Quantitative Autoradiography with Radiopharmaceuticals, Part 2: Applications in Radiopharmaceutical Research: Concise Communication

P. Som, Y. Yonekura, Z. H. Oster, M. A. Meyer, M. L. Pelletteri, J. S. Fowler, R. R. MacGregor, J. A. G. Russell, A. P. Wolf, I. Fand, W. P. McNally, and A. B. Brill

Brookhaven National Laboratory, Upton, New York, and State University of New York at Stony Brook, New York

We describe the application of macroautoradiography, a relatively simple, quantifiable method for the evaluation of positron-emitting and gamma-emitting radiopharmaceuticals. We have investigated the response properties of two types of film to positron (F-18) and negatron (C-14) emitters. Variations in the response of film to increasing film-to-source distance are described, along with the effects of different intensifying screens and mounting tape. Digitization of whole-body autoradiograms (WBARG) in small animals was performed by using a videodensitometry system (videocamera interfaced to a computer). Quantitation was derived from analysis of a series of step-wedge standards that covered the range of radioactivities in the sample. By using a close-up lens on the videocamera, a 2- by 2-cm field is digitized as a 128 × 128 array, each pixel representing 156 × 156 μm. The effect of chlorpromazine (CPZ) on glucose metabolism in mice was studied by giving C-14 2DG followed by CPZ and F-18 FDG in the same animal. Muscle activity decreased and brown-fat activity increased. The high spatial resolution of this technique enables quantification in structures as small as the basal ganglia in mice. The use of dual-nuclide ARG permits each animal to be its own control, which greatly increases the utility of this method.

J Nucl Med 24: 238–244, 1983

The increasing use of cyclotron-produced radionuclides for positron emission transaxial tomography requires the design and development of new radiopharmaceuticals that will provide quantitative metabolic information in normal and diseased states. Conventionally, the evaluation of a new radiopharmaceutical is performed by excision and direct counting of selected organs or portions of them. External imaging methods such as scintigraphy or tomographic scanning are also used. The disadvantages of sampling by excision are obvious. Since not all organs and tissues are sampled, accumulation of the compound in nonsampled sites will be missed. The distribution of the radiopharmaceutical may vary in the different histological components of the tissue included in the sample. Thus, information is spatially coarse and lacks details on the distribution of the tracer within an organ or within a diseased tissue. The limited spatial resolutions of the gamma camera or the positron-emission tomographic devices is also known, and they enable visualization of the whole body or organ uptake only on a global basis. With these techniques it is almost impossible to resolve discrete structures in a small organ.

Whole-body autoradiography (WBARG) was developed by Ullberg (*1*,*2*) and has since been refined and applied in many fields of biomedical research. The present-day technique permits ARG of small animals or whole organs of large animals, and can provide very-high-resolution images of the distribution of radiolabeled compounds (*3*). In addition, when whole-body sections

Received Aug. 10, 1982; revision accepted Nov. 1, 1982.

For reprints contact: P. Som, Medical Department, Brookhaven National Laboratory, Upton, NY 11973.

are studied, comprehensive information on the global distribution pattern can be obtained. Therefore, the study is not limited to certain preselected samples, but permits localization to be observed at unexpected sites in the various tissues represented in the ARG sections.

Double-tracer studies using this method allow the simultaneous delineation of the distribution of a radiopharmaceutical under development compared with a reference compound (labeled with C-14 or H-3) in the same animal. Moreover, by using double or multiple tracers of the same compound (for example, C-14 2-deoxy glucose and F-18 2-fluoro-2-deoxy-D-glucose) one could study in the same animal the effects of chemical, pharmacological, or physical interventions. The radiation dosimetry of a new compound can also be calculated from distribution data derived from whole-body autoradiograms.

In recent years, whole-body autoradiography has been used extensively in the field of pharmacology and toxicology to map the distribution patterns of various substances (*4–7*). Significant progress was made when these film images were quantified by using various types of manually operated densitometers (*8,9*). Although the spatial resolution of an ARG is far better than can be obtained in vivo by external detection (gamma cameras, positron-emission tomography), the procedures for quantitating ARGs are less well established. Computer-aided in vivo imaging systems in routine use provide useful numerical estimates of activity in different organs as well as quantitative changes of activity with time. In order to obtain similar information from autoradiography, it is essential to be able to quantify the distribution of activity in an ARG. By quantifying data from animals killed at different times after tracer injections, one can estimate the temporal kinetics of tracer distribution. Quantitation with special emphasis on positron-emitting radiopharmaceuticals was accomplished with a computerized videodensitometer (*10–12*). We describe the application of this method for short-lived positron emitters, as well as for gamma emitters and the more conventional beta emitters for simultaneous multiple-tracer studies.

MATERIALS AND METHODS

Animal studies. Radiopharmaceuticals were injected intravenously in Hale-Stoner strain BNL mice and the animals were killed by cervical dislocation. After freezing in liquid nitrogen, body hair was removed and the animals were embedded in methylcellulose. The cryomicrotome* used permitted the cutting of sections 5–200 μm thick through the whole body in mice and rats. The sections from C-14-injected mice were mounted on transparent tape. After freeze-drying for 3 days to prevent chemography, the sections were placed directly on x-ray film. Since long periods of freeze-drying are not feasible with short-lived nuclides, chemography was prevented in this case by placing the tape-mounted sections on the x-ray film with the tape side facing the film. Thus, the mounting tape served as a barrier between the section and the film. Intensifying screens were also used to compensate for the rapid decay of F-18 FDG.

Three sets of experiments were carried out: (a) single- and double-tracer experiments on the comparative distributions of C-14 2-deoxy-D-glucose (C-14 2DG) (2.5 μCi, specific activity 282 mCi/mmol) and of F-18 2-fluoro-2-deoxy-D-glucose (F-18 FDG) (800 μCi), synthesized by a method reported earlier (*13*); (b) uptake of C-14 2DG (2.5 μCi) in animals carrying a spontaneous transplantable mouse tumor of mammary origin; and (c) the study of glucose metabolism in sedated and nonsedated animals. This last was studied as follows: two groups of animals were given C-14 2DG, and 45 min later one group received a sedative dose (12 mg/kg) of chlorpromazine (CPZ) intraperitoneally, whereas the control group was given an equal volume of normal saline. Subsequently, both groups received F-18 FDG (800 μCi), and the animals were killed 45 min after the second injection. In a fourth experiment, the comparative spatial resolutions of a positron-emission tomographic system (PETT III) and the autoradiographic technique were studied by injecting a sheep with 5 mCi F-18 FDG. After imaging on the PETT III system, the animal was killed by overdose of barbiturate. The brain was sectioned (1 cm thick) for ARG (overnight exposure) and histology at the same levels as used for the PETT III sections.

Film response properties. We explored the response properties of a double-coated film XRP1† (X-OMAT) and a single-coated film SB5† to positron (F-18) and negatron (C-14) emitters as follows. Squares of filter paper were soaked in eight increasing dilutions of the standard F-18 solutions. The dried step-wedge standards were first counted in a well-type scintillation counter and subsequently sandwiched between the XRP1 and SB5 films for exposure to take place. The exposure time was four hours for F-18 FDG and 10 days for C-14 2DG. For C-14, commercial standards, precalibrated to 20 μm thickness were used. Mean optical density (O.D.) was determined with a densitometer and calculated from eight measurements for each dilution. Standard response curves were made for XRP1 and SB5 films by plotting the logarithm of the activity as abscissa and the optical density as ordinate. The same procedure was repeated using the commercially available blue intensifying screen lining the x-ray cassette in which exposure took place.

Effects of intensifying screens and scotch tape. In the set of experiments described below, the role of intensifying screens and the attenuation of Scotch tape were determined when using short-lived, positron-emitting radionuclides such as F-18. In this situation it will be beneficial to collect as many photons as possible in the

shortest time, which rules out the time-consuming drying of the sections. Chemography is, therefore, prevented by interposing a layer of Scotch tape between the section and the film. The effect of these two factors was determined by using a series of filter-paper strips, soaked in step-wedge dilutions of F-18, in the following manner:

a. With blue intensifying screen, but no Scotch tape.

b. With blue intensifying screen and Scotch tape.

c. No intensifying screen, Scotch tape interposed.

Double-tracer ARG. In double-emitter studies, discrimination between the radionuclides was made on the basis of their different physical half-lives. Sections containing F-18 and C-14 imaged for several hours following preparation will represent the distribution of F-18. Exposure after the decay of F-18 will depict the distribution of the longer-lived C-14.

Quantitation of autoradiograms. Autoradiograms were scanned with a videocamera‡ interfaced to a mini-computer. Digitized readings of light intensity from the WBARG were correlated with step-wedge standards of the different dilutions. A full description of the system is to be found in reference 14.

RESULTS

Film response characteristics. The response curves of two types of film to F-18 positrons are shown in Fig. 1. The response curve of XRP1 (X-OMAT) double-coated film has a steeper slope in the linear region compared with the single-coated SB5, indicating higher sensitivity of the former. This is true at high count rates, whereas at lower count rates the XRP1 response curve falls below the linear region of SB5, indicating lower sensitivity at these activity levels.

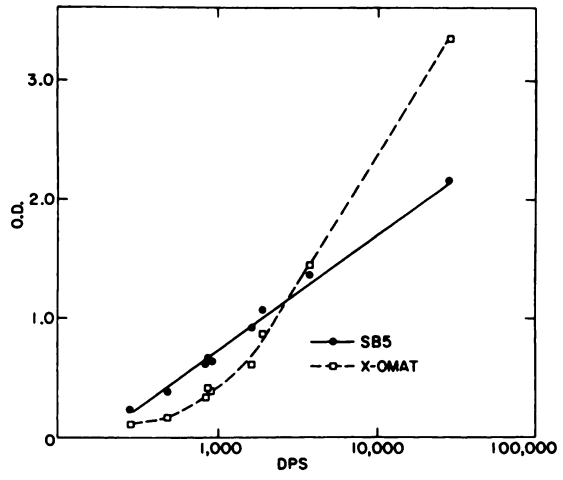

FIG. 1. Film response to positrons from F-18, plotted as mean optical density (O.D.), is shown as a function of number of disintegrations per second (DPS). Notice that, at higher count rates, XRP1 (X-OMAT) film is more sensitive than SB5, and that the opposite is true at lower count rates.

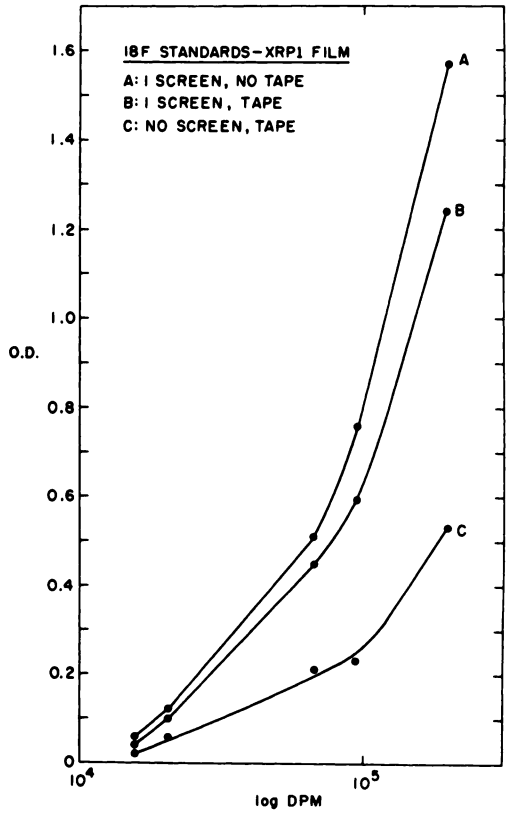

FIG. 2. Effects of intensifying screens are seen by comparing curve A with curve C. Small difference between curves A and B minimal indicates attenuating effect of Scotch tape.

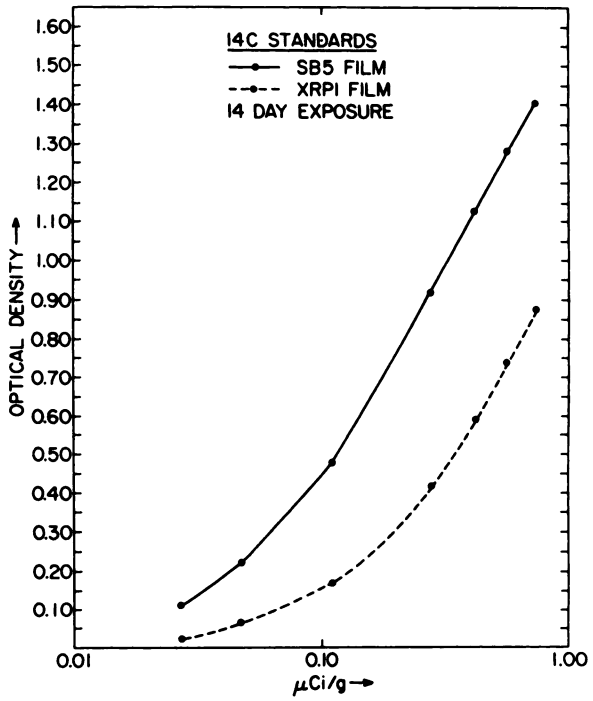

FIG. 3. Response of SB5 and XRP1 (X-OMAT) films (in O.D.) as a function of radioactivity (as μCi/g) of C-14 2DG step-wedge standards.

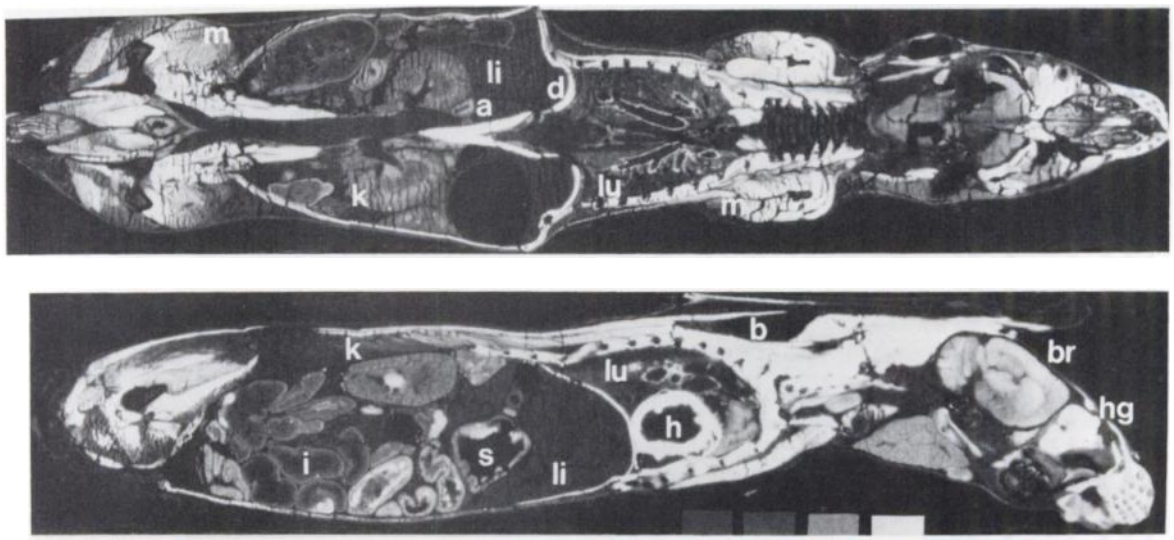

FIG. 4. Frontal section (above) and sagittal section (below) WBARG of mouse after C-14 2DG. Organs with high uptake are: heart (h), skeletal muscle (m), diaphragm (d), and Harder's gland (hg). Moderate uptake is seen in brain (br), upper part of stomach lining (s), kidneys (k), and adrenals (a). Lower uptake is found in liver (li) and lungs (lu). There is also high uptake in blood-vessel lining in thorax. Different loops of intestines (i) had variable uptake, some with more than most. No uptake is seen in brown fat pad (b).

Figure 2 shows the effects of intensifying screens and the attenuating effect of the Scotch tape used for mounting the tissue sections to prevent chemography with short-lived emitters. The difference between curves A and B is small, indicating that attenuation by Scotch tape is not very large for F-18. However, exposure to

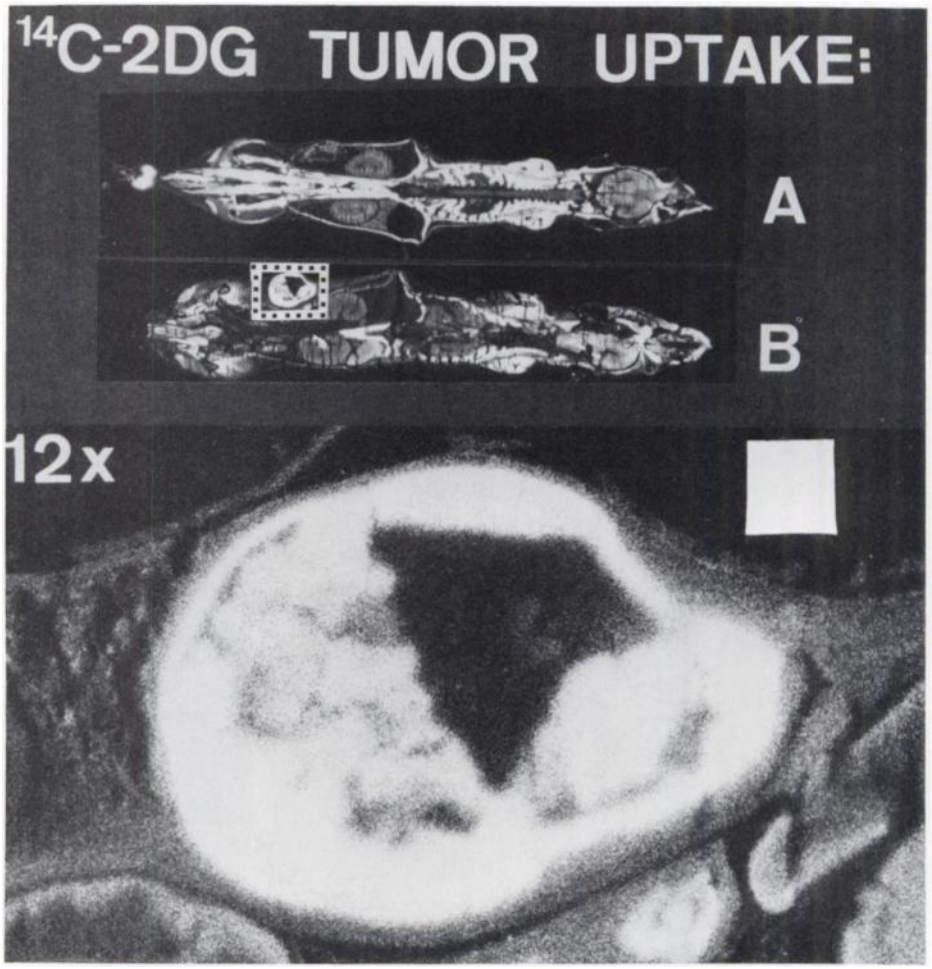

FIG. 5. Whole-body ARG showing the distribution of C-14 2DG in normal mouse (A) and tumor-bearing mouse (B), with magnified (×12) section of tumor at bottom. Notice lack of C-14 2DG uptake in center of necrotic area of tumor.

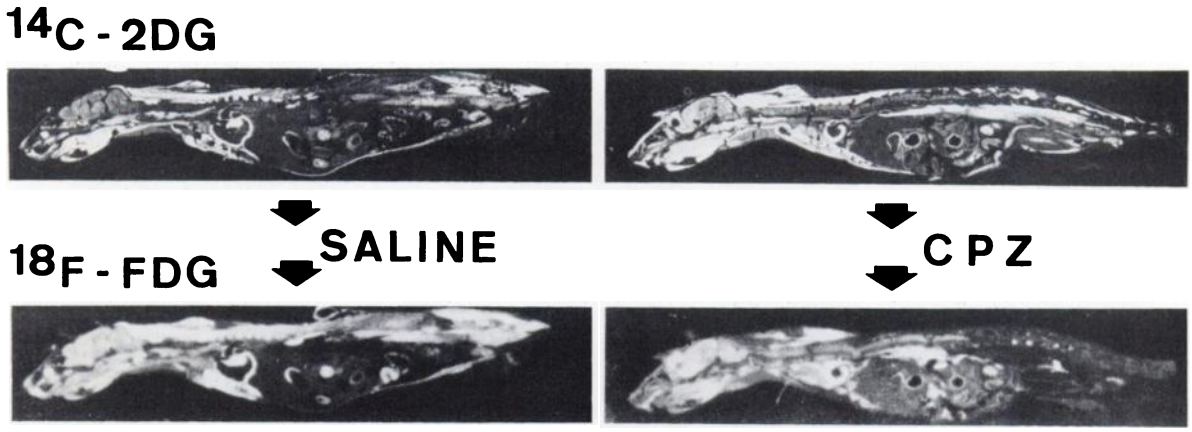

FIG. 6. Double-tracer studies with C-14 2DG and F-18 FDG, showing effect of CPZ. Note decrease in muscle uptake and increase in brown fat following CPZ sedation.

sections containing F-18 without the use of intensifying screens decreases the O.D. significantly, as seen in curve C. It seems therefore, that the use of intensifying screens is useful in autoradiography with short-lived positron emitters. It was found that a double-coated film (X-OMAT) in conjunction with a blue intensifying screen was most effective for the high-energy positron emissions of F-18. For the lower-energy negatron emissions from C-14, a single-coated film with no intensifying screen was suitable, providing improved sensitivity and resolution (see Fig. 3).

Animal studies. Figure 4 depicts sagittal and frontal whole-body ARG sections of mice containing C-14 2DG. Quantitative analysis of these autoradiograms revealed that the organs with highest concentration were the heart, Harder's gland, skeletal muscle including the diaphragm and bladder (the last not shown in these sections), and the brain (*14*). Harder's gland is a tan, crescent-shaped organ located in the orbit over the supero-medial aspect of the eyes. It is found in all vertebrates except primates, and occurs very rarely in man as a congenital anomaly (*15*). Organs showing moderate concentration of C-14 2DG were: some (but not all) intestinal loops, upper (glandular) portion of the stomach, adrenal cortex, and renal cortex. The distribution in the brain is not uniform. Cerebral and cerebellar cortices, thalamus, and basal ganglia contain more radioactivity than the hypothalamus and hippocampus. The liver, lungs, and vascular spaces had very low activity. Since it was shown earlier that F-18 FDG concentrates in a variety of tumors in animal models (*16*) and in humans (*17,18*), C-14 2DG whole-body autoradiograms of tumor-bearing mice were performed. The relative distributions of C-14 2DG in a normal animal, compared with an animal carrying tumor, are shown in Fig. 5, including a ×12 magnification of the tumor. Quantitative

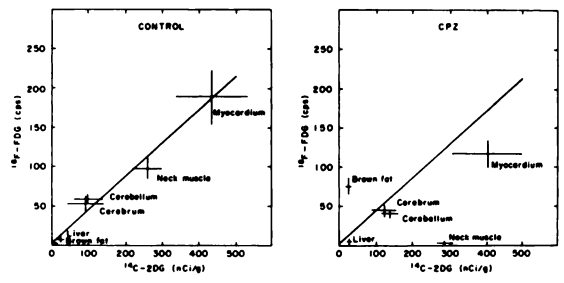

FIG. 7. Correlation of C-14 2DG and F-18 FDG distributions in chlorpromazine-sedated mice and controls, as calculations from digital videodensitometry of ARG (cf. Fig. 6).

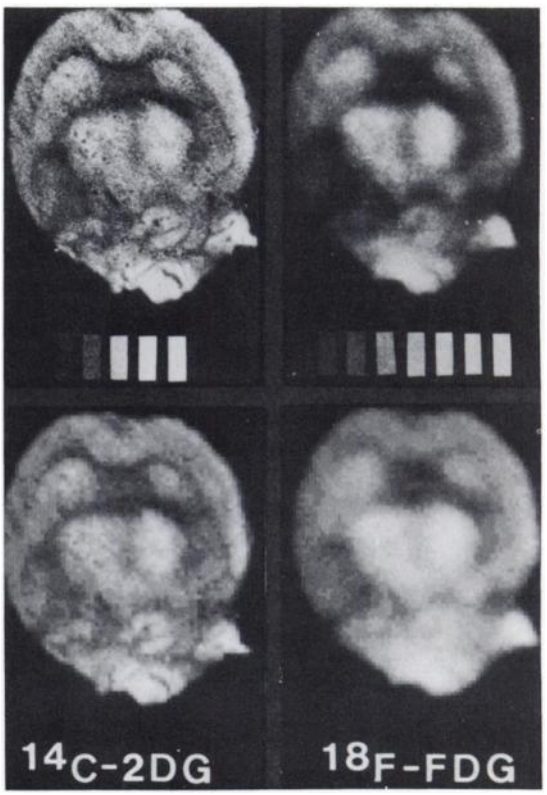

FIG. 8. Comparison of C-14 2DG and F-18 FDG in mice injected simultaneously with tracers and killed 30 min later. Upper panels show analog images with step-wedge standards; lower panels show digitized images. High uptake is seen in caudate, basal ganglia and gray matter.

digitized videodensitometry of different regions of tumors showed a concentration of 449 nCi/g in the viable tumor tissue compared with 188 nCi/g in the periphery of a necrotic area, and 25 nCi/g in the center of the necrotic area (Fig. 5).

Figure 6 shows the autoradiograms of two mice. Both were given C-14 2DG, which was followed in one by CPZ and in the other by saline; then both were given F-18 FDG. In the animal given a sedative dose of CPZ, a significant decrease in muscle and increase in brown fat activity can be seen (Fig. 7).

A comparison of simultaneous studies with C-14 2DG and F-18 FDG is shown in Fig. 8. In the mouse brain, the small details are visible and even the caudate nucleus, basal ganglia, and gray matter can be recognized.

DISCUSSION

Whole-body autoradiography (WBARG) as developed by Ullberg (*2*) has become a well-established method in biomedical research. It is particularly suitable for the study of diffusible substances, since the animal is quickly frozen in liquid nitrogen and the sections are dried. Thus new, metabolizable radiopharmaceuticals can be studied because diffusion is prevented promptly after the animal has been killed. In the design and evaluation of new radiopharmaceuticals, WBARG offers many advantages over routine studies of tissue distribution because no arbitrary organ sampling is necessary and the distribution of the radionuclide within and between organs can be identified because of the higher spatial resolution. Until recently measurements of the distribution of radioactivity was achieved by using spot measurements of optical density over a few locations in the tissue section and relating these readings to those from an autoradiogram of step-wedge dilutions of the standard solution (*19*). More recently a computer-assisted quantification method was described utilizing a rotating-drum digital densitometer (*20*). This method was applied only to rat brain ARG using C-14 2DG.

The quantification system used in this study consisted of a dedicated nuclear medicine computer to which a few relatively inexpensive video-input and digitizing components were added. The resolution of the whole system is 400–500 lines per image size (*14*), which is comparable to that of the more elaborate system used by Goochee et al. and is adequate for WBARG quantification (*20*). We could identify and delineate structures as small as the basal ganglia in the mouse, and the quantitative results after appropriate film calibration were reproducible and had low statistical spread (*14*). In contrast, when using an in-vivo imaging device (PETT III), no structural localization of F-I8 FDG could be seen even in the brain of an animal as large as the sheep.

By using digitized videodensitometry, quantitation of ARG can be performed, as shown in Table 1. Concentrations of C-14 2DG and F-18 FDG are highest in the frontal cortex, cerebellum, and thalamus. The relative distribution of the two compounds in the various brain structures is similar. The absolute values, however, cannot be directly compared because the reference standards for each compound were different. Carbon-14 2DG was calibrated with methylmethacrylate(C-14) (nCi/g) and F-18 FDG with filter-paper standards (cps).

The advantages of multiple-tracer studies, in which an animal can serve as its own control, are well established. The chlorpromazine experiment shows that with this method multiple-tracer experiments can be carried out. The administration of CPZ, with its sedating effect causing decreased movement in the animal, is followed by decreased glucose metabolism of skeletal muscle and increased uptake of glucose in the brown fat. It is known that norepinephrine stimulates brown-fat mitochondria to oxidize substrates and produce heat rather than ATP (*21*). Since CPZ decreases muscle activity and heat production, thermoregulation is maintained by heat produced in brown fat through increase in glucose uptake.

The WBARG technique not only enables one to register the total radioactivity in the body without loss or migration, but also permits one to determine the chemical form of the compound in various tissues. The extraction of part of the labeled compound from desired tissue sections, followed by their chromatographic separations, permits one to determine the various active

TABLE 1. CONCENTRATIONS OF C-14 2DG AND F-18 FDG IN MOUSE BRAIN*

Compound	Frontal cortex	Caudate nucleus	White matter	Thalamus	Cerebellum
C-14 2DG†	162.4 ± 15.4	145.7 ± 23.5	53.8 ± 13.9	183.4 ± 14.5	166.7 ± 25.7
F-18 FDG‡	143.9 ± 35.5	129.9 ± 19.1	53.2 ± 11.3	160.6 ± 10.4	148.8 ± 22.5
F-18/C-14	0.89	0.89	0.99	0.88	0.89

* Quantitation by digitized videodensitometry from double-isotope study shown in Fig. 8.
† Calibrated by methylmethacrylate standards (nCi/g).
‡ Calibrated by filter-paper standards (cps).

intermediates and metabolites in various tissue structures (*22*). Moreover, using "low temperature" ARG, it is possible to monitor the distribution of volatile and non-volatile metabolites in the different tissue compartments (*23*).

Preliminary experiments have shown that the method is also suitable for gamma emitters with various energies such as: I-131, I-125, Sn-117m, Tc-99m, and Tl-201 (*14,24-26*).

Quantitative WBARG has the potential to become a very useful tool in the development of new radiopharmaceuticals. It complements the existing methods and provides more comprehensive and detailed data on biodistribution, metabolic pathways, and radiation dosimetry.

FOOTNOTES

* LKB 2250 PMV cryomicrotome (PMV 450 MP).
† Eastman Kodak Company, Rochester, N.Y.
‡ Hamamatsu Systems, Inc., Waltham, MA.

ACKNOWLEDGMENTS

We thank Professor Sven Ullberg, Dept. of Toxicology, University of Uppsala, Sweden, for his helpful suggestions and discussions. The authors also thank Mr. D. Sacker for technical assistance and Mrs. E. Roche for secretarial help.

Research supported under the auspices of the US Department of Energy Contract #DE-AC02-76CH00016.

The US Government retains a non-exclusive royalty-free license to publish or reproduce the published form of this contribution or allow others to do so for US Government purposes.

REFERENCES

1. ULLBERG S: Studies on the distribution and fate of ^{35}S-labeled benzylpenicillin in the body. *Acta Radiol* (Suppl. 118): 1-110, 1954
2. ULLBERG S: Autoradiographic studies on the distribution of labelled drugs in the body. *Proc. Second UN Int. Conf. Peaceful Uses of Atomic Energy*, Geneva, 24:248-254, 1958
3. *Whole body autoradiography.* CG Curtis, SAM Cross, RJ McCullich, GM Powell, Eds. Academic Press, London, New York, Toronto, Sydney, San Francisco, 1981, pp 38-48
4. BERGMAN K: Whole-body autoradiography and allied tracer techniques in distribution and elimination studies of some organic solvents: Benzene, toluene, xylene, styrene, methylene chloride, chloroform, carbontetrachloride and trichloroethylene. *Scand J Work Environ Health* 5 (Suppl 1): 1-263, 1979
5. BERLIN M, ULLBERG S: Accumulation and retention of mercury in the mouse. *Arch Environ Health* 6:589-616, 1963
6. COHEN EN, HOOD N: Application of low temperature autoradiography to studies of the uptake and metabolism of volatile anesthetics in the mouse. *Anesthesiology* 30:306-314, 1969
7. BUSCH U: Whole body autoradiography (WBARG): Use for pilot studies of pharmacokinetics in rats. *Acta Pharmacol Toxicol* 41:28-29, 1977
8. CROSS SA, GROVES AD, HESSELBO T: A quantitative method for measuring radioactivity in tissues sectioned for whole-body autoradiography. *Int J Appl Radiat Isotop* 25: 381-386, 1974
9. MASUOKA DT, PFEIFFER E: An in vitro brain autoradiographic technique and a digital microdensitometer for the quantitation of autoradiograms. *Acta Pharmacol Toxicol* (Suppl. 1) 41:54-55, 1977
10. SOM P, MEYER M, BRILL AB, et al: Quantitative autoradiography with radiopharmaceuticals. *J Nucl Med* 22:P66, 1981
11. YONEKURA Y, MEYER M, BRILL AB, et al: Quantitative digital autoradiography by videodensitometry. *J Nucl Med* 22:P14, 1981
12. YONEKURA Y, BRILL AB, SOM P: Quantitative analysis of multiple isotope autoradiography. In *Proceedings of the Third World Cong. Nucl. Med. Biol.*, C. Raynaud, Ed., Vol IV, Paris, France, Pergamon Press, 1982, pp 3236-3239
13. FOWLER JS, MACGREGOR RR, WOLF AP, et al: A shielded synthesis system for production of 2-deoxy-2-(^{18}F)Fluoro-D-glucose. *J Nucl Med* 22:376-380, 1981
14. YONEKURA Y, BRILL AB, SOM P, et al: Quantitative autoradiography with radiopharmaceuticals. Part 1: Digital film analysis system by videodensitometry. *J Nucl Med* 24: 231-237, 1983
15. *The Anatomy of the Eye and Orbit.* E. Wolff, Ed. H.K. Lewis and Co., Ltd., 1954, p 459.
16. SOM P, ATKINS HL, BANDOPADHYAY D, et al: A fluorinated glucose analog, 2-fluoro-2-deoxy-D-glucose (F-18): Nontoxic tracer for rapid tumor detection. *J Nucl Med* 21: 670-675, 1980
17. YONEKURA Y, BENUA RS, BRILL AB, et al: 2-deoxy-2-(F-18)Fluoro-D-glucose accumulation in metastatic liver tumor. *J Nucl Med* 23:P8, 1982
18. PATRONAS NJ, DICHIRO G, BROOKS R, et al: Grading of cerebral gliomas by positron emission tomography (PET) using F-18 fluorodeoxyglucose (FDG). *J Nucl Med* 23:P6, 1982
19. IRONS RD, GROSS EA: Standardization and calibration of whole-body autoradiography for routine semiquantitative analysis of the distribution of ^{14}C-labeled compounds in animal tissues. *Tox Appl Pharm* 59:250-256, 1981
20. GOOCHEE C, RASBAND W, SOKOLOFF L: Computerized densitometry and color coding of (^{14}C) deoxyglucose autoradiographs. *Ann Neurol* 7:359-370, 1980
21. DANFORTH E, JR: Thermogenesis, obesity and thyroid hormones. In *Thyroid today.* J.H. Oppenheimer, Ed. Travenol Lab, Deerfield, Ill., 4(6):1-6, 1981
22. D'ARGY R: Tissue pieces from whole body sections used for chromatographic separation of metabolites. *Acta Pharmacol Toxicol* (Suppl 1) 41:16-17, 1977
23. RHODES C, LONGSHAW S: Autoradiographic distribution study of a short acting anaesthetic ICI 35, 868. *Acta Pharmacol Toxicol* (Suppl 1) 41:132-133, 1977
24. SOM P, YONEKURA Y, SRIVASTAVA SC, et al: Autoradiographic (ARG) and scintigraphic studies on regional distribution of N-isopropyl-p-iodo amphetamine (M-123 Acetate). *J Nucl Med* 23:P57, 1982
25. SOM P, SRIVASTAVA SC, OSTER ZH, et al: Autoradiographic studies of tin-containing compounds as potential bone agents. *J Nucl Med* 23:P78, 1982
26. OSTER ZH, SOM P. BRILL AB, et al: Enhancement of radiopharmaceutical excretion by chemical interventions. In *Proceedings of the Third World Congress of Nuclear Medicine Biology.* Raynaud C, Ed. Vol IV, Paris, France, Pergamon Press, 1982, 3257-3261