A computerized autoradiographic technique for the simultaneous high-resolution mapping of myocardial blood flow and metabolism

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By using both radioactive particulate and diffusible tracers, combined with computerized processing and analysis of generated autoradiographs, a new technique for the high-resolution mapping of organ blood flow and metabolism has been developed. In this paper the technique has been evaluated for the myocardium.

Introduction

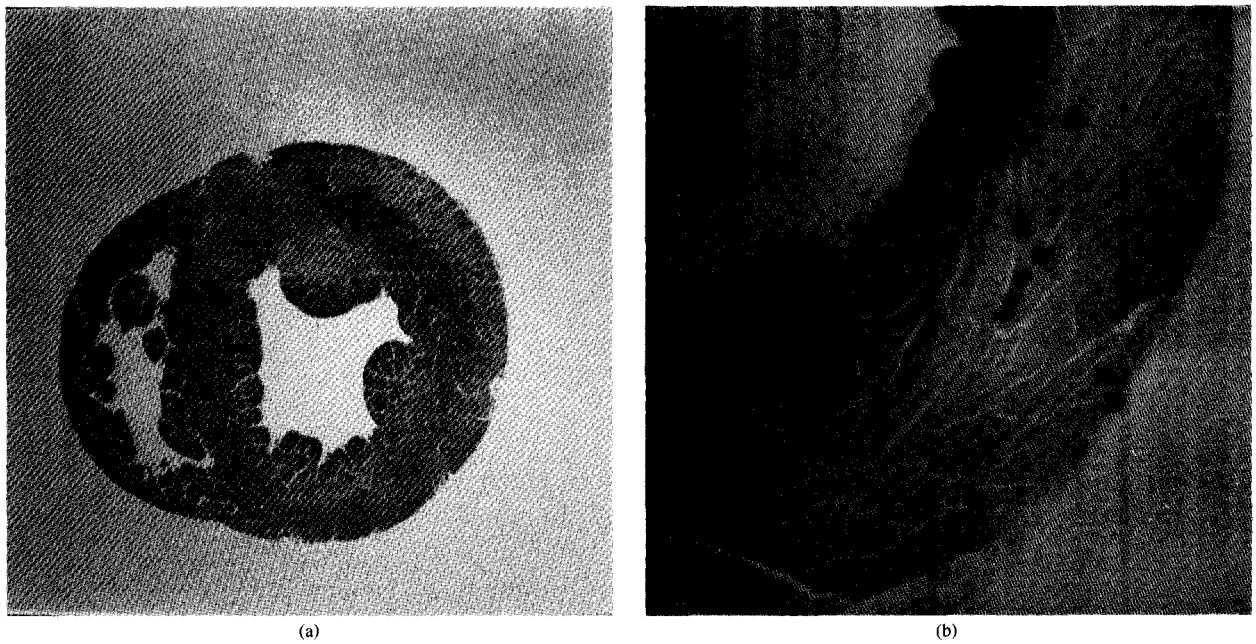
Myocardial blood flow distribution can be measured with radioactive microspheres using well-counting or autoradiographic techniques.

When using the conventional well-counting technique, more than one blood flow measurement can be performed simultaneously with different isotopes. However, only limited spatial resolution can be achieved because of the small number and specific activity of the microspheres and the relatively large size of the tissue specimen [1–5].

The conventional autoradiographic method yields higher resolution but is limited to only one blood flow distribution measurement because of the inability of the radiographic film to discriminate among isotopes of different energies.

Our group previously developed a multitracer autoradiographic technique for the simultaneous evaluation of myocardial metabolism and blood flow using particulate and nonparticulate tracers and isotopes with different half-lives [6]. In those studies only one type of particulate tracer was used.

The present study is intended to demonstrate the feasibility of discriminating among one diffusible and two particulate radioactive tracers in order to obtain a metabolic map in one specific hemodynamic state and two blood flow



(a) (b)

Figure 1

Digital image of an autoradiograph (a); different sizes of the two types of microspheres (b).

maps with high spatial resolution. We also wish to evaluate the possibility of quantifying absolute flow by autoradiographic assessment of microsphere content in reference blood samples.

Experimental method

A total of four to six million Gd-153- and Ce-141-labeled microspheres, 15 μm in diameter, were injected into the left atrium of an anesthetized experimental animal. This was almost double the quantities previously employed [1–3] and did not alter the systemic and coronary hemodynamics [2, 5] because of the relatively low number of beads compared with the estimated high number of vessels [7–9]. The two types of injected microspheres produced spots of different sizes on the autoradiographs.

First, Gd-153-labeled microspheres were injected into the left atrium. These microspheres enter the coronary circulation, as well as all the other vascular areas of the body, and stop at the precapillary level in quantities proportional to the blood flow. Their distribution in the myocardium maps the blood flow in the basal condition.

After this first injection, cardiovascular parameters were varied and a second injection of Ce-141-labeled microspheres was performed in order to obtain a second blood flow mapping relative to the new hemodynamic state. For the set of experiments presented here, intervention was the eliciting of hemodynamic changes by sympathetic nerve stimulation.

In order to quantify absolute regional myocardial blood flow, reference blood samples were withdrawn from a peripheral artery at a constant pump rate during the microsphere injections.

At the time of the second microsphere injection, 0.50 millicuries of 14C-deoxy-glucose were given intravenously. Different uptakes of this substance enable myocardial regions with different glucose metabolic activity to be identified.

In previous work by our group, we documented a higher deoxy-glucose uptake in the subendocardial layer of the left ventricle, where a higher metabolism and function had also been documented, as well as in the right ventricle when a pressure load was applied to this chamber [10].

At the conclusion of this experiment, the heart was placed in a cardboard box, surrounded by a support of cellulose, and immediately frozen by liquid nitrogen.

Three vertical radioactive markers were positioned at one anterior and two posterior corners of the box to provide reference points for alignment of the autoradiographic images. The heart was sliced into one-centimeter-thick transverse sections.

Each transverse section was sliced into 20 consecutive microslices 40 μm thick using an LKB microtome kept at −20°C. The microslices were dehydrated inside the microtome for 24 hours, labeled, and mounted on a radiographic film which was then exposed for 15–20 days.

The reference blood samples obtained during each injection of microspheres were counted in a gamma counter to obtain counts per ml per min of blood flow; samples of 0.1 ml of blood containing different amounts of radioactivity were smeared on a rigid plastic sheet and exposed on a radiographic film.

Digitization of autoradiographs

A digital image of an autoradiograph is shown in **Figure 1(a)**. The greatly magnified part of the autoradiograph in **Figure 1(b)** demonstrates that the isotopically labeled microspheres can easily be seen relative to the metabolic background and can be distinguished from each other on the basis of size.

The autoradiographs were digitized with an optical scanner having the capability of 256 grey levels and various spatial resolutions. A linear resolution of 100 μm in both the x and y directions was used for this analysis. Since the minimum size of the spot produced by a microsphere on the autoradiograph was about 0.30 mm, all microspheres could easily be resolved at this resolution.

An area of 80 mm × 80 mm was digitized for both blood smears and microslices, producing an 800 × 800-pixel image with 256 grey levels.

Alignment problems were minimized by careful registration of the reference points on the film and the special holding frame in the scanner. Digital images were coded and stored on disk.

Processing and analysis

Three different features are present in the digital image: the two types of microspheres and the metabolic background.

A software package was developed for the IBM 7350 [11] image processing system, taking advantage of its internal special computational capabilities to separate and evaluate these features.

To describe how the algorithm works, we introduce the concept of a "section process." A section process is the sequence of operations necessary to process all the twenty digital images of the microslices sampled from one of the transverse sections of the heart.

A section process can be logically divided into three phases:

1. Image preprocessing
2. Image processing
3. Image analysis and evaluation

• *Image preprocessing*

A preprocessing phase is used to remove possible alignment errors, which could have been introduced at the time of digitization, to separate the real image from the background, and to evaluate the mean value of the various pixels in the image.

Because of the high contrast between specimen and background, a simple thresholding operation is sufficient to force all pixels of the background to a value of 255. After histogramming, the mean value of the pixels of the image was computed for later use.

Using the three radiographic markers on the first image of the series for registration, subsequent images of the section were displayed and translated using an interactive procedure in order to register their markers with the reference.

• *Image processing*

The first step of the processing phase consists of identifying and counting the two different types of microspheres.

The algorithm utilizes the *a priori* knowledge that i) the microspheres appear as dark globules which can easily be separated from the rest of the image; ii) the two types of microspheres have distinctive sizes; and iii) the probability of having clusters of microspheres is very low, due to the thickness of the microslice and the relatively low number of beads compared to the number of vessels [7–9].

For Ce-141, the average size is between 6 and 8 pixels wide, and for Gd-153, it is from 2 to 3 pixels wide.

A sequence of thresholding and convoluting operations using Laplacian masks [12] is used to separate the microspheres from the rest of the image (A). The result of these operations is a binary image (B) in which microspheres are assigned a pixel value of 255 and background pixels are set to 0.

The binary image (B) is processed by a 7 × 7 smoothing operator and a thresholding operation to yield a binary image (C) containing only the Ce-141 microsphere image; these microspheres are assigned a value of 1 in this image.

The smaller Gd-153 microspheres are located by adding the Ce-141 localized image (C) to the combined image (B). In this manner, the Ce-141 globules are given a value of 0 by virtue of the original choice of pixel value. After thresholding, the pixels corresponding to the Gd-153 microspheres are assigned a value of 1 in this image (D).

The microspheres in images (C) and (D) are counted by applying a sequence of smoothing filter and thresholding operations to generate two new images (E and F) where all of the microspheres have the shape of a standard square (7 pixels/side for Ce-141 and 5 pixels/side for Gd-153).

These images are histogrammed, and the total number of pixels with a value of 1 is divided by the area of the standard square to obtain the total number of microspheres present in the image.

The procedure described is applied to all the images of the section, obtaining two series of images (E and F).

All the images of each series are summed pixel by pixel to generate two images (G and H) representing the section distribution maps of the two types of microspheres.

The metabolic image without microspheres present is obtained by combining the input image (A) with images

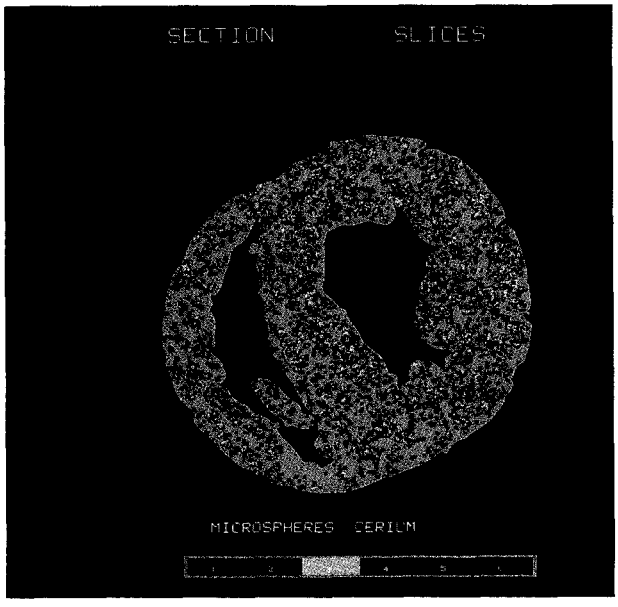


Figure 2

Distribution map of Ce-141 microspheres of Section 4. Different microsphere densities are shown using the color scale displayed (from 1, red, to 6 or more, blue).

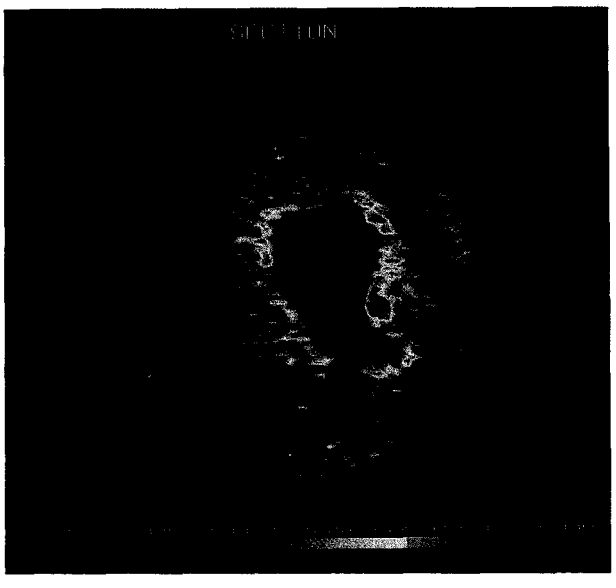


Figure 3

Distribution map of deoxy-glucose of Section 4. Different uptake values are shown using the color scale displayed.

(C and D) to generate an image (I) where the pixels corresponding to the microspheres are replaced by the mean value of the pixels of the image calculated in the preprocessing phase.

This image (I) is processed with a sequence of smoothing filters to reduce the noise introduced by the previous operation, and a new image (J) is generated.

By adding all the images (J) of the section pixel by pixel and dividing the obtained values by the number of added images, an image (K) representing the metabolic map of the section is produced.

With a simple operation of thresholding, forcing all the pixels of the image (K) to a selected value, an image having the shape of the section is obtained. This image is used as background to images (G and H) in order to have the microsphere distribution maps included in their anatomical frame.

Images (G), (H), and (K) can be displayed in color to give a general idea of the microsphere distribution maps and metabolism (see **Figures 2** and **3**).

Images (G) and (H) can be displayed together, using different color scales for the two types of microspheres, in order to have a macroscopic idea of the possible different distributions (refer to **Figure 4**).

Additionally, for a general overview, several sections can be displayed simultaneously, as shown in **Figures 5** and **6**.

All the described operations are performed using the internal processing capabilities of the IBM 7350; the processing time is about 35 seconds per image.

• *Image analysis and evaluation*

A procedure similar to the one described above has also been used to evaluate the number of microspheres contained in the autoradiographs of blood smears.

By using the values obtained by the gamma counter from the blood samples withdrawn during microsphere injection and the number of microspheres detected in the blood smears, it is possible to obtain a correlation between the radioactivity per unit of volume and the number of microspheres of each type per unit of volume. The absolute value of blood flow is obtained starting from the standard formula used to calculate flow by conventional well-counting:

$$Q_c = D_c/D_b,$$

where

Q_c is the flow in a myocardial sample region expressed in (ml/min)/g,
D_c is the radioactivity in a myocardial sample region expressed in cpm/g, and
D_b is the radioactivity in the sampled blood expressed in cpm/(ml/min).

By substituting for the radioactive dose D the corresponding number of microspheres N, regional flow can be computed by the formula

$$Q_c = [N_c/(A \times h)]/N_b,$$

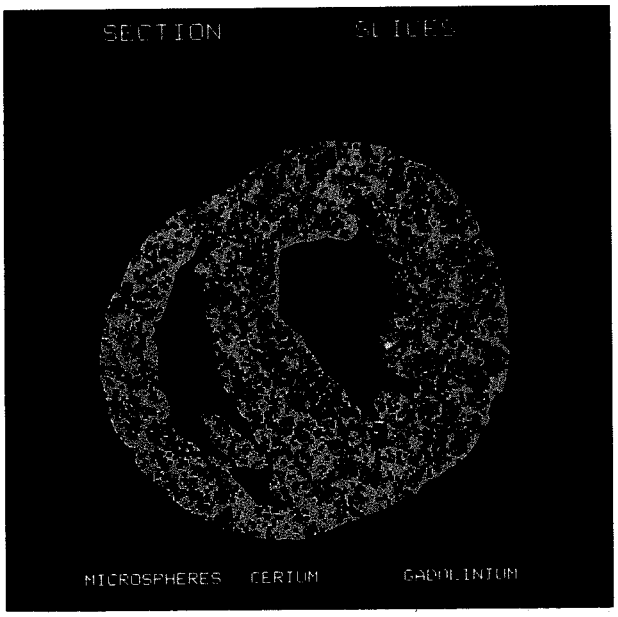


Figure 4

Distribution map of Ce-141 and Gd-153 microspheres of Section 4.

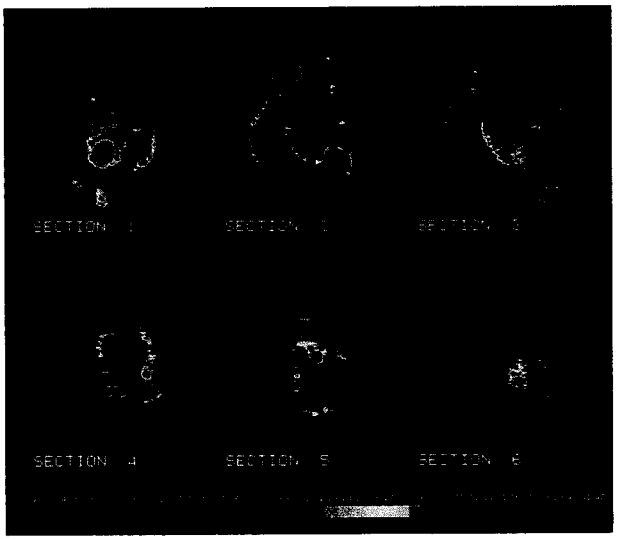


Figure 5

Deoxy-glucose uptake maps of different sections.

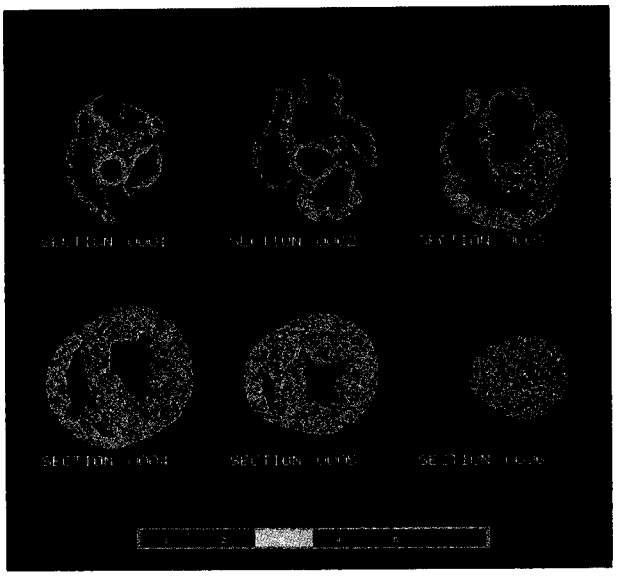


Figure 6

Distribution maps of Ce-141 microspheres of different sections.

where

N_c is the number of microspheres in a myocardial region,
A is the area of the region (number of pixels divided by 100, each pixel representing an area of 0.01 square mm),
h is the thickness of the region given by the product of the number of microslices and their thicknesses, and
N_b is the number of microspheres in the blood sample expressed in N/(ml/min).

The spatial resolution of the flow maps is given by the value of $A \times h$. The number of microspheres N_c is given relative to volume units, which are converted to weight (g), assuming 1.063 g/ml as myocardial specific density [13, 14].

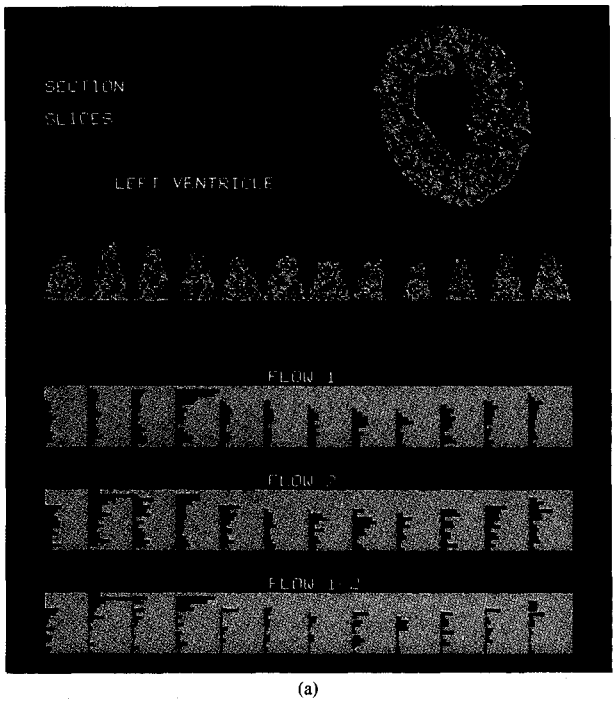
For deoxy-glucose distribution, an arbitrary density scale is used to obtain a semiquantitative mapping for each experiment.

The analysis can be performed separately on the two ventricles; image splitting is performed by means of an interactive procedure.

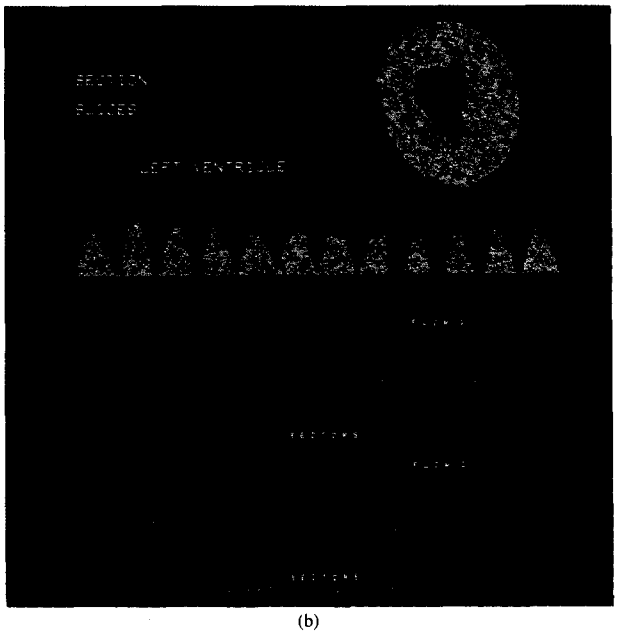
After the splitting, the interactive procedure allows one to divide the selected ventricle into a number of wedges (from 4 to 36). A wedge is that part of the ventricle obtained by inscribing the ventricle image in a circle and dividing it into circular sectors all of the same size.

To make the analysis easier, wedges are rotated, by means of a resampling process, in such a way as to have, for each wedge, the external part of the ventricle aligned on a horizontal line. Each wedge can be analyzed by superimposition of a variable grid; by varying the pass of the grid in one or both directions, a very large set of maps and histograms (in display and numerical form) can be obtained with different resolutions.

Comparative analysis can be performed between the two blood flow maps; absolute flows in the same region as well as frequency distribution of flows in a single slice and in the entire ventricle can be compared for the two types of microspheres.



(a)



(b)

Figure 7

Comparative analysis (left ventricle) of the two blood flows using 12 wedges: (a) shows a line-by-line histogram of the two blood flows and the histogram of the difference of the two flows; (b) shows the histograms of the two blood flows obtained by dividing wedges into three levels.

In addition, relative deoxy-glucose uptake in a single region and a slice can be compared with corresponding blood flow distribution.

Examples of various types of possible analysis are shown in **Figures 7**, **8**, and **9**.

Discussion

A great deal of work has been done in the last twenty years on this matter, and the microsphere technique is considered at present the standard technique for perfusion studies of the heart as well as of any other organ [1–5].

Alternative methods are based on the use of diffusible rather than particulate radionuclides, such as isotopes of K, Rb, and Tl, which are delivered to tissue according to flow and "deposit" into the cell according to the K^+ pool [15]. Although not invasive, and for this reason widely applied to clinical studies, compared to microspheres this approach has several drawbacks: Tracer uptake is affected by Na^+-K^+ pump function, K^+ intracellular content, and other metabolic factors which prevent the uptake from always being proportional to flow [16, 17].

Angiographic methods for regional perfusion studies are under development. They are based on densitometric measurement of "tissue" contrast from X-ray images [18]. However, the fast washout rate of the intravascular contrast medium, the nonlinear relationship between density and blood concentration of dye, and the need for high-speed tomographic devices represent severe limitations. The great advantage of the microsphere technique is that a dynamic situation such as perfusion can be frozen at the time of injection and analyzed afterwards.

The aim of our study was to map absolute flows and metabolic tracer distribution within an organ, taking advantage of the high spatial resolution of autoradiography and overcoming at the same time its major limitation, which is the lack of discrimination power among different isotopes.

Computerized autoradiography has the obvious advantage of facilitating microsphere detection and mapping.

In our case, the additional advantage of detecting two types of microspheres and of measuring absolute blood flow rather than relative perfusion must be emphasized.

One of the limitations of the present technique is due to the amount of time required: The steps that consume most of the time are cutting of microslices and film exposure.

The former is related to the microsphere content and to the requested blood flow spatial resolution. The latter depends on the type of isotopes and is inversely related to specific activity of the tracer and film sensitivity.

Of course, the limitations of the procedure can be balanced by the quality and amount of information provided. These are related both to the spatial resolution and to the number of isotopes which can be used in the same experiment.

In contrast, the densitometric acquisition and the computer processing are relatively fast procedures when adequate equipment is available.

Relative to the conventional microsphere technique, the routine procedure cost is mainly related to radiographic film.

The possibility of using one type of microsphere simultaneously with two diffusible tracers has already been documented [6, 19].

This study represents a further development of the previous approach, extending application of the procedure to studies in which more than one flow measurement is required.

Since microspheres labeled with various isotopes produce, according to the energy and the exposition, autoradiographic spots of variable size and/or density, it is reasonable to anticipate the possibility of identifying more than two microspheres by further extending the computerized procedure.

The physiological interest in high-spatial-resolution tracer mapping is obvious. The application of this technique to the simultaneous study of perfusion and metabolism in the heart and the possibility of correlating these two parameters on a regional basis is only one example of the potential of this method; furthermore, the use of this technique can be extended to the studies of other organs.

Finally, since tomographic nuclear techniques are becoming available today for clinical studies of organ perfusion and metabolism, experimental techniques like the one described here could be of great interest for basic research in this field.

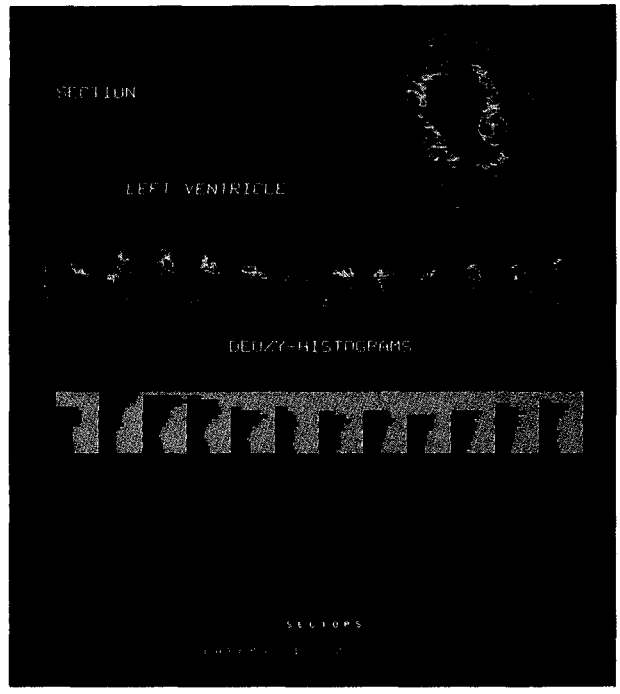


Figure 8

Analysis of the deoxy-glucose uptake (left ventricle); line-by-line histogram and histogram obtained by dividing wedges into three levels.

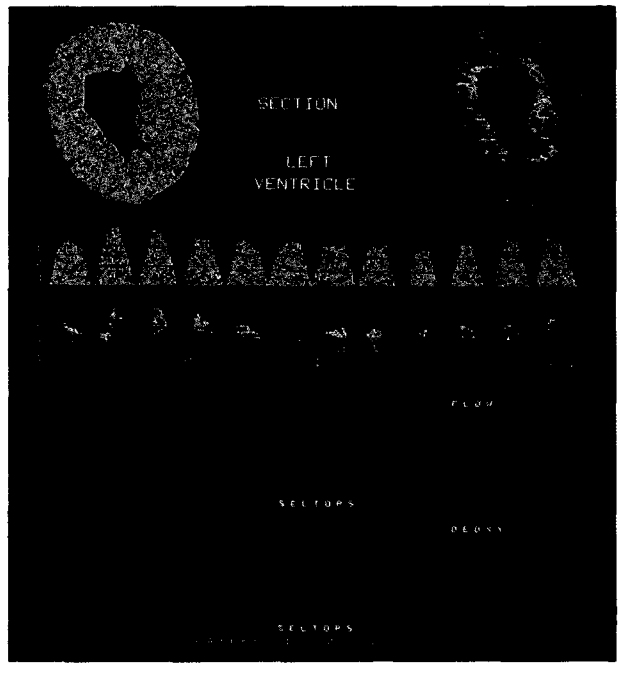


Figure 9

Comparative analysis (left ventricle) between deoxy-glucose uptake and the corresponding blood flow map.

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