Works in Progress

Quality Control of Radiochemical Purity by a Rapid Feedback System

W. B. Hladik III, K. T. Study, J. H. Gallagher, and B. A. Rhodes*

University of Kansas Medical Center, College of Health Sciences and Hospital, Kansas City, Kansas

By imaging gel column chromatograms of ^{99m}Tc-labeled radiopharmaceuticals it becomes possible to assess radiochemical purity within a few minutes after preparation. The images can be compared to images of pure compounds to determine the amounts of common radiochemical impurities such as free pertechnetate, insoluble hydrolyzed ^{99m}Tc, as well as various ^{99m}Tc complexes. If the images are obtained with a scintillation camera interfaced to a computer, automatic analysis of up to six compounds can be made. The computer can be programmed to report the percentages of the various radiopharmaceuticals and impurities and give a decision as to whether each preparation is acceptable for clinical use. The quality control testing of the radiopharmaceutical can be integrated with other daily quality control tests of the scintillation camera to give a "go" or "no go" decision as to the acceptability of the total imaging system. Such a quality control system is proposed and preliminary considerations and feasibility studies are reported.

The need for an expedient yet accurate means of quality control has surfaced because of the increased utilization in recent years of short-lived radiopharmaceuticals prepared in the hospital—particularly the ^{99m}Tc-labeled compounds which are used in 85–90% of all in vivo nuclear medicine diagnostic tests—and because of earlier, busier, and longer clinic hours.

To some extent the introduction of commercially available kits which are labeled with ^{99m}Tc within the radiopharmacy at the time of dispensing has minimized the problem of quality assurance. Regardless, however, of how rigid the standards may have been on the manufactured kit, the purity of the final product cannot be guaranteed without further quality control checks by the radiopharmacist before injecting the patient. The failure of ^{99m}Tc-pyrophosphate preparations to provide acceptable bone scanning images (Fig. 1) in several nuclear medicine facilities across the United States illustrates this point clearly *(1)*.

Much work has already been completed on the sterility and pyrogenicity testing in radiopharmacy *(2,3)*, but considerably more needs to be studied in order to streamline the quality control testing of radiochemical purity. Methods such as thin layer, paper, and column chromatography are presently available for this aspect of radiopharmaceutical quality control. However, column chromatography with fraction collection is extremely

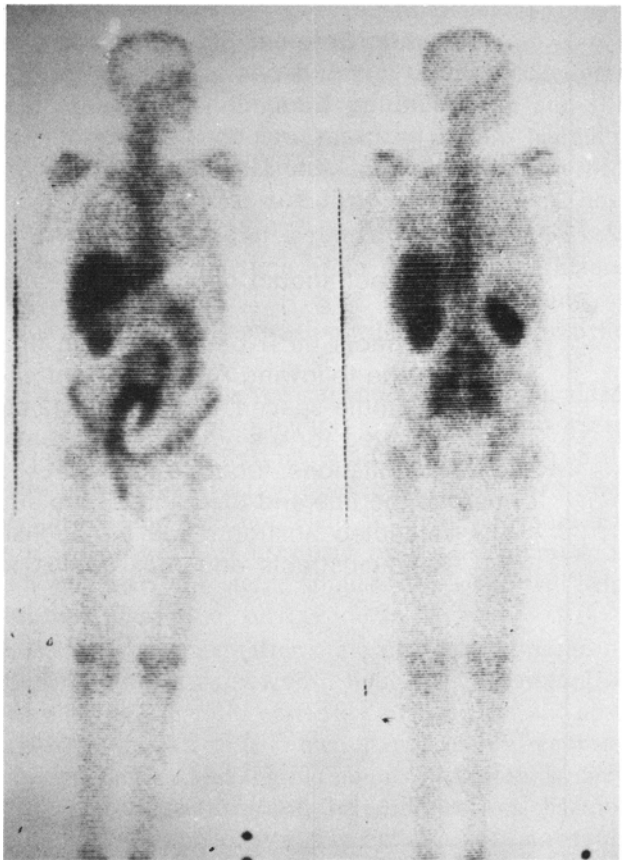

FIG. 1. Poor-quality ^{99m}Tc-pyrophosphate bone scan.

For reprints contact: K. T. Study, Dept. of Nuclear Medicine, College of Health Sciences, University of Kansas Medical Center, Kansas City, KS 66103.

*Present address: University of New Mexico, Albuquerque, NM 87131.

time consuming, while simple thin layer and paper chromatography are limited to determinations of free pertechnetate and hydrolyzed activity rather than total radiochemical purity *(4)*. Complete quality control data are, therefore, usually available only after the first patients in the clinic have been imaged. If the first scan for each diagnostic agent proves satisfactory clinically, then the need for quality control is diminished, and the value of after-the-fact chromatography testing is markedly reduced. But if the first scan proves unsatisfactory, valuable time, patient safety, and money have been sacrificed. Therefore, a rapid procedure for quality assurance of radiochemical purity by which results can be obtained before any patients receive the radiopharmaceuticals is desirable.

Looking at the problem in more detail, there are several reasons why before-the-fact quality control testing for radiochemical purity is necessary. First, not only may there be variation in nonradioactive kits from lot to lot and from company to company, but there are some radiopharmaceuticals for which there are no kits available commercially, including those for investigational use such as ^{99m}Tc-penicillamine and ^{99m}Tc-leukocytes. The entire process of preparation and labeling must be done at the site of dispensing, and it is with these compounds that quality control may take on added importance. Secondly, there are those radiopharmaceuticals that are inherently difficult to label or to keep labeled, e.g., ^{99m}Tc-diphosphonate, and those compounds that are known to vary in physical characteristics with the passing of time, e.g., ^{99m}Tc-sulfur colloid. Therefore it is essential to be capable of monitoring the effects of time, laboratory atmosphere, and technical manipulations which may lead to oxidation, ligand exchange, and aggregation of particles (Table 1). Thirdly, there is a daily need for checking the purity of the solution that is eluted from the molybdenum generator to confirm that there are no unwanted reduced states of ^{99m}Tc which may result in poorly labeled compounds.

TABLE 1. Common Radiochemical Impurities for Various Radiopharmaceuticals

Radiopharmaceutical	TcO^2	TcO^4	Tc-chelate(s)	Particle aggregation
^{99m}Tc-pyrophosphate	X	X	X	
^{99m}Tc-DTPA	X	X		
^{99m}Tc-HSA	X	X	X	
^{99m}Tc-sulfur colloid		X		X
^{99m}Tc-penicillamine	X	X	X	
^{99m}Tc-human albumin microspheres	X	X	X	X*

**undectectable by GCS method*

Literature Review—The GCS System

The solution to this problem of slow feedback of total quality control data to the radiopharmacist may be provided by the practical application of a recently developed method for determination of radiochemical purity. This method, known as gel chromatography column scanning (GCS), was introduced in Sweden by Persson et al. *(5)*. The GCS method utilizes gel filtration which can be described in the following manner.

"Molecules larger than the largest pores of the swollen (gel), i. e. above the exclusion limit, cannot penetrate the gel particles and therefore they pass through the bed in the liquid phase outside the particles. They are thus eluted first. Smaller molecules, however, penetrate the gel particles to a varying extent depending on their size and shape. Molecules are therefore eluted from a (gel) bed in the order of decreasing molecular size" *(6)*.

Samples of each radiopharmaceutical are placed on the column and developed using just enough fluid so as not to elute any radioactivity, i.e., by replacing the void volume of the column. (Dextran Blue can be used to visually determine the elution volume.) Then the filtration is stopped and the distribution of radioactivity is measured, preferably using a scintillation camera. Each radiopharmaceutical and its common impurities occupy characteristic zones on the column, which are reproducible and can thus be identified (Fig. 2).

The majority of the investigational work executed by Persson's group has resulted in specific knowledge of the system itself, but little, if any, work has yet been directed toward the clinical application of this GCS system. Therefore, the purpose of this proposed research is to determine how to establish and evaluate gel chromatography column scanning for daily radiochemical purity testing of commonly used ^{99m}Tc-labeled radiopharmaceuticals: ^{99m}Tc-pyrophosphate, ^{99m}Tc-DTPA, ^{99m}Tc-sulfur colloid, ^{99m}Tc-human albumin microspheres, ^{99m}Tc-penicillamine, and ^{99m}TcO_4^-.

Preliminary Considerations and Testing

Prior to formulating a proposal for daily application of the GCS system, several experiments were performed to

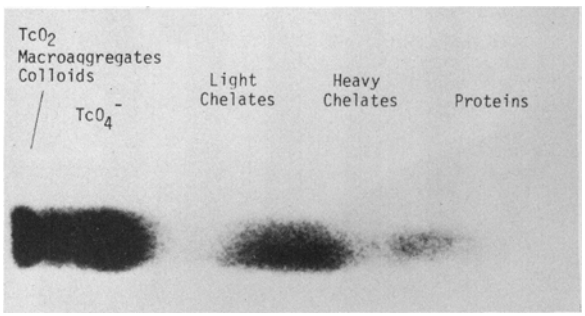

FIG. 2. Various zones of the gel column to which radiochemicals migrate.

investigate what alterations should be made in the system in order to best adapt it to clinical use.

The design of the chromatography equipment for use in the final system for clinical application is of considerable importance. It is essential that the columns be assembled in a manner to allow for the most rapid means of analysis. Previously the columns had been analyzed using a modified radiochromatographic scanner which could examine only one column at a time. For use in a practical situation, this method would be slow. Therefore several columns were arranged into one holder that would permit imaging and analysis of all columns simultaneously.

An arrangement of six cylindrical columns 30 cm long by 15 mm inside diameter was constructed and enclosed in a plexiglass holder. The columns were filled with a gel bed 20 cm in length (Fig. 3). The gel was prepared and the columns packed following the recommendations of Pharmacia, Inc., with only minor revisions *(7)*. The packing of freshly prepared gel into a nondisposable column has proven to be extremely time consuming and potentially may lead to inconsistencies in void volume from column to column. These problems can be overcome by using disposable prefilled gel columns standardized with a constant void volume which are now

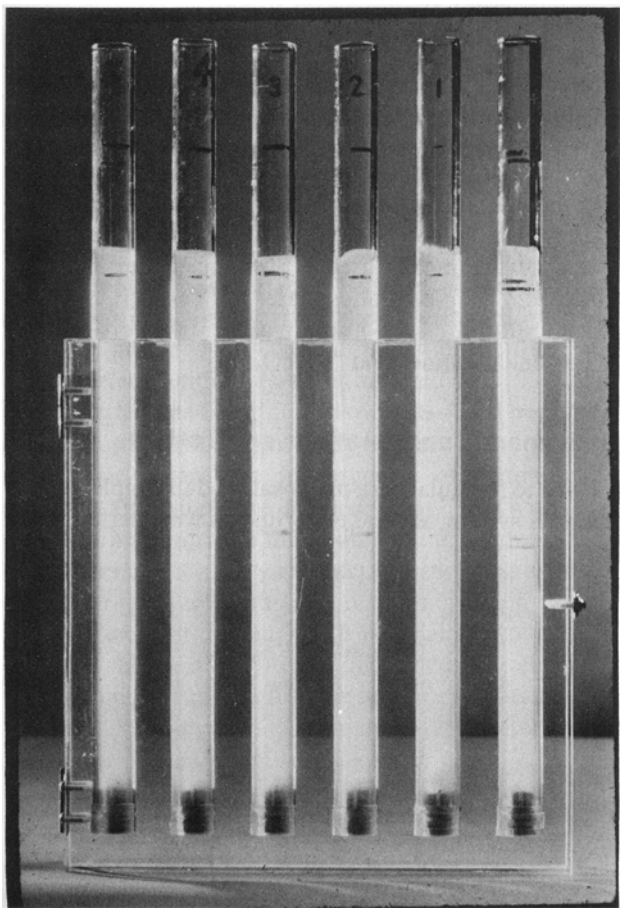

FIG. 3. Gel columns in plexiglass holder.

TABLE 2. Type of Gel and Eluting Solvent to be Used With Several Radiopharmaceuticals

Radiopharmaceutical	Type of gel	Eluting solvent
^{99m}Tc-DTPA	Sephadex G25	Saline, distilled water
^{99m}Tc-penicillamine	Bio Gel P10	Penicillamine soln. 31 mg/ml
^{99m}Tc-pyrophosphate	Sephadex G25	Pyrophosphate soln. 2 mg/ml
^{99m}Tc-sulfur colloid	Sepharose	Saline, distilled water
^{99m}Tc-HAM	Sephadex G25	Saline, distilled Water

under development. The disposable columns may also eliminate the problem of microbial growth in the gel, which can cause a slowing of the flow rate through the columns, thus increasing significantly the time of development and possibly reducing resolution. The column and gel bed dimensions given here may be varied without changing the principle involved in separation of components by gel filtration, although resolution of the developed compounds may be altered.

An additional consideration in column designing is that of marking the location of the origin of the gel so that it is visible both to the naked eye and to a nuclear activity detection device in order to establish a constant point of reference for identification of radioactive zones. A cesium point source imbedded in a lead hemisphere or tube which is implanted into the plexiglass column holder serves this purpose.

As was mentioned previously, the elution volume must be sufficient to develop the column with good resolution but just to the point that no radioactivity is eluted from the column. Determination of the endpoint can be achieved by placing a high-molecular-weight compound, e.g., a ^{99m}Tc-protein, on the column and allowing it to advance to the final (bottom) zone of the column. This assures that no radiochemical impurities of this molecular weight or less are lost from the column during the development process.

Another factor considered in preliminary testing procedures was to ascertain which solutes are appropriate to use for the development of the various radiopharmaceuticals (Table 2). Note that with several of the radiopharmaceuticals the parent compound is used as the eluant in order to avoid an irreversible action of the radioactive sample with the solid phase of the gel bed, i.e., some gels (Sephadex, Pharmacia, Inc.) will tend to dissociate weaker ^{99m}Tc-labeled radiopharmaceuticals (^{99m}Tc-pyrophosphate), and prevent their separation. Three types of gel are used to separate components of the radiopharmaceuticals: Sephadex G25, Sepharose 2B (Pharmacia, Inc.), and BioGel P10 (BioRad Labs., Inc.)

Sensitivity testing was carried out to determine whether or not the GCS method reveals subtle differences in radiochemical composition of radiopharmaceuticals (Fig. 4). In addition, samples of various radiopharmaceuticals were developed on the columns to visualize separation capabilities of the gel bed (Fig. 5).

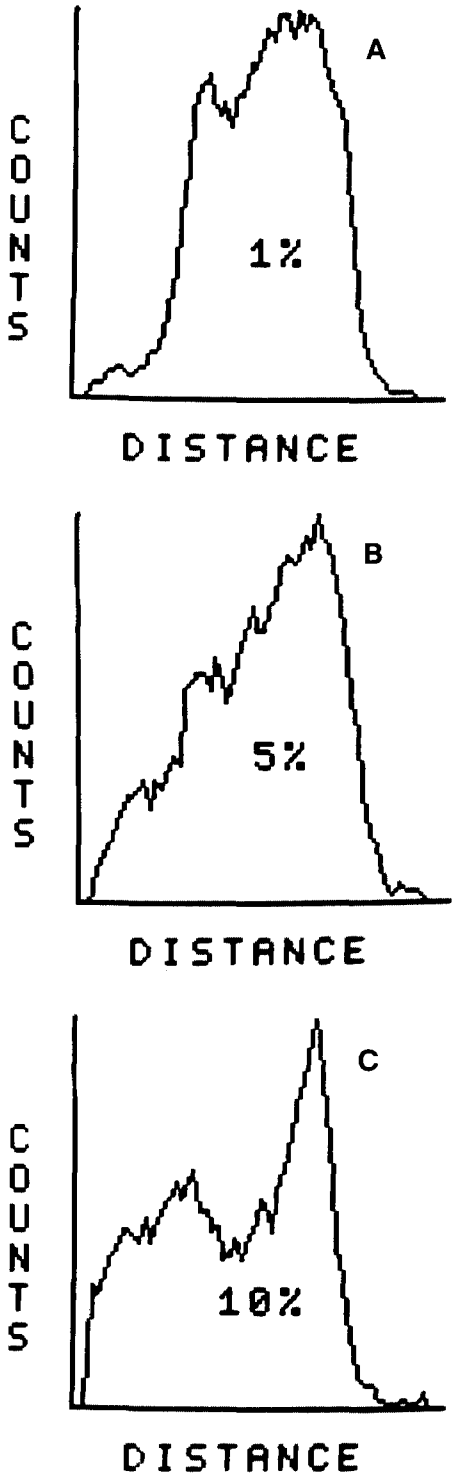

FIG. 4. Sensitivity testing: various known quantities of free pertechnetate (peak to extreme left) are detected here by scintillation camera method with computer analysis. Percentages indicate percent of total activity along column that is present as pertechnetate. (A) 1%; (B) 5%; (C) 10%.

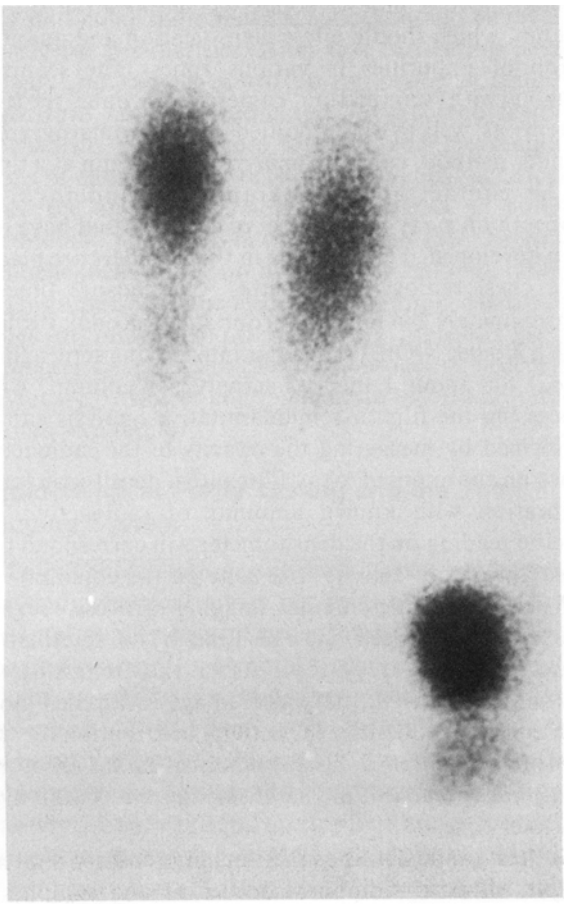

FIG. 5. Separation capabilities showing from left to right $^{99m}TcO_4$, ^{99m}Tc-DPTA, and ^{99m}Tc-pyrophosphate.

Imaging of the Chromatograms and Analysis of the Resulting Data

Keeping in mind that the GCS system should be made workable in all nuclear medicine diagnostic centers regardless of the sophistication of the resources available to them, four distinct methods of recording and analyzing data have been developed. Nearly all radiopharmacy departments should be capable of accommodating at least one of the four methods described below, although some are quicker and more practical to use than others.

These four methods use (A) scanning scintillation camera and data processing system, (B) modified radiochromatographic scanner, (C) contact autoradiography with densitometer analysis, and (D) whole column imaging with scintillation camera and computer analysis.

The scanning method uses a slit-collimated scanning scintillation camera to record the radionuclide activity along the column and store the distribution in the data processing system. The data are then played back in histogram mode to give the distribution as a function of position rather than time since the camera scans at a constant speed. The scanning camera is functioning as a rectilinear scanner with graphing capabilites. The scantillation camera/data processing equipment that we have used is the Ohio Nuclear series 75 system.

The modified radiochromatographic scanner method *(5)*, as the name implies, uses a modified paper or thin layer radiochromatographic scanner with a NaI crystal which has been adapted to scan gel columns. These two

methods give a plot of counts versus location on the column, which should allow identification and quantification of impurities in various zones. The scanning method with scintillation camera and data retrieval system, as well as the modified radiochromatographic scanner method, can only analyze one column at a time.

The contact autoradiography method involves exposure on x-ray film of a set of columns that have just been developed. The columns in their holders are placed on a chest radiograph cassette. An exposure time of approximately 2–4 min is appropriate for Kodak PR film with a Kodak X-Omatic regular intensifying screen (high speed) for about 1 mCi of activity per column. After processing the film, a semiquantitative analysis can be performed by measuring the opacity of the radioactive zones on the exposed x-ray film with a densitometer. By calibration with known amounts of radioactivity, a specific reading on the densitometer will correspond to a given amount of activity in a zone on the column.

With the whole-column imaging process, several columns are imaged at one time by a scintillation camera/computer system. After the image of the columns is acquired, the digital image is sampled along each column to quantify the activity distribution for that particular gel column. These numerical curves are stored on computer readable media (disk, tape, etc.) for analysis at a later time.

To test and develop this quality control system, samples of pure radiopharmaceuticals and samples of compounds with known amounts of impurities are developed and the resulting data are saved for reference.

Analysis of Gel Column Radiochromatograms

Two principles regulate the analysis of radiochromatograms. The area under a peak on a radiochromatogram is proportional to the amount of radiopharmaceutical constituting that peak; and the position of a peak from the origin of the gel bed is proportional to the logarithm of the mean molecular weight of the material of the peak.

Qualitative, semiquantitative, and quantitative techniques are available for analyzing the radiochromatograms obtained by one of the four methods.

A practiced observer can estimate the area under various peaks of the radiochromatogram to assess the relative amounts of impurity. The position of the impurity peak is highly suggestive of the identity of the impurity. The most common impurities (Table 1) will fractionate to zones of the column (Fig. 2) and may be readily identified. When impurity peaks are incompletely resolved [Fig. 6(A) and (C)], purely qualitative assessment of the peak will prove unsatisfactory for estimating the amount of the impurity, but this analysis technique can be used in all four methods and should be accurate to about 20%.

From three of the methods a hardcopy radiochromatograph is obtained (Polaroid picture from the scanning camera, and a graph from the modified radiochromatographic scanner and a scanning autoradiographic densitometer). By using a planimeter, the areas under peaks can be measured. Peak position can be graphically assessed to give estimates of impurities accurate to

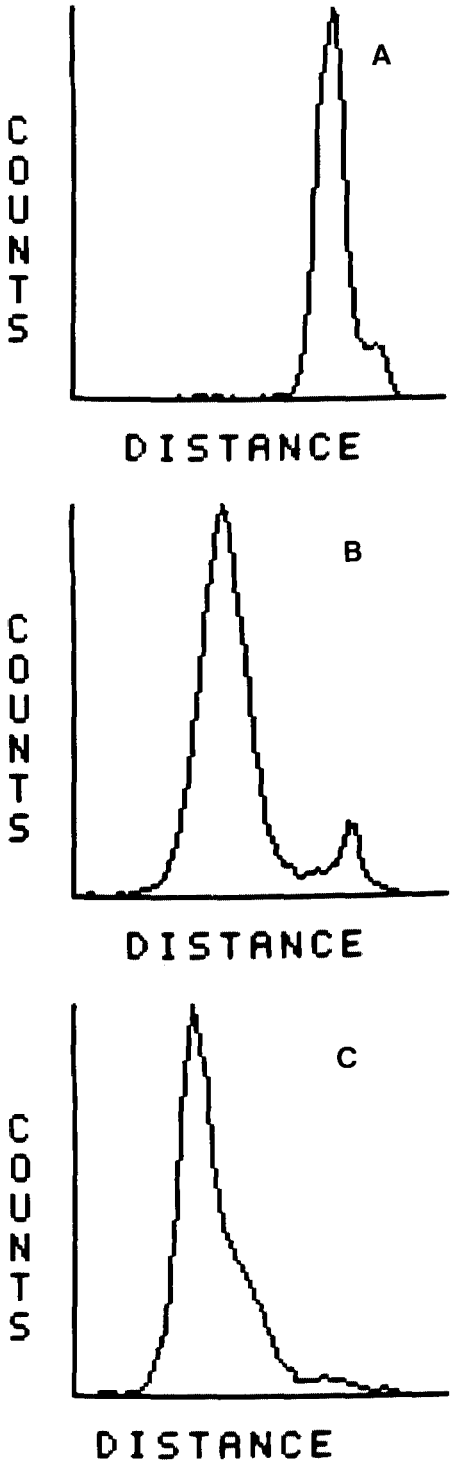

FIG. 6. Radiochromatograms of three radiopharmaceuticals using scintillation camera with computer analysis. These radiochromatograms represent the three preparations shown in Fig. 5. In Fig. 4(A), 4(B), and 4(C) the origin of the x axis corresponds to the bottom of the gel column. (A) $^{99m}TcO_4$; (B) ^{99m}Tc-DTPA with $^{99m}TcO_4$ impurity; (C) ^{99m}Tc-pyrophosphate.

approximately 5%. Incompletely resolved peaks are also not handled by this technique.

The numeric radiochromatogram obtained from the scintillation camera/computer data retrieval system lends itself to a fully quantitative analysis. Radiochromatographic peaks of samples with low viscosity *(8)* have a Gaussian distribution *(9)*. Each radiochromatographic peak is curve fit to a three-parameter function via the technique of least squares analysis. The three parameters are the area of the peak, the mean value of the peak, and the width of the peak, which is the standard deviation of the Gaussian distribution.

One term of the three parameters is included for each peak in the radiochromatogram. By iterative least squares fit, the computer can precisely measure the area and position of each peak. This quantitative technique can assess the amount and mean molecular weight of each component accurate to about 1%, even when radiochromatographic peaks are incompletely resolved.

Daily Clinical Operation of the GCS System

The practical application of this GCS method will be initiated by using it on a daily basis with five ^{99m}Tc-labeled radiopharmaceuticals. The plan includes daily computer analysis and use of a decision-making algorithm to feed back quality-control data on radiochemical purity to the radiopharmacy prior to dispensing the first dose of each preparation. A computer program (the algorithm) is presently being developed to evaluate radiopharmaceutical samples and then accept or reject each according to standards incorporated into the program.

Each column will be analyzed daily by fitting Gaussians to the radiochromatogram data. The percentage of each impurity is the ratio of the area of one peak to the area under the total radiochromatogram. When the percent activity of impurity peaks is plotted against the logarithm of the mean molecular size of the radioimpurity of that peak, a scattergram results. A scattergram of many days of analysis will form groups or clusters of data points. When a particular day's data for one radiopharmaceutical do not appear on the scattergram near previous data points, that day's radiopharmaceutical is suspect and is a candidate for rejection. By taking into account such factors as patient safety, image quality, cost of the contaminated radiopharmaceutical, and cost of the lost time to remake the radiopharmaceutical, a decision boundary can be established on the scattergram so that whenever a gel column has data above the decision boundary, that particular radiopharmaceutical is rejected.

A correlation must be made between a documented clinical problem with diagnostic images as detected by nuclear medicine physicians and the quality control data for the radiopharmaceutical used on the day that the problem occurs. Conversely, when radiochemical purity data show an abnormal variation, the scans done with that particular radiopharmaceutical should be reviewed closely for peculiarities. After this correlation between images and radiochemical purity testing is made, the algorithm can be readjusted so that the feedback mechanism will be more sensitive and meaningful in terms of clinical rather than just theoretical significance. Once the algorithm is adjusted to detect all potential problems, the system should be able to assure radiochemical purity on a regular and automatic basis.

This type of daily procedure can be applied to the other types of imaging and recording methods besides computer analysis, but the decision-making process will obviously be slower and much more inconsistent due to human variability.

Radiochemical Purity Testing and the Total Imaging System

To put things into perspective, it must be understood that radiochemical purity testing is only one section of the total quality control procedures for the scintillation imaging system *(10)*. A complete daily quality control test of the scintillation imaging system includes the evaluation of field uniformity, resolution, and radiochemical purity of the tracers. For this we propose a set of column chromatograms along with a field flood and a bar phantom. The field flood test for field uniformity and the bar phantom test for resolution are already in routine clinical use; however, the gel chromatography column scanning test for radiochemical purity is still under development (Fig. 7).

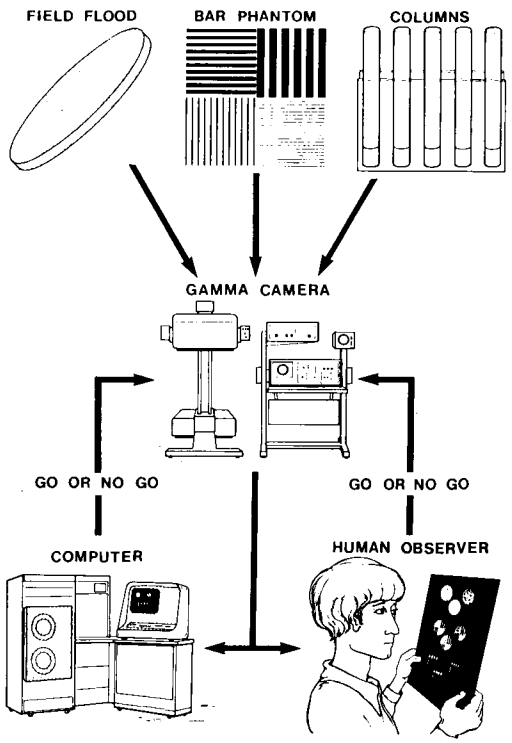

FIG. 7. Scheme of quality control for total scintillation imaging system.

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