need to be considered. In the [^{11}C] ACPC, "pale zone" is much lower than usual. Both scans suggest perihilar abnormality, especially the one made after Ga-67; this area is somewhat obscured by cardiac activity in [^{11}C] ACPC scan. In the latter scan, activity seen in region of left clavicle and axilla is most likely due to artifact; that in left upper thigh may result from biopsy of left femoral node. [^{11}C] ACPC shows typical views of upper extremities.

this was not because of decay, since all counting statistics were adequate. Although the number of cases included in this "gamma mode" investigation is small, the results indicate that in general Ga-67 citrate is definitely superior to [^{11}C] ACPC as a tumor-localizing agent, both in terms of numbers of lesions found and in the target-to-nontarget ratios observed (Fig. 1). In three instances, however, [^{11}C] ACPC showed apparent lesions not detected with Ga-67 citrate; unfortunately, none of these has yet been confirmed histologically. In an additional case, an extensive abdominal tumor was clearly shown by the [^{11}C] ACPC, whereas the Ga-67 scan was difficult to interpret because of uncertainty about intestinal activity. It appears that [^{11}C] ACPC could be useful in searching for and evaluating lesions in the lower abdomen where Ga-67 is often unsatisfactory.

The normal distribution of [^{11}C] ACPC includes a prominent liver image, uptake in the salivary glands and nasopharynx, but is faint and variable in spleen, myocardium, and kidney. Bone and marrow were never seen, and the background activity of soft tissues in torso and extremities was very homogeneous.

In a very limited experience, infectious lesions and areas of previous surgery were visualized faintly. At least two inflammatory lesions showed no concentration of [^{11}C] ACPC. It is too early to conjecture on whether this preparation might help differentiate neoplastic from nonneoplastic processes.

The present study is based on scans made in the single-photon detection mode. There is strong reason to believe that when new instruments (involving positron computerized axial tomography) are available to take advantage of the positron emission, greatly improved volume of interest-specificity (*14*) should result.

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FOOTNOTES

* Oak Ridge National Laboratory, Oak Ridge, Tenn.

‡ Limulus amebocyte lysate, Difco Laboratories, Detroit, Mich.

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