MASTER

COMPREHENSIVE PROGRESS REPORT

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EVALUATION OF ENERGY SPECTRAL INFORMATION IN NUCLEAR IMAGING
AND INVESTIGATION OF PROTEIN BINDING OF CATIONIC
RADIONUCLIDES BY LACTOFERRIN.

PREPARED FOR

THE UNITED STATES DEPARTMENT OF ENERGY

UNDER

DOE Contract No. DE-AS02-78-EV04625.A002

This report includes an overall review of progress to date on the main research objectives as well as a more detailed discussion of progress on objectives for the contract year commencing October 1, 1979.

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288

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CONTENTS INCLUDE

ABSTRACT.....	3	
SCOPE OF INVESTIGATION.....	4	
PLANS FOR CONTINUATION OF PRESENT OBJECTIVES AND NEW OBJECTIVES (ABSTRACTED FROM RENEWAL REQUEST).....	12	2 1
FINANCIAL STATEMENT.....	19	Removed
BIBLIOGRAPHY.....	22	
PRESENT STATE OF KNOWLEDGE.....	25	
APPENDICIES.....	27	
ANNOTATED BIBLIOGRAPHY.....	38	Removed -

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PROGRESS REPORT

ABSTRACT

We have completed construction of and are currently using an Anger camera-computer system which allows collection of both the position and energy signals from events detected by the scintillation camera. The system allows correction of energy response non-uniformity of the detector and facilitates research related to effects of energy discrimination in radionuclide scintigraphy. The system consists of electronic hardware to transmit and digitize the energy signal, software to record and process that signal in conjunction with spatial positioning signals and additional hardware for recording the processed images so that they can be evaluated by observers. Our preliminary results indicate that the system is useful in evaluating clinical images. Assymetric (eccentric) energy windows do improve image quality and are of value in improving detection of lesions on liver scintigraphs.

We have also elucidated the mechanisms by which Ga-67 is taken up in infection and tumor. We have studied uptake of radiogallium in micro-organisms as a function of its interaction with siderophores. The primary function of these low molecular weight compounds is to trap ferric ion. However, gallium may be substituted for ferric ion and becomes trapped within the micro-organism. We have also studied the uptake of radiogallium by neutrophils and the role that lactoferrin plays in both intracellular localization of radiogallium and subsequent deposition of the radionuclide at sites of infection. Investigation of ferric ion analogs reveals definite differences in the affinity of these metals for binding molecules which helps explain their biologic activity. While ferric ion has the strongest affinity for such molecules, gallium has very high (almost competitive) affinity for siderophores, moderate affinity for lactoferrin and lower affinity for transferrin. The relative affinity of indium for these molecules is in approximately the reverse order.

We have also demonstrated that tumor tissue specimens from patients with Hodgkins Disease and Burketts' lymphoma and tumor cell cultures from melanoma contain significant quantities of lactoferrin. These studies provide insight into the similarities of gallium and ferric ion metabolism and suggest pathways for future therapeutic interventions.

SCOPE OF INVESTIGATION AND SIGNIFICANT RESULTS

Project A: Energy Spectral Information

Goal I: Construction of a combined spatial distribution and energy spectrum interface for an Anger camera and PDP gamma 11/34 computer.

We have completed construction of and successfully tested a system which allows collection of both the position and specific energy signals from events detected by an Anger scintillation camera and records this information in a computer. The system works in the following manner.

Hardware: In addition to the usual x and y signal analog to digital converters (ADC's), the system includes an ADC for the energy signal. This signal is amplified and buffered (delayed 4 microseconds) and digitized so that it is recorded in the computer with the appropriate x and y signal. Each event detected by the scintillation camera is recorded in the computer in a two word list format, the first word containing the x and y coordinates and the second containing the digital energy coordinate.

Software processing is then carried out as follows (see also figure 1).

In order to collect and reference events, an "F-Energy Program" is used, which is a modification of the Digital Equipment Corporation's F-GAMMA assembler program and allows the events to be recorded in a two word format.

E-1 program: Since the energy signal response over the face of the Anger camera is not uniform for input photons of identical energy, a correction program is needed. The E-1 program uses a field flood of 2.5 million events. These events are analysed on a 32x32 matrix and the energy peak for each pixel is determined and an energy offset matrix is produced. The energy off-set matrix is used prior to all subsequent image processing to restore energy uniformity. The E-1 program uses a 32x32 matrix to insure a statistically adequate energy spectrum and peak determination. Patient and phantom data is collected in a 64x64 matrix.

E-2 Program. This program uses the energy off-set matrix to correct subsequent patient or phantom study data for energy non-uniformity and then creates a study file with the corrected energies.

E-3 Program: This program allows the events detected from any patient or phantom study to be sorted in up to 5 energy windows. The events which fall within these energy windows are then re-organized into frame mode in a new study file with x and y words only. The study can then be recorded, displayed or otherwise processed using B-GAMMA, the general program available from Digital Equipment Corporation for processing nuclear medicine images.

OPERATION OF ENERGY - SPATIAL DATA COLLECTION AND CORRECTION SYSTEM - SOFTWARE

A hardware interface has been constructed between the computer and a Searle microdot display system which allows the digital images to be recorded on transparent film with a gray scale of up to 16 shades.

Goal II & III: Develop optimum methods of scatter rejection using this system and investigate optimum collimator designs to be used in conjunction with these systems.

These studies are in progress. Because of a delay in construction of the interface, initial scatter rejection studies were conducted using an on line microprocessor controlled energy correction circuit (Picker Micro-Z).

Based on phantom studies using a modified Rollo phantom with movable "cold spots" a 15% eccentric window with base line at approximately 136 keV was selected as an optimal scatter rejection window. Comparison was made using a specially constructed "ultra-ultra fine" low energy collimator which produced approximately the same decrease in sensitivity as the eccentric window.

Three sets of anterior and right lateral images were obtained on a series of randomly selected patients (first scheduled clinical liver scan of the day). The Picker 4/15 large field camera with Micro-Z correction circuit was used for the study. Following injection of 5 millicuries of ^{99m}Tc sulfur colloid, an initial set of images was obtained using a symmetric 15% energy window, conventional high resolution low energy collimator (Picker Ultrafine) and a count density of 6,000 cts/cm².

Following this, a second set of anterior and right lateral images was produced using identical imaging time and symmetric 15% window but substituting a higher resolution collimator (specially constructed Picker "ultra-ultra fine" collimator) which reduced the count density to approximately 4,000 cts/cm². A third set of images was then obtained by using the conventional collimator (Picker ultrafine), but now establishing an asymmetric energy window of 15% with base line of 136keV. The count density for these images was slightly less than 4,000 cts/cm². Efforts were made to obtain similar film density for all images.

While a total of 48 studies were recorded, problems with uniform film density or other technical features limited the total studies available for comparison. Two experienced observers were asked to judge the relative diagnostic quality of the images, looking at sets of either two or three images but not knowing which technique was used to produce the image.

Preliminary results from this study revealed a statistically significant preference on the part of the observers for the eccentric window and higher resolution collimator studies but no clear preference between the higher resolution collimator and the eccentric window.

Following completion of this preliminary analysis, the computer based energy correction and windowing system became available as a complete system including acquisition, processing and display modes and it was elected to conduct a somewhat modified but similar analysis using this system. The major modifications involved comparing only eccentric vs symmetric window images, using a somewhat lower count density and equal counts for the images (300,000), including a low (118-140 keV) as well as high (137-162 keV) eccentric window, a clinical standard for evaluating positivity or negativity of scans and a receiver operating characteristic curve mode of data analysis. Preliminary results from this study indicate a trend toward improvement in diagnosis with decreasing scatter fraction. These preliminary results are discussed in greater detail in the Appendix (Appendix A and C).

Both of these studies are ongoing and data collection will be completed before the end of the contract year. Since the studies require clinical follow-up, final reporting of results in the literature will be somewhat later.

However, based on this preliminary data it seems likely that eccentric energy windowing to improve image quality will be no more effective than higher resolution collimation, given equal trade-offs in sensitivity. We are, therefore, planning to work in conjunction with Beck and associates to explore the optimum-weighted multichannel (energy) imaging approach as an alternative to simple scatter rejection.

Goal IV: Develop programs which allow recording of relative isotope distributions of two or more radionuclides in vivo.

This goal has been achieved in the design of the interface and associated software programs discussed in Goal I. The E-3 program is designed to allow up to 5 separate energy windows. These can be added, subtracted or factored to allow determination of relative distribution of various radionuclides. We have not had the opportunity to use this capability of the system in either clinical or laboratory studies.

Project B: Ga-67 Association with Lactoferrin and other Binding Proteins

Goal V: Confirm association of Ga-67 with lactoferrin

We have achieved this goal by demonstrating the association of Ga-67 with lactoferrin in human milk, tear fluid and neutrophils. We have also quantitated the relative binding affinity of gallium, indium and iron to transferrin, lactoferrin and desferrioxamine.

Our basic methodology for the confirmation of relative gallium-67 activity bound to lactoferrin has been the use of affinity chromatography. Sepharose columns have been prepared with anti-human lactoferrin antibody and anti-human transferrin antibody respectively. The anti-human transferrin column serves as a control. Test specimens are eluted through the columns and the relative amount of activity retained in the column is determined. As a further control against non-specific binding, a dissociating agent, 3.5 M solution of $MgCl_2$, is used to dissociate the antibody-antigen complex. The activity which is eluted is compared to the protein content of the eluent to confirm that the activity is, in fact, protein bound. The methodology for determination of relative binding affinity of various proteins for iron, gallium and indium is the use of dialysis chambers. In one set of experiments, the metal in radioactive form is added to one side of the chamber while the protein is placed in the other chamber. In the competitive binding studies, the metal-protein complex on one side of the dialysis chamber is challenged with either a second metal or binding agent on the other side of the chamber.

As expected, iron binds to iron transport proteins and siderophores more avidly than either gallium or indium. Gallium, however, does have strong affinity for siderophores and its binding affinity is within two orders of magnitude of that iron. The relative binding affinity of gallium for the molecules tested in order of decreasing affinity is siderophore (Desferrioxamine), lactoferrin and transferrin. By comparison, indium bound most avidly to transferrin and least avidly to Desferrioxamine.

We have demonstrated that radiogallium taken up in human neutrophils is bound primarily to lactoferrin. Neutrophils (PMN's) were incubated with gallium-67 citrate. The relative uptake was highly variable and dependent to some extent on citrate content. However in all cases the vast majority of gallium-67 taken up by the cells was bound to lactoferrin as determined by affinity chromatography. Viability of the cells was confirmed by trypan blue exclusion and demonstration that this was not merely cell surface binding was achieved using a multiple cell washing method.

We have demonstrated, in fungi, that siderophores may play the principal role in mediating radiogallium uptake. The fungus model U. Sphaerogena was used for these studies because siderophore production in this organism has been studied extensively and its siderophore content is well characterized. Using this organism, we have shown that gallium-67 bound to siderophore is avidly taken up by growing cells in a manner similar to the iron siderophore complex. Further, when gallium-67 citrate is added to the culture media, the radiogallium is accumulated in the cell in the form of gallium-siderophore complex. These studies also confirm the strong binding affinity of gallium for siderophores. Since most microorganisms produce siderophores, these results may well explain the previously observed direct bacterial uptake of radiogallium.

We have also studied the effects of various conditions including pH and heat on bacterial uptake of radiogallium. We have, in addition, demonstrated that radiogallium taken up by neutrophils is actually incorporated in intracellular lactoferrin rather than interacting with the cell surface only.

All of these studies are either published, accepted for publication, have been presented at meetings and been submitted for publication or are currently being submitted for presentation. They are noted in the bibliography and included as appendices to this report.

Goal VI: Search for lactoferrin in tumors known to localize Ga-67.

We have demonstrated, by immunofluorescent methods that lactoferrin is present in tumor tissue from patients with Hodgkins' Disease, Burkitts' Lymphoma and melanoma.

Tumor specimens from two patients, one with Hodgkins Disease and one with Burkitts Lymphoma were studied for lactoferrin content. Both patients had gallium-67 scans which revealed tumor uptake of the radionuclide. The tissue specimens were studied using fluorescein labeled anti-human lactoferrin and appropriate controls. Significant lactoferrin content was demonstrated in both tumors.

We have also studied a small number of tumor specimens from patients with melanoma who had gallium-67 scans within 3 to 5 days of biopsy. In one specimen we were able to demonstrate that at least 16% of radiogallium activity in the specimen was bound to lactoferrin as determined by affinity chromatography. In the second specimen the activity was inadequate to accurately quantify.

We have also studied cell cultures of melanoma cells from patients. Based on immunofluorescent analysis, these cells contain significant amounts of lactoferrin. Our studies with melanoma tissue are preliminary and will require further confirmation. Therefore, we have developed plans to evaluate the lactoferrin content in melanoma more extensively. We had intended to initiate these plans this year. However, budgetary constraints have delayed our implementation schedule. We now hope to initiate these studies within the next three months and to expand these studies in subsequent years as noted in our contract proposal.

Goal VII: Determine binding characteristics of other metals in human milk.

The chief metal binding protein in human milk is lactoferrin. Studies evaluating the binding affinity of lactoferrin for various metals, specifically gallium, indium and iron, under various conditions are described in Goal V and in the accompanying appendices.

VIII Additional Goals:

In addition to the specific goals outlined in the original contract proposal, a number of ancillary projects have been investigated. These were usually related to one of the major goals and were enumerated in the yearly contract proposals. Most of these projects have been published in the scientific literature and are noted in the bibliography. Others have proved less fruitful.

a) Evaluation of Desferal as a clearance agent for radiogallium. We investigated use of a commercially available biologic siderophore, Desferal, for improvement of radiogallium imaging in detection of tumor and abscess. We demonstrated that, in animals, Desferal administered 6 to 24 hours after gallium-67 resulted in decrease in blood pool activity of radiogallium without affecting activity retained in the lesion. The net effect was improvement in lesion to blood ratios. These results have been reported.

Following appropriate clearance from the Human Investigation Committee, we conducted a series of human studies to determine if this effect would result in improved lesion detection. Following informed consent, patients with suspected inflammatory lesions received 6 millicuries of gallium-67 citrate and were imaged at 24 hours. Blood specimens were obtained and Desferal (1 gram) was administered intramuscularly. Repeat blood samples were obtained at approximately 4 and 24 hours after Desferal administration. Repeat images were also obtained 6 to 24 hours after Desferal. Five patients were studied. In most patients there was a noticeable fall in radiogallium activity following administration of Desferal.

Three patients had detectable lesions on scan. In none of these patients was the post-Desferal image significantly improved over the original image i.e. the lesion more easily detected.

We determined that most of the background activity observed on clinical radiogallium scans is from tissues other than blood and that larger doses of Desferal would be required if the studies were to be continued. We concluded that the larger doses would increase the risk of clinical reactions with only minimal likelihood of achieving improved clinical results. Since we were aware that Larson and associates were conducting a similar study on patients with tumors, using large doses of Desferal, we elected to suspend our clinical study in inflammatory lesions pending further determination of potential toxicity from larger doses used in Larson's study.

b) Iron deprivation and heat: Briefly, we proposed to study the effect of iron deprivation, achieved by Desferal administration, in combination with hyperthermia, on inhibition of tumor growth in a known gallium-67 avid tumor. A Cloudman S-91 melanoma implanted in a DBA-2J mouse was the model system we studied. The rationale for

the study was 1) the known enhancement effect of these two agents (heat and Desferal) on inhibition of bacterial growth, 2) the suggested increased iron needs of gallium avid tumors and 3) preliminary demonstration of growth inhibition in a cell culture model.

Our results were inconclusive. While a trend was noted ($0.2 > p > 0.1$) toward inhibition of tumor growth, our model proved difficult to work with. Heating of the small animals resulted in high mortality (approximately 40%) and tumor growth rates also varied from one group of transplants to another. A more stable tumor-host system was considered but the expense involved was beyond the resources of the contract.

Therefore Dr. Daniel Kapp, of the Therapeutic Radiology Department at Yale, has agreed to continue cell culture growth studies in his laboratory (which is primarily concerned with drug effects under conditions of hyperthermia). These studies will be supported by Dr. Kapp's laboratory. Our experimental protocol and results are summarized in the Appendix (Appendix B).

The following projects have not yet been fully implemented.

1) Studies of the effects of scatter rejection in Thallium-201 imaging. A commercially available phantom for these studies has been identified (New England Nuclear) and a tentative order placed. Once this phantom is received, we will begin investigations into use of eccentric energy windows for improved lesion detection as outlined in the current contract year proposal.

2) Providing availability of the energy interface and software information to other interested investigators. An advertisement is being placed in the Journal of Nuclear Medicine. Cooperative plans to study weighted multichannel (energy) imaging using a modification of the current software program have been initiated with Beck and associates at the University of Chicago.

PLANS FOR CONTINUATION OF PRESENT OBJECTIVES AND NEW OBJECTIVES

Project A: Energy Spectral Information

Our original interest in spectral analysis of radionuclide images was directed toward using this information to improve images by selective elimination of scatter. These efforts were directed to improvement in lesion detection of "cold" lesions i.e. situations in which scatter played a major role in contrast degradation. Our initial clinical analysis, which will be completed by the end of the contract year, suggests that while scatter rejection is useful in improving lesion detection under such circumstances, the improvement is modest and similar degree of improvement can be achieved by collimator modifications. Better results may be achieved using an optimum weighted multichannel imaging system originally proposed by Beck et al. (1). Conventionally, photons detected from radionuclides distributed within a patient are categorized as either photopeak events or scatter events. The photopeak events are considered "desirable" and the scatter events "undesirable". The limited energy resolution of the NaI detector prevents perfect separation of these two categories of events and dictates a certain compromise between elimination of scatter and maintenance of sensitivity. The optimum weighted multichannel imaging concept considers that, for any specific energy or small energy range recorded by the detector, there will be a "mix" of photopeak and scatter events. Moreover, the scatter is not a single entity but consists of photons which have scattered at various angles. The energy of the detected scattered photon is dependent on the angle of scatter. Thus each energy channel consists of photopeak events and scatter events of various angle of scatter. If a weighting factor is assigned to each energy channel which is a function of the effects of this combination of events, the resultant image will be better than that achievable using only a single energy "window". While some energy channels will have positive weighting factors, others may actually have negative factors.

Since the basic features of our computer based system are well suited to collecting images for and evaluating this concept, we propose to work with Beck to determine the clinical value of this approach to image improvement as an extension of our earlier asymmetric energy window approach.

Another important new area for evaluation of the effects of scatter is quantitative imaging as it relates to cardiac evaluation.

Use of radionuclides for non-invasive evaluation of cardiac function has greatly improved the ability of physicians to diagnose and treat heart disease (2,3,4,5,6,7). The most widely used cardiac function study is the ejection fraction determination. This study involves the use of a blood pool label. Images of the heart are

obtained in a gated acquisition mode, each image representing a fraction of the cardiac cycle. The left ventricular area is then defined either manually or by an edge detection algorithm and a background area similarly selected. The activity in the area of interest (the edge being modified for each phase of the cardiac cycle) is then calculated and the "background" activity subtracted. This background is determined by counting from a small region of interest adjacent to the ventricle and extrapolated for an area of equal size to the ventricular region of interest. The difference of the activity counted in the end diastolic frame compared to the end systolic frame divided by the end diastolic frame activity gives the ejection fraction (EF). The EF determined by this method correlates well with EF determined by geometric angiographic methods.

It has generally been appreciated that background has a strong influence on the EF determination and careful attention has been placed on proper background selection. Little attention however has been paid to attenuation and scatter. It has been assumed that these factors remain constant for the end systolic and end diastolic images and therefore their influence is cancelled out in the final EF determination.

Recently, however, there has been interest in actually quantitating ventricular volumes. Since these determinations generate an absolute rather than a relative number, it is assumed that attenuation must be compensated for. Ironically, however, Slutsky (8) has shown that as the volume of a ventricular phantom increases, the count rate detected by an Anger camera over a region of interest defined by the edges of the activity increases in linear proportion. This finding is surprising because self attenuation within the increased volume should cause the count rate to increase more slowly than the volume. Both Slutsky (8) and Dehmer (9) have also shown that the best correlation between radionuclide determined ventricular volume and angiographically determined volume is achieved when attenuation factors are disregarded or assumed to be constant.

Since these findings make little sense from the physics point of view, it is probable that some factor is acting to compensate for the effects of tissue attenuation. We speculate that the compensating factor is scatter. The depth of the left ventricular chamber with regard to the detector is determined primarily by two factors, the thickness of the chest wall and the size of the ventricle itself. If the patient has a thin chest wall and the ventricle is small, the mean ventricular depth will be minimal. Attenuation will be lowest under these circumstances as will scatter. If the chest wall is thick and the ventricle large, the depth of the mean ventricular position will be maximized. This should result in a greater attenuation effect and underestimation of ventricular volume. However these are also the circumstances which

will result in a greater amount of scatter being included in the region of interest. The increase in activity due to scatter would, in turn, compensate for the attenuation effect. The situation is further complicated by the effect of the size of the region of interest. If a small source is located in scattering material (i.e. a small ventricle) it will produce as much scatter as a larger source in a similar geometric configuration. The amount of scatter included in a small area of interest defining the borders of the smaller source will, however, be less than that included in a larger area of interest i.e. the borders of the larger source (see Fig. 2).

The interaction of three factors, attenuation, scatter and the definition of regions of interest, may play a strong role in influencing quantitative estimates of radionuclide activity in the ventricular cavity but these factors may compensate each other in such a way that their importance may not be readily apparent. We therefore propose, over the next three years, to study the effects of scatter as they relate to depth and region of interest in ventricular volume studies using radionuclide blood pool agents. We hope to develop a better understanding of the role of the various factors which influence radionuclide volume determination and to use this information to improve the accuracy of these determinations.

Our efforts will be directed at the following three goals as noted in our contract renewal request.

Goal I: Modify the current energy and spectral acquisition system to facilitate optimum weighted multichannel imaging and also the spectral analysis of quantitative cardiac images.

Goal II: Evaluate optimum weighted multichannel imaging to determine potential improvement in lesion detection initially in phantom models and subsequently in clinical studies.

Goal III: Study the effects of scattered radiation on ventricular volume and ejection fraction estimates .

Project B: Gallium-67 Binding

We have spent much of our effort attempting to elucidate the manner in which Ga-67 localizes in infection and tumor. The results of these investigations may be summarized as follows.

In a carrier free environment, radiogallium localizes in body tissues in a manner which is very similar to the ferric ion. There are, however, certain significant differences. First, radiogallium cannot be reduced in vivo and therefore remains bound to iron carrier molecules rather than becoming incorporated into metabolically active molecules such as heme. Second, the affinity of radiogallium for iron binding molecules is not identical to iron. While gallium binds to transferrin (TF) much more weakly than iron, it binds to lactoferrin (LF) with somewhat greater avidity and its binding to siderophores appears almost competitive with ferric ion.

Spectrum from small volume
1 mCi ^{99m}Tc source in water (depth
5 cm) with region of interest
including source only.

Spectrum from same small volume
1 mCi ^{99m}Tc source in water (depth
5 cm) with region of interest
expanded beyond edges of source –
this simulates a distributed source.
Scatter fraction determined to be
20% of photo peak in a symmetric
20% window.

It has been demonstrated that both neutrophils and bacteria themselves play a significant role in gallium localization in inflammatory lesions. We have expanded our understanding of these mechanisms by demonstrating that lactoferrin (LF) plays a major role in the neutrophil mediated mechanisms of Ga-67 localization while siderophores (S) seem to play a role in direct uptake in micro-organisms.

Our results thus far have been circumstantial rather than direct. We have demonstrated the Ga-67-lactoferrin complex in neutrophils and the siderophore mediated mechanism of Ga-67 uptake in microorganisms. It remains for us to actually demonstrate that these mechanisms are responsible for the radiogallium uptake in inflammatory lesions. We propose to do this in our future research by extracting radiogallium taken up by siderophore producing versus non-siderophore producing bacteria, comparing distribution of radiogallium with bacterial distribution and, perhaps most importantly, isolating Ga-67 bound to inflammatory lesions and attempting to identify the binding molecules.

We also wish to investigate the clinical significance of these findings by studying radiogallium uptake in a non-infected versus infected bone model. We have selected bone as our model system because it is an area in which gallium will continue to be useful and in which a more extensive understanding is needed into the patterns of localization in both infected and non-infected lesions.

In the area of tumor uptake of gallium, our understanding is much more primitive. Recent work by Larson (10) and Fernandez-Pol (11) suggests that while radiogallium is also acting as ferric ion analog in this situation, there are a number of potential mechanisms of localization including specific transferrin receptors and siderophore like factors produced by tumor. Our studies reveal that another iron binding protein, lactoferrin, is also present in increased concentration in some tumors. It is unclear if this protein is a major mechanism for radiogallium localization or merely one of a variety of iron binding proteins which are present in increased number in tumors. Therefore, we are also proposing additional studies into the presence of lactoferrin in the radiogallium avid tumor, melanoma, in conjunction with a parallel effort to assay these tumors for proteins to which radiogallium has bound.

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Activities of the Principal Investigator

The Principal Investigator, Paul B. Hoffer, M.D. was originally scheduled to devote 20% of his professional effort to the contract with 5% financial compensation. He has, on average, devoted approximately 20% of his efforts to contract related activities and anticipates continuing to do so for the remainder of the contract year.

The Contractor, to the best of his knowledge, has complied with the terms of the current contract agreement with the United States Department of Energy.

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BIBLIOGRAPHY (Published or submitted)

This bibliography is arranged in approximate reverse chronologic order. The notation in parenthesis at the end of the citation indicates the type of article: (Ab) = abstract; (C) = complete article; (R) = review article based on contract related work; (T) = thesis; (B) = book.

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Bibliography (Unpublished)

This section includes results of experiments discussed in the progress report but not yet consolidated or submitted for publication.

- A. Simon TR, Gottschalk A, Hoffer PB: Improvement in liver scan images using high resolution collimation versus scatter rejection: Preliminary report of clinical study - Appendix A.
- B. Shansky C, Weiner R, Hoffer PB: Effect of iron deprivation and hyperthermia on growth of gallium avid tumor (Cloudman's S-91 melanoma) in vivo: Preliminary results. Appendix B.
- C. Simon T, Browne D: Effect of various energy window settings on lesion detection in liver scans: A preliminary report. Appendix C.

*Thesis and book not included in annotated bibliography due to their length. A summary of Dr. Browne's thesis is included as Appendix C.

Present State of Knowledge

Project A: Energy Spectral Information

The study of spectral data and its contribution to the overall imaging process remains a neglected area of research. Originally, when rectilinear devices were used for radionuclide imaging, spectral information was relatively easily collected and initial determinations regarding acceptable window settings and levels of scatter rejection were determined.

With the introduction of the Anger camera and its subsequent ascendance to the role of the major imaging device in nuclear medicine, there was a marked decrease in efforts in this area. The Anger camera had uniformity limitations which became the principal determinant of acceptable energy window settings. Also, it was difficult to obtain spectral data from the Anger camera. The energy spectrum is still very important, however, in both imaging and quantitative data derived from nuclear detection devices used in medicine. We feel that application of our spatial and energy interface to spectral oriented experiments will not only expand our knowledge of the imaging process but also reap direct benefits in improved quantitative and imaging data being obtained from nuclear medicine studies i.e. better diagnosis of disease.

Project B: Ga-67 Binding

The understanding of the mechanisms by which Ga-67 localizes has major biologic implications. Over the past few years our laboratory as well as others has provided convincing evidence that radiogallium localizes in biologic systems as a ferric ion analog. We have also demonstrated the distribution of iron binding proteins in previously unsuspected tissues and elucidated the potential role of these molecules in Ga-67 localization in infection and tumor. Currently the major need in this field is to confirm these findings and determine their quantitative significance with regard to gallium localization. These results, if confirmed, may have much broader implications. First, they can provide the basis for better tumor and infection imaging agents. Second, they suggest the need for more extensive investigation of both gallium and iron as environmental agents associated with bacterial growth and tumor development. Finally, they may provide useful information about methods for interrupting tumor growth.

PRESENT DIVISION OF FEDERAL SUPPORT FOR OVERALL RESEARCH PROGRAM

All of the work described in this program is totally supported by DOE Contract DE-AS00-78EV04625. A002 with the following exceptions.

a) The studies conducted by Dr. Thomas Emery working at Utah State University in Logan Utah were supported in part by NIH Grant A109580-08.

b) Dr. Ronald Neumann currently receives 20% salary support from NIH Contract NO-1-HR-9-2904 and 40% from NIH Contract 5-RO-1-HL-22470-03.

The above cited exceptions represent only minor support elements in our overall effort, contributing, in aggregate, less than 5% of total costs.

Appendix A

Improvement in liver scan images using high resolution collimation versus scatter rejection: Preliminary report of clinical study. Simon TR, Gottschalk A, Hoffer PB.

The study was designed to determine if asymmetric peaking (scatter rejection) or "ultra" high resolution collimation can improve hepatic scintigraphy. The results reported here are preliminary.

All studies were performed using 5 millicuries of Tc-99m sulfur colloid as the imaging agent and a large field Anger Camera (Picker 4/15) equipped with a microprocessor for energy and uniformity correction as the imaging instrument. Anterior and right lateral views were collected under the following conditions and for equal imaging times:

- 1) 15% symmetric window, "routine" high resolution collimator (6,000 cts/cm²)
- 2) 15% symmetric window, "ultra" high resolution collimator (approximately 4,000 cts/cm²)
- 3) 15% asymmetric window (approximately 140-160 keV) "routine" high resolution collimator (approximately 4,000 cts/cm²)

Forty-eight patients were studied. Technical limitations due to inexact density matching as well as other factors limited the number of pair comparisons. Studies were judged with regard to "image quality" rather than diagnosis due primarily to the limited clinical follow-up period.

Forty-eight liver/spleen studies were compared without clinical preselection. A 6,000 count/cm² anterior view was obtained on a Picker 4/15 using a high resolution parallel hole collimator. An equal time right lateral view was then made. The same time was then used to obtain an anterior and right lateral view using (1) a window asymmetrically peaked to improve scatter rejection and (2) "ultra" high resolution parallel hole collimator. The "ultra" high resolution collimator has 75% of the sensitivity of the high resolution collimator. The resolution of the "ultra" high resolution collimator is 2mm (surface) and 6mm (at 100mm) compared to 5.2mm (surface) and 9.8mm (at 100mm) for the high resolution collimator. Hole size and wall thickness are similar. The asymmetrically peaked and higher resolution collimator images had approximately 4,000 counts/cm² due to the diminished sensitivity. The mean counts per image were 640,000, 340,000 and 460,000 for the high resolution collimator, asymmetrical peak and "ultra" high resolution collimator, respectively. Each view required approximately 90 seconds. Those views lacking appropriate intensity on the photographic film were excluded from the study.

Two experienced observers then viewed the images to evaluate image quality without knowing which technique was used. The images were graded by a consensus opinion regarding anatomic detail. Where a consensus could not be obtained, grades were averaged. The results appear in Table 1. The data were subjected to binomial pair analysis.

The observers significantly preferred the higher resolution collimator ($p < 0.001$) and asymmetric window ($p < 0.01$) studies to those obtained using conventional methods. There was however, no significant preference for either of the two methods of image improvement with regard to each other.

TABLE 1

<u>COMPARISON</u>	<u>NUMBER OF TECHNICALLY SATISFACTORY STUDIES*</u>	<u>NUMBER OF STUDIES JUDGED EQUIVALENT</u>	<u>PREFERENCE IN THE REMAINING STUDIES</u>
UltraUltrafine vs. Ultrafine	39	19	UltraUltrafine 17 Ultrafine 3**
Asymmetrical peak vs. Ultra- fine	28	12	Asymmetrical peak 15 Ultrafine 1***
UltraUltrafine vs. Asymmetrical peak	27	15	UltraUltrafine 8 Asymmetrical peak 4

*Total of 48 patients. Relative intensity differences made it necessary to exclude some views from analysis.

**p < .001

***p < .004

APPENDIX B

Effect of iron deprivation and hyperthermia on growth of gallium avid tumor (Cloudman's S-91 melanoma) in vivo: Preliminary results. Charles Shansky, Ronald Weiner, Paul B. Hoffer.

It has been previously observed that when bacterial cultures are subjected to hyperthermia and iron deprivation, growth is inhibited to a greater extent than would be anticipated from either factor alone (Kluger MJ and Rothenberg BA, Science 203: 374, 1979). Since bacteria incorporate iron via a siderophore mediated mechanism and some tumors may incorporate iron by a similar mechanism (Fernandez-Pol JA, Febs Let. 88: 345, 1979), we speculated that the combination of heat and iron deprivation might also inhibit tumor growth to a greater extent than heat alone. We further speculated that tumors which incorporate Ga-67 are likely candidates to be sensitive to iron deprivation since their uptake of radiogallium suggests the presence of an augmented iron uptake mechanism.

Preliminary experiments with tumor cell cultures (Chinese Hamster ovarian tumor-CHO) revealed that iron deprivation by use of siderophores in culture media did augment cell killing with hyperthermia (Kapp, unpublished data, Yale 1979). The augmented killing effect is not unique since many agents produce a similar effect. The effect of the siderophore was unique however in that it was not totally dose dependent. This suggested that an ideal dose could be achieved which would have maximum tumor inhibition effect without significant toxicity for normal tissues.

Preliminary studies using microinjectors in mice revealed a dose of 0.012 mgm/gm body weight administered continuously for 144 hours was the maximum dose at which no toxicity (as measured by weight loss) was observed.

The protocol for these studies was basically as outlined in the FY 1980 contract proposal. The initial tumor studied was the Cloudman S-91 melanoma, transplanted subcutaneously in DBA 2J mice. This tumor model is known to take up radiogallium with tumor blood ratios of approximately ten to one at 24 hours (Hoffer PB, JNM 20: 248, 1979).

Tumors were implanted in animals subcutaneously using minced tumor tissue in 0.1 cc volume diluted with saline. The experiments were started at 10 days post tumor implantation at which time tumor growth was confirmed by a palpable subcutaneous mass. Small continuous injectors (ALZET) containing 170 microliters of either sterile water or 43 mgm Desferal in water were implanted subcutaneously. The injectors administered approximately 1 microliter of fluid per hour for 7 days. Three days following implantation of the injectors, heat treated animals were subjected to a hot water bath at 39.5°C for one hour. The animals were held in place by putting them in a 50cc plastic centrifuge tube with

the tip end removed. The tubes were partially submerged in the water bath so that the animals heads were above water and the rest of their bodies, including the tumor, were submerged. This provided a relatively comfortable but adequately confining holder. The heated animals were subjected to 1 hour heating for five days. All animals were sacrificed at 10 days following implantation of the injectors. Total body weight was determined, tumors were resected and tumor weight determined. (All animals had also been weighed prior to implantation of injectors). Weight comparisons included subtraction of weight of the spent injector.

The four groups of animals studied were:

- 1) Controls, water injector only, no heat
- 2) Desferal only, no heat
- 3) Water injector, heat
- 4) Desferal and heat

Animal studies were performed in groups of eight to twenty animals over the course of eight weeks.

Results

No statistically significant difference was noted in tumor size between any pair of groups. There was however a trend (0.2 P 0.1) for the Desferal plus heat treated tumors to be smaller than the control untreated tumors. The composite data are summarized below:

	Control		Desferal	
	No Desferal No Heat	Desferal No Heat	No Desferal Heat	Desferal Heat
Number	24	26	21	19
Mean Tumor Weight (gm)	0.64	0.51	0.60	0.40
S.D.	0.62	0.44	0.49	0.37

A relatively high mortality was observed (40%) in all heat treated groups and great difficulty was encountered in maintaining uniform animal heating effect. Moreover, confirming animal temperature by rectal probe also occasionally resulted in colonic perforations. Tumor size varied greatly from one group of transplantations to another, yet efforts to achieve uniform growth rates by injection of known concentration of tumor cells resulted in a low percentage of tumor "takes". Due to these factors, we lacked confidence in achieving reliable results even if the animal studies were extended. Consultation with an experienced investigator using hyperthermia in animal studies (Kapp D., personal communication), indicated that highly variable mortality was frequently encountered using small animals for such studies. We felt that the high cost associated with the use of a larger animal host would be beyond the

resources of the contract. Therefore, the studies are being continued on a more limited scale using cell cultures under the supervision of Dr. Daniel Kapp. Our primary interest is in studying a gallium avid versus gallium non-avid model for tumor inhibition effect. The major financing for these studies is through Dr. Kapp's laboratory and not currently through our contract.

APPENDIX C

EFFECT OF VARIOUS ENERGY WINDOW SETTINGS ON LESION DETECTION
IN LIVER SCANS: A PRELIMINARY REPORT.

Report prepared by Theodore Simon

Based on Thesis by Daryl Browne

(This report contains the significant findings discussed in the
Thesis document, but in a much abbreviated presentation)

ABSTRACT

The effect of scattered radiation upon the diagnostic value of liver-spleen scintigraphy was measured using receiver operator characteristic analysis. Twenty-five patients were prospectively examined. Identical studies were subjected to 3 energy windowing algorithms that created high (136-162 keV), medium (129-151 keV), and low (118-140 keV) energy window images of equal counts. The images were evaluated by 2 observers for the presence of hepatic parenchymal disease, metastatic disease involving the liver and overall hepatic disease. In general, both observers performed better interpreting low scatter (high energy window) images, although differences in performance were modest.

INTRODUCTION

The distribution of photon energies recorded by the Anger camera is determined by three factors, 1) the uniformity of energy resolution of the detector, 2) the uniformity of energy response of the detector and 3) the relative proportion of scatter versus photopeak events impinging on the detector. Since scattered radiation usually acts to degrade resolution it is usually limited by the establishment of imaging energy windows. These windows are, however, also limited by sensitivity and detector uniformity. We have designed a special camera-computer interface which records both energy and position signals and corrects for detector non-uniformity (with regard to energy response). This system has allowed us to store patient studies and subsequently analyze these data with regard to selected energy windows.

METHODS

Patients were prospectively selected by including the first liver/spleen study scheduled each day at Yale-New Haven Hospital from September 1979 to February 1980. The series was interrupted as necessary for technical reasons. The 25 patients collected with technically satisfactory studies included 2 with hepatic parenchymal disease, 5 with hepatic involvement in a metastatic process and one with both parenchymal and metastatic disease of the liver.

Patients were imaged in the anterior and right lateral positions using a modified Searle LFOV Anger camera. The position and energy signals for each count were channeled to a BEC model 6911 analog to digital converter, formatted into list mode on a DEC PDP 11/34 computer and stored on DEC RK06 magnetic discs. A 2,500,000 count field flood image from a Tc-99m sheet source was obtained daily using the identical system.

Processing consisted of two steps. Firstly, the patient studies were corrected for system drift and flood non-uniformity. An energy spectrum was obtained for the flood and the peak energy recorded. The difference between this value and 140 keV was ascribed to drift and subsequent windowing was corrected for the shift in this peak. The flood was then formatted into a 32x32 pixel matrix. A separate energy spectrum was constructed for each pixel. Peak values for each pixel were then compared to the peak of a central pixel and the difference recorded as an integer in a 32x32 correction matrix. Each energy event for any patient was then examined for its pixel location and shifted by the value in the pixel location of the corresponding correction matrix. The corrected study was stored on tape (DEC TS-93). Secondly, the corrected studies were subjected to energy acceptance windowing, count equalization, and framing. Three images were created for each patient view. The "high energy" image was an asymmetric window, 136-162 keV including only minimal

scatter. The "middle energy" image corresponded to the standard 15% symmetric window used in this laboratory, 129-151 keV. The "low energy" image was an asymmetric window, 118-140 keV including a relatively large scatter fraction. Each count with appropriate energy address within the designated window was recorded in the appropriate image 64x64 matrix according to its location. All images for any patient had an equal number of counts, about 300,000. All counts in any image in excess of 300,000 were ejected. Each image then underwent a 9-point smooth and an interpolation to a 128x128 format. The images were displayed on a Searle microdot screen and recorded on Kodak NMB-1 transparency film. The pictures were randomized and mounted.

The diagnostic interpretation utilized receiver operating characteristic (ROC) analysis. Two nuclear medicine physicians (TRS, RN) scored each image on a 5-point scale for the presence of hepatic parenchymal disease, metastatic disease to the liver and any hepatic abnormality. These scores were compared to biopsy results, when available, or to at least 3 month clinical follow-up. The areas under the ROC curves were computed by trapezoidal approximation (1).

RESULTS

The ROC curves for each observer are included in the thesis document. The areas under these curves appear in Table 1.

In general both observers performed better on the high energy window images (low scatter) than on the middle or lower energy windows. The one exception was the performance of observer 2 on the parenchymal disease images.

Interpretation of the significance of these results should be made with extreme caution. The group of 25 studies included only 9 abnormalities. An adequate method of expressing the significance of results using the ROC method of analysis has still not been established. Nonetheless it is obvious that these findings demonstrate only a trend and must be expanded before their significance can be accurately assessed.

DISCUSSION

The preliminary results from a pilot study using various energy windows and determining their effect on diagnosis of liver scans suggests that asymmetric windowing with increased scatter rejection improves observers ability to detect clinically significant lesions. The current report is preliminary and is limited in a number of significant aspects.

Due to limitations in instrumentation, all images were recorded for equal counts rather than equal time. The later is the more desirable standard. It was not used in this study because equal

TABLE 1. Area under the ROC curve

Condition	Window	Area under the ROC curve	
		Observer I	Observer 2
Metastatic Disease	High (137-162)	100	94
	Middle (129-151)	88	85
	Low (118-140)	89	79
	All windows	92	86
Abnormal Study	High	92	97
	Middle	90	86
	Low	81	94
	All windows	87	91
Parenchymal Disease	High	82	73
	Middle	57	56
	Low	63	87
	All windows	68	70

time images would have resulted in some studies having count densities of lower than 2,000 counts per cm^2 , which was judged to be unacceptable. Also, no study was performed using a higher resolution collimator to determine its effect on lesion detection. However, a parallel study to measure this parameter is in progress. Finally, and most importantly, the number of patients and lesions studied is inadequate to draw any definite conclusions. We are currently expanding this series.

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