Imaging of Spontaneous Canine Tumors with Ammonia and L-Glutamine Labeled with N-13

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Ammonia and L-glutamine, labeled with N-13, were tested as imaging agents for a variety of spontaneous canine tumors. The imaging capabilities of these compounds were compared with each other, with other scanning agents, and with radiologic and pathologic procedures. Good agreement between positive gamma images and postmortem findings occurred in 11 of 15 cases with [^{13}N] ammonia as the localizing agent. Eight of the nine scans using [^{13}N] glutamine as the imaging agent showed positive correlation with postmortem findings. In cases where both ammonia and glutamine were used to image the same lesion, no qualitative differences in tumor uptake were found between the two.

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Radioactively labeled amino acids are incorporated into some tumors more than into normal tissue (*1,2*). Amino acids labeled with an appropriate tracer for external visualization may thus be useful as tumor-seeking agents. Indeed selenomethionine labeled with selenium-75 has been reported to have an affinity for a wide variety of tumors (*3*). The long half-life of this nuclide, and the consequent radiation dose, limit the amount of activity that can be administered, thus reducing the scanning sensitivity and effectiveness of this agent. The recent development of procedures for enzymatically synthesizing amino acids labeled with short-lived positron-emitting radionuclides—e.g. 10-min N-13 and 20.4-min C-11—has resulted in several studies testing their effectiveness as tumor-visualizing agents. The significance of such studies is emphasized by the presence of nitrogen and carbon in all amino acids, as well as by the desirable decay properties of N-13 and C-11, which may permit quantitative measurement and tomographic visualization of tissues at low radiation exposure to the patient.

Carbon-11-labeled aspartic acid was used to image an implanted Walker carcinoma in the thigh of a rat (*4*). Implants of Morris hepatoma in the flank of a rat were well visualized 30–40 min after injection of [^{13}N] glutamic acid or [^{13}N] ammonia (*5*). One hour after injection of [^{13}N] glutamic acid, the N-13 concentration in the tumor averaged 70% of that in the liver. In a study comparing tumor uptake of [^{13}N] glutamine, [^{13}N] glutamic acid, and [^{13}N] ammonia in several types of implanted mouse tumors, Spolter, et al. (*6*) reported that [^{13}N] glutamine showed good tumor localization, which varied from 53% of liver uptake in mice with fibrosarcoma to 123% of liver uptake in mice with polyoma. [^{13}N] glutamic acid and $^{13}NH_3$, however, were concentrated only 20% as much as in liver. The authors suggest that [^{13}N] glutamine is incorporated at a greater rate because of a relative lack of the enzyme glutamine synthetase in the implanted tumors.

We have been studying the abilities of N-13-labeled compounds to image spontaneous tumors in

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dogs. The similarities between dog and man in classification and behavior of spontaneous neoplasia, and in the frequency of like response to therapy, suggest that our approach is superior to implant studies in the rodent as a model for evaluating tumor-scanning agents (*7,8*). Our earlier communications have described the imaging of a thyroid carcinoma with [^{13}N] ammonia (*9*) as well as studies in a few spontaneous dog tumors using [^{13}N] ammonia and [^{13}N] glutamine as the scanning agents (*10*). The present investigation extends these studies to a survey of a larger variety of spontaneous tumors in dogs, and compares [^{13}N] glutamine and [^{13}N] ammonia with each other as visualizing agents, and with other tumor-seeking agents as well as with radiographic and pathologic findings.

MATERIALS AND METHODS

The experimental models for these studies were dogs with spontaneously occurring neoplasms. These were selected from the canine case load in the Animal Medical Center's Section of Oncology. Each dog was injected intravenously with approximately 10 mCi of [^{13}N]-tagged ammonia or glutamine. Tumor imaging was begun 5 min after injection. Scintiscans were obtained using a scintillation camera with a high-energy collimator (*11*), and a dual-detector rectilinear scanner equipped with collimators whose response (sensitivity and resolution) is essentially constant for sources at various depths in tissue (*12*). Scan data were stored on magnetic tape and processed in a computer. Scintiscans were displayed in a 64 $\times$ 64 array on a storage oscilloscope in dot-density-modulated fashion using 16 equally spaced count levels. To allow areas of low counts to be displayed where regions of high activity lay within the camera's field of view, the above density scale was recycled to a 32-level presentation.

In all cases radionuclide images were checked against pathologic, radiographic, and postmortem findings. In one case, a comparative scan was carried out with gallium-67-citrate, in which case 2 mCi of the tracer were injected intravenously 3 days before the scan. In several cases with neoplasia involving bone, Tc-99m polyphosphate was used as a comparison agent (5 mCi, injected 2–3 hr before scanning).

[^{13}N] ammonia was produced with the Sloan–Kettering cyclotron either by deuteron bombardment of methane gas (*13*) or by irradiation of water with protons and the subsequent reduction of the generated [^{13}N] nitric acid. The [^{13}N] ammonia was collected in a saline solution for intravenous injection, or in a buffer solution for enzymatic conversion to [^{13}N] glutamine. We have reported the details of the synthetic procedure, including organ-distribution studies of the N-13-labeled compounds in normal dogs (*14*).

RESULTS

Table 1 summarizes the results of 15 cases of canine neoplasia that were scanned with [^{13}N] ammonia. A positive scan indicates visible uptake of radioactive label in the tumor area. Good agreement between a positive scan and postmortem findings were seen in 11 of the 15 cases. Two of the scans were inconclusive because the lesion occurred in an area of high [^{13}N] ammonia uptake in a normal dog (heart in dog 6 and liver in dog 7) and the tumor was therefore obscured by label in that organ. In one case of osteosarcoma (dog 13) the primary lesion was well visualized in the distal radius but a false-positive diagnosis of metastasis to the humerus was made. In one case of colonic adenocarcinoma (dog 10) neither primary nor metastatic lesions could be visualized. In the case where [^{13}N] ammonia was compared with Ga-67 citrate (dog 2), both of the compounds were taken up in the primary tumor as well as in a large axillary metastasis. A lung metastasis, however, was not visible with [^{13}N] ammonia because of high myocardial activity. The uptake of gallium in the heart was minimal and this permitted better visualization of the lung fields. The inhomogeneous distribution of [^{13}N] ammonia in the liver, with filling defects in particular areas of the organ, suggested the possibility of space-occupying disease. To test this further 2 mCi of Tc-99m sulfur colloid was injected and the liver was scanned. Voids in uptake of this agent in the liver gave further indication of liver involvement.

Results of the [^{13}N] glutamine studies are summarized in Table 2. Eight of the nine glutamine scans showed positive correlation with postmortem findings. A normal scan was obtained in a case of osteosarcoma of the proximal humerus (dog 8, Table 2). Three dogs were scanned with [^{13}N] glutamine and Tc-99m polyphosphate. In two of the cases (dogs 5 and 9, Table 2), the labeled amino acid imaged as well as Tc-99m polyphosphate. In the third case [^{13}N] glutamine was not taken up in the tumor although Tc-99m polyphosphate imaged the tumor satisfactorily. In those cases where [^{13}N] ammonia and [^{13}N] glutamine were compared as scanning agents in the same animal, the tumor's uptake of the [^{13}N] compounds was similar. The difference in effectiveness between [^{13}N] ammonia and [^{13}N] glutamine as tumor-scanning agents was based on differences in the background contributed by normal organs: [^{13}N] ammonia is taken up in the myocar-

TABLE 1. RESULTS OF DOGS SCANNED WITH [^{13}N] AMMONIA

Case	Roentgenograms	[^{13}N] ammonia scan findings	Other agents used	Postmortem	Tumor type	Correlation of $^{13}NH_3$ and post
1. Mix F, 9 yr	Soft tissue mass, cervical area	Uptake area of thyroid; chest not scanned	None	Yes	Thyroid adenocarcinoma	+
2. Germ. shepherd F, 8 yr	Large mammary mass with axillary and lung masses	Uptake in mammary mass and chest, voids in liver	Ga-67 citrate, Tc-99m sulfur colloid	No; surgical biopsy	Mammary adenocarcinoma	+
3. Mix F, 14 yr	Large soft-tissue mass on lip	Uptake in oral area and corresponding mandibular lymph nodes	None	Yes	Lymphosarcoma on lip and mandibular lymph node	+
4. Mix F, 11 yr	Undefinable uniform density in chest	Uptake in chest with definite nodule, anterior thorax	None	Yes	Mesothelioma throughout chest	+
5. Bull mast M, 5 yr	Mass, proximal tibia	Uptake in prox. tibia and sublumbar area	None	Yes	Rhabdomyosarcoma	+
6. Kerry blue F, 11 yr	Chest nodules; prev. amputation	Questionable uptake in chest	None	Yes	Osteosarcoma metastasis in chest	Inconclusive
7. Germ. shepherd M, 8 yr	Enlarged spleen	Questionable uptake in area of spleen	None	Yes	Lymphosarcoma of spleen	Inconclusive
8. Mix F, 11 yr	Mammary and thoracic masses	Uptake in chest and mammary glands	None	Yes	Mammary adenocarcinoma with pulmonary metastasis	+
9. Doberman F, 13 yr	Large soft-tissue mass off cranium	Uptake on cranium	None	Yes	Myosarcoma and small mammary adenocarcinoma	+
10. Mix M, 13 yr	Sublumbar tumor with lung masses	Uptake in posterior abdomen and chest	None	Yes	Colon adenocarcinoma with lung metastasis	—
11. Mix M, 7 yr	Enlarged spleen	Uptake in prescapular, anterior abdominal, and mandibular lymph nodes	[^{13}N] glutamine	Yes	Generalized lymphosarcoma	+
12. Mix F	Soft-tissue mammary mass	Uptake in inguinal mammary area	[^{13}N] glutamine	Yes	Mammary adenocarcinoma	+
13. Germ. shepherd M, 2 yr	Osteogenic tumor, distal radius	Uptake in distal radius and distal humerus	[^{13}N] glutamine, Tc-99m polyphosphate	Yes	Osteogenic sarcoma of distal radius	False-positive
14. Germ. shepherd M, 10 yr	Lack of parenchymal detail anterior right abdomen with lung masses	Uptake in lumbar area, chest, and abdomen	[^{13}N] glutamine	Yes	Hemangiosarcoma in lumbar muscles, spleen, liver, and lung	+
15. Beagle M, 8 yr	Generalized lymphadenopathy	Uptake in peripheral lymph nodes	[^{13}N] glutamine	Yes	Generalized lymphosarcoma	+

dium of the dog while [^{13}N] glutamine is not (*14*). Thus in Case 4, Table 2, diagnosed as generalized lymphosarcoma, the caudal mediastinum was partially obscured by the high uptake of [^{13}N] ammonia in the myocardium (Fig. 2A). (The anatomic areas in the scintiscans of this and the succeeding 2 cases are sketched in Fig. 1.) Figure 2B demonstrates improved mediastinal visualization when the tracer is [^{13}N] glutamine because of lack of masking activity in the heart region. Both compounds are also taken

TABLE 2. RESULTS OF DOGS SCANNED WITH [^{13}N] GLUTAMINE

Case	Roentgenograms	[^{13}N] glutamine scan findings	Other agents used	Postmortem	Tumor type	Correlation of [^{13}N] glutamine or surgical biopsy
1. Poodle M, 9 yr	Mammary mass with lung masses	Uptake in mammary tumor and chest	None	No	Mammary adenocarcinoma with lung metastasis	+
2. Mix M, 9 yr	Enlarged spleen	Uptake in mandibular lymph node	$^{13}NH_3$	Yes	Generalized lymphosarcoma	+
3. Germ. shepherd M, 10 yr	Enlarged spleen and liver	Uptake, mandibular and femoral lymph nodes; questionably enlarged spleen	None	Yes	Generalized lymphosarcoma	+
4. Mix F	Soft-tissue mammary mass	Uptake in inguinal mammary area	$^{13}NH_3$	Yes	Mammary adenocarcinoma; adrenocortical adenoma	+
5. Germ. shepherd M, 2 yr	Osteogenic tumor, distal radius	Uptake in distal radius	$^{13}NH_3$; Tc-99m polyphosphate	Yes	Osteogenic sarcoma of distal radius	+
6. Germ. shepherd M, 10 yr	Lack of parenchymal detail anterior right abdomen with lung masses	Uptake in dorsal lumbar area, spleen, and chest	$^{13}NH_3$	Yes	Hemangiosarcoma, in lumbar muscles, spleen, liver, and lung	+
7. Beagle M, 8 yr	Generalized lymphadenopathy	Uptake in sternal, mediastinal, and prescapular lymph nodes	$^{13}NH_3$	Yes	Generalized lymphosarcoma involving spleen, liver, lung, lymph nodes	+
8. Labrador M, 2 yr	Bony erosion of proximal humerus	Slight uptake in shoulder area	Tc-99m polyphosphate	No	Osteogenic sarcoma of proximal humerus	−
9. St. Bernard F, 3 yr	Large bony erosion of distal radius	Uptake in distal radius	Tc-99m polyphosphate	No	Osteogenic sarcoma of distal radius	+

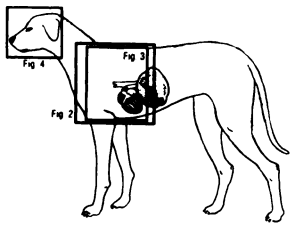

FIG. 1. Sketch of representative dog, outlining anatomic areas that were imaged in scintiscans of the succeeding figures.

up in the liver and would thus not be effective in imaging tumors in that area.

A comparison between radiographic, scintigraphic, and postmortem results can be seen in Fig. 3. An 11-year-old female dog of mixed breeding (Case 3, Table 1) was admitted with a history of gradual onset of dyspnea which had become most severe over the previous 24 hr. Radiographic studies of the chest showed an accumulation of fluid which obscured the detail of the thoracic structures (Fig. 3A). The lungs could be seen dorsally in the thorax and were almost completely collapsed. [^{13}N] ammonia was injected and camera scintiscans of the same area (Fig. 3B) showed displacement of the heart posteriorly and an apparent mass in the anterior mediastinal area. The entire chest showed abnormally high concentration of the tracer. On the basis of these scintiscans, a tentative diagnosis of thoracic neoplasia could be made. Necropsy (Fig. 3C) following euthanasia revealed a large mesothelioma with pleural, pulmonary, mediastinal, and pericardial involvement. This extensive mass had caused the lung lobes to collapse and had pushed the heart back in the thorax. A large component of the mass extended into the anterior mediastinum. The scintigrams with [^{13}N] ammonia allowed more complete visualization of the thoracic disease than did the radiographs.

A third representative case is that of a 10-year-old

German shepherd dog with generalized lymphosarcoma (Case 3, Table 2). Peripheral lymph nodes were enlarged, as were the liver and spleen. Figure 4 shows a lateral camera image of the dog's head. There is increased uptake of [^{13}N]-labeled glutamine in the mandibular lymph nodes, just below the normally visualized salivary gland. Necropsy confirmed lymphosarcoma of the mandibular lymph nodes.

DISCUSSION

Several reports dealing with amino acids labeled with short-lived nuclides have described the increased uptake in implanted tumors of rodents (*4–6*). Spontaneous tumors in dogs were used as our experimental material because scanning studies with a large animal more closely simulate technical procedures involving clinical conditions. Furthermore, spontaneous canine tumors were thought to resemble human neoplasia more closely with respect to growth and development. Thus the results that have been obtained by imaging the canine tumors with [^{13}N]-tagged ammonia and glutamine are encouraging. The rapid blood clearance of these labeled compounds (*14*) indicates that this imaging procedure may prove useful for rapid diagnosis—particularly when compared with gallium-67, where imaging is usually carried out 2–3 days after administration.

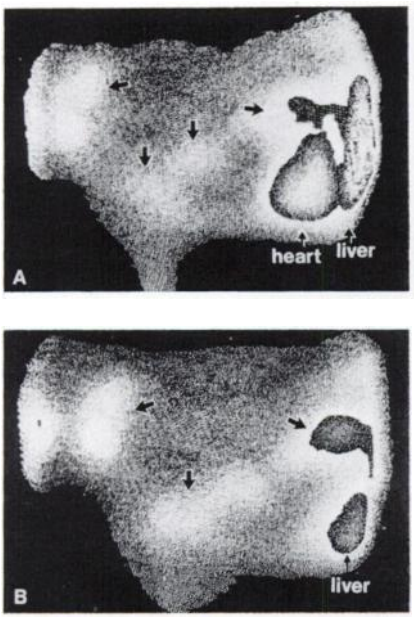

FIG. 2. Effect of normal organ distribution of [^{13}N] ammonia and [^{13}N] glutamine on tumor-imaging abilities of these labeled compounds. Storage oscilloscope is used, with a dot-density-modulated display in which sixteen levels of contrast are available. Increasing whiteness indicates increasing radioactivity. When level of contrast greater than sixteen is required, the levels will recycle. (A) [^{13}N] ammonia scintiscan of lateral thoracic region (Case 4, Table 2). Anterior part of chest is to left. Areas with highest uptake are heart and liver (narrow arrows) which have recycled. Increased activity is also seen toward the neck, as well as in the midchest regions (wide arrows). Postmortem examination confirmed these areas of uptake as tumors. (B) [^{13}N] glutamine scintiscan of the same area in same dog. Absence of overlying activity in the heart region enhances the image of the mediastinal involvement seen in Fig. 2A.

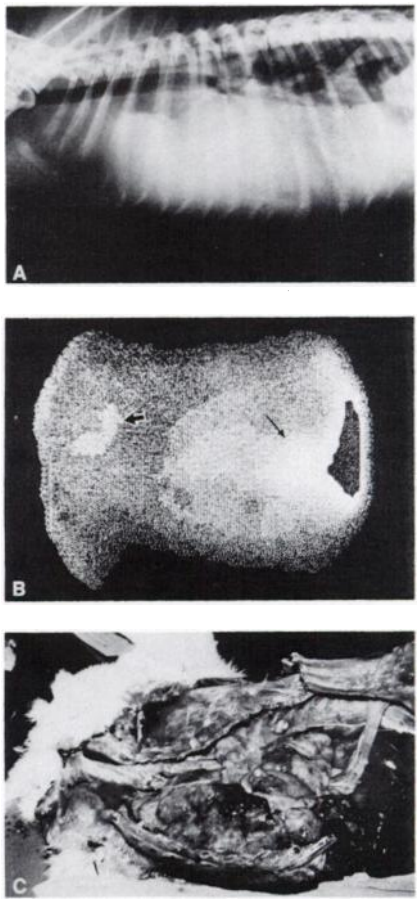

FIG. 3. Comparison between radiographic, scintigraphic, and postmortem results of a case of thoracic neoplasia (Case 4, Table 1). Radiographic studies (A) of this dog showed pleural effusion that obscured detail of thoracic structures and caused partial lung collapse. (B) Scintiscan of area 10 min after injection of 10 mCi of [^{13}N] ammonia. There is general increase of N-13 activity in thoracic cavity, displacement of heart caudally in thorax (narrow arrow), and dense activity in anterior mediastinum (wide arrow). (C) Postmortem photograph showing posteriorly displaced heart caused by a large diffuse mesothelioma occupying much of the chest.

The random selection of the type and anatomic location of these tumors has allowed evaluation of a wide variety of neoplastic lesions. However, few cases of each individual type of tumor could be collected, and thus definitive conclusions as to specificity of these agents in imaging particular tumors cannot be made. In general, enlarged lymph nodes and other organomegaly associated with lymphosarcoma, as well as mammary adenocarcinoma and its metastases, were consistently well visualized with [^{13}N]-tagged ammonia and glutamine. Primary and metastatic bone tumors, however, were inconsistently visualized with both agents.

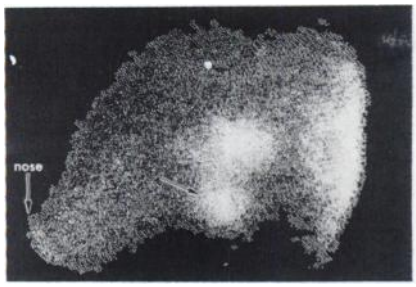

FIG. 4. Lateral camera scintiscan of head of a dog with generalized lymphosarcoma (Case 3, Table 2). There is uptake of [^{13}N] glutamine in the mandibular lymph nodes (arrow) below the salivary gland.

The choice between the two labeled compounds as potential tumor-imaging agents should be based primarily on the organ distribution of the compound in the normal animal. In the dog, [^{13}N] ammonia is taken up by such organs as the liver, brain, kidney, heart, salivary glands, and urinary bladder. [^{13}N] glutamine is heavily concentrated in the liver (*14*), and is also taken up in the kidney and bladder. Thus glutamine proves to be more valuable in thoracic imaging because of the absence of the cardiac uptake that occurs after [^{13}N] ammonia injection.

In those cases where both agents were used to scan tumors, no qualitative differences were found in imaging properties between [^{13}N] ammonia and glutamine. Thus the present study did not reveal specific types of spontaneous canine tumors that preferentially take up [^{13}N] glutamine, as had been reported for implant tumors of mice (*6*). Such selective imaging of tumors by [^{13}N] glutamine, if present, might provide an in vivo means of distinguishing neoplastic cells that lack the ability to synthesize glutamine, and hence might be responsive to glutaminase therapy. The mechanism by which these [^{13}N]-labeled compounds are rapidly taken up in tumors is not known, and uptake of [^{13}N] ammonia into tumors by ionic exchange or by incorporation into metabolites other than glutamine might obscure the inability of a specific tumor to synthesize glutamine. Our current research is directed toward a more extensive survey of tumor imaging with [^{13}N] glutamine as well as other [^{13}N]-labeled amino acids, such as asparagine and glutamic acid, in order to detect qualitative biochemical deficiencies in tumors that could be exploited by enzyme therapy. A study describing [^{13}N] glutamic acid as a satisfactory imaging agent for tumors involving bone has been reported recently (*15*). In two of the four cases presented, [^{13}N] ammonia and glutamine were also evaluated. Ammonia did not image either case well, while glutamine produced a favorable image in only one case. The mechanism of this differential uptake in tumors involving bone is not yet understood.

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