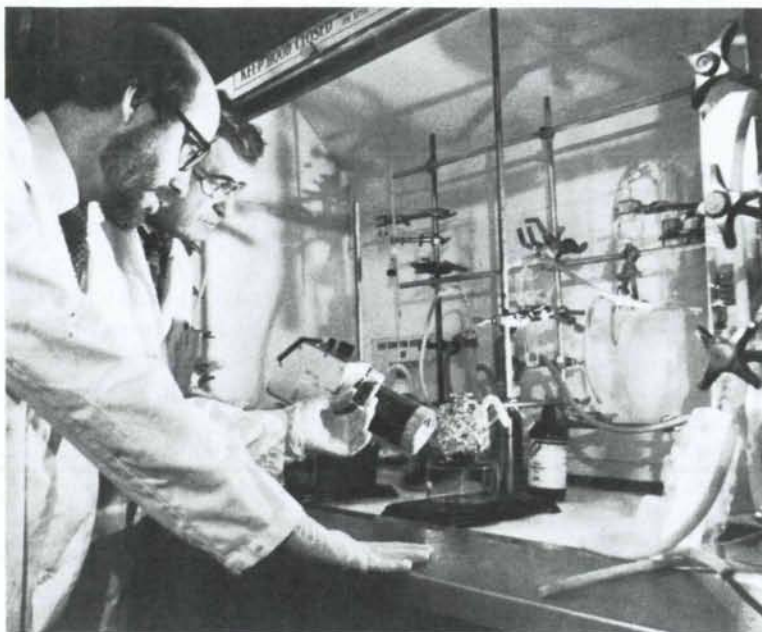




New agents to detect heart disease

A report on work at the Oak Ridge National Laboratory in Tennessee

by F.F. Knapp

In the United States, heart disease, and related cardiovascular disorders, is the leading cause of death. It includes the conditions that lead to heart attacks, which usually occur when the coronary arteries are narrowed by the buildup of arteriosclerotic plaque — the fatty, yellowish deposit composed largely of cholesterol. Too much plaque can cause a blockage in a coronary artery, significantly decreasing or cutting off the blood supply to one region of the heart muscle. Partial blockage leads to a condition known as ischemia, where a region of the heart muscle (myocardium) is partially deprived of oxygen and nutrients. Ischemia leads to angina, the acute pain suffered during a heart attack (coronary). In more severe cases the myocardium is totally deprived of nutrients and oxygen and suffers irreversible damage (infarction).

Fortunately, two of three Americans who have heart attacks recover because the myocardium is not irreversibly damaged. However, to keep a patient's heart disease under control, a physician must determine the extent of damage to the myocardium. This information guides the physician in selecting the proper treatment to restore the normal blood supply to the affected region. Treatment can range from a coronary bypass operation to a therapeutic regimen combining exercise with drugs that restore blood flow to affected regions of the myocardium.

Dr Knapp is Group Leader, Nuclear Medicine, in the Health and Safety Division at Oak Ridge National Laboratory (ORNL) in Tennessee, USA. He recently was a visiting scientist at the Institute for Clinical and Experimental Nuclear Medicine, University of Bonn, in the Federal Republic of Germany. This article is based on one previously published in the *ORNL Review*.

Radioisotopes for diagnosis

An effective means of evaluating heart damage is to inject patients with a radioisotope that is selectively absorbed by the heart. The most desirable imaging agents emit gamma-ray photons that are efficiently detected by "gamma cameras". At the same time, because these agents have short physical half-lives, they expose patients to only a small amount of potentially hazardous radiation. One radioisotope that is used in the USA for an estimated four to five million patients a year is thallium-201, which is produced in cyclotrons by particle bombardment of thallium-203.

Even though thallium-201 and other radioisotopes are valuable diagnostic tools, researchers throughout the world continue to develop imaging agents that are potentially safer for patients, less expensive, and able to provide clearer images in the earliest stages of heart damage. At the Oak Ridge National Laboratory (ORNL), the Nuclear Medicine Group in the Health and Safety Research Division has spent more than 5 years developing new agents for evaluating heart disease. Some of these radioactive agents, such as the fatty acids labelled with iodine-123, have a strong affinity for the heart and remain there long enough to provide a very clear image of tissue damage.

Because of the unique physiological and metabolic properties of the myocardium, the group has had an opportunity to develop new types of compounds labelled with radioactive materials that can be used effectively in the heart.* Work progressed to the point where the

* See "Images of the Heart: ORNL Makes Isotopes for Disease Detection", *ORNL Review*, Fall (1981).

Photo: Under a special hood, a radiiodinated fatty acid is synthesized and purified before investigation of its usefulness in evaluating heart disease in animals. (Credit: ORNL)

group proved that these agents work in animals, and clinical studies with some of these new heart imaging agents have been initiated.

Modified fatty acids

Since 1979 the Group has worked on designing and developing radiolabelled fatty acids. It chose fatty acids because the heart is unique in its need for long-chain fatty acids as its primary energy source (other organs derive their energy chiefly from oxidation of glucose obtained from the blood). That fatty acids can be used as natural carriers of radioisotopes to the heart is well known. However, because the heart breaks down, or metabolizes, fatty acids, the goal was to develop structurally modified fatty-acid analogues that would be picked up, but not metabolized, by the heart. The group sought to make analogues that would be "trapped" by the heart long enough to provide a picture of the extent of damage. After designing fatty-acid agents with the right biological properties and testing these agents in animals, the group found that, indeed, the modified analogues behave like natural fatty acids and are trapped by the heart. The group calls this property "metabolic trapping."

Fatty acids are concentrated, or extracted, by the myocardium from the blood plasma. Under normal conditions, the cardiac output (or fraction of the total blood pumped from the heart that flows through the heart muscle) is about 3% to 5%. The remainder of the blood is pumped to the other body organs. Thus, if a radio-pharmaceutical agent, such as radiolabelled fatty acids, is totally extracted in the first pass through the heart muscle, a maximum of 4% of the injected material can be localized in the heart. However, if part of the heart muscle has reduced blood flow because of an arterial blockage, the uptake of the radioactive agent will be lower than for the rest of the heart — because of reduced uptake, the camera will detect less radioactivity and thus can pinpoint where the damage occurred.

To solve the problem of the heart metabolizing fatty acids, the group sought to introduce into the fatty-acid molecule a structural feature that does not effect or decrease uptake from the blood but which would interfere with its metabolism. Such a goal represents a major conceptual and synthetic challenge because drastic structural modification could lead to a molecule that would no longer resemble a fatty acid and thus would not be extracted efficiently from the heart muscle.

Early studies

Early studies performed at ORNL involved inserting the tellurium-123m radioisotope into the fatty-acid chain. Tellurium-123m in the molecule has two important functions: (1) it is a source of gamma photons and thus makes the fatty-acid chain a diagnostic tool, and (2) it blocks the heart's attempt to metabolize the fatty-acid chain. The group found in animal studies that the

tellurium-labelled fatty acid shows outstanding trapping properties. In collaboration with Dr H. William Strauss and his co-workers at Massachusetts General Hospital, this agent was evaluated in conjunction with other analogues in which the tellurium heteroatom had been inserted into other positions of the chain.

The early studies were significant because they demonstrated for the first time that such a modified fatty acid would still have a strong affinity for the heart muscle (myocardial specificity). More importantly, this was the first demonstration that the modified fatty acid would remain in the heart for a much longer time, presumably because of the metabolic blocking action of the tellurium.

Importance of longer retention

This prolonged retention is important for the new generation of imaging instruments that use the technique of single photon emission computerized tomography (SPECT), which requires long imaging periods so that the computer can reveal a three-dimensional structure based on the numerous images taken from different angles by the moving detector.*

Because of the prolonged retention (only 10% to 15% loss from the heart muscle within 24 hours after injection) of this modified fatty-acid agent, the radioactivity tends to stay in one place and not be readily redistributed. This minimal redistribution is important for SPECT analyses because the greater the change of the distribution pattern during the imaging period, the larger the error that will be introduced into the final image.

Although fatty acids labelled with tellurium-123m were crucial for developing the experimental concept of trapping, the radioisotope is not ideal as a clinical diagnostic tool. It has a long physical half-life (120 days), a high production cost, and a low specific activity (amount of radioactivity per unit weight). Therefore, the group has attempted to retain tellurium in its non-radioactive form as a blocking agent but to attach to the same molecule a radioisotope with more suitable properties for diagnostic purposes — namely, iodine-123.

Iodine-123 is a more attractive radioisotope for use in nuclear medicine than thallium-201 because when iodine-123 decays, it emits gamma rays at an energy level (159 keV) that can be efficiently detected by today's equipment. It also can be obtained in high specific activity, which makes it safer than radioactive tellurium: less mass needs to be injected to obtain a detectable level of radioactivity. It is more practical than tellurium-123m because it has only a 13-hour half-life, which is just long enough to permit the isotope's commercial distribution from accelerators to hospitals. A major advantage of

* Photons of light are emitted by electron excitation produced when gamma rays from the radioactive agent in the patient's body impinge on a sodium iodide crystal in the detector: the emerging pattern of photon intensities is proportional to the spatial distribution of the radioactivity, which gives a picture of the extent of tissue damage.

using iodine-123 is the tremendous versatility of chemical methods for introducing iodine into a wide variety of tissue-specific molecules, such as the fatty acids.*

Applications demonstrated

One major application of this second generation of modified fatty acids is to evaluate the amount of myocardial tissue that can be salvaged after an ischemic attack. These agents have been extensively evaluated in animal studies and appear well suited for SPECT imaging equipment because of their prolonged retention and

* Inserting non-radioactive tellurium and attaching iodine-123 to the terminus of the ORNL group's modified fatty acids required some difficult, sophisticated chemistry. The preparation involved stabilizing iodine-123 on model tellurium fatty acids using two different chemical methods. In the experimental fatty-acid chains, iodine-123 was stabilized as either an iodophenyl or iodovinyl group. These special groups were designed to minimize iodide losses that occur because of the chemical susceptibility of the carbon iodide bond attached to the fatty acids.

minimal redistribution and the attractive properties of iodine-123.

As described earlier, ischemia is localized tissue hypoxia (deprivation of oxygen and nutrients) caused by reduced blood flow in a blocked artery. Usually after a patient has a heart attack resulting from reduced blood flow to regions of the heart muscle, drugs are administered to increase the flow to these damaged regions. Hence, there is also a need for a diagnostic agent to determine how well the drug treatment is working by revealing changes in blood flow to the heart.

The unique ability of the fatty-acid agent to detect and characterize ischemia and changes in blood flow patterns as a result of drug therapy has been demonstrated in dogs by Dr J.A. Bianco at the University of Massachusetts Medical Center. Working with ORNL's group in a medical co-operative programme, Dr Bianco has removed and dissected the heart of each experimental dog and determined the distribution of the fatty-acid agent by measuring the radioactivity from the small sections of the heart muscle.

Hypertension studies

Recent studies have demonstrated the promise of fatty acids labelled with iodine-123 for evaluation of aberrations in regional fatty-acid uptake that may occur in hypertensive heart disease (high blood pressure).

The studies were performed in an ORNL medical co-operative programme in collaboration with Dr A.B. Brill, director of the nuclear medicine programme at the US Brookhaven National Laboratory, and Dr H. William Strauss at Massachusetts General Hospital. They involved autoradiography, a technique whereby tissues from experimental animals are removed after intravenous administration of radiolabelled agents, frozen, sliced into ultrathin slices, and placed on photographic film. In this technique, which offers very high resolution, the degree to which the radioactivity exposes the film indicates the distribution of radioactivity in the tissue slice. By using quantitative dual tracer autoradiography, Dr Brill and his colleagues monitored the relative distribution of two different agents.

For example, the fatty acids (BMIPP and DMIPP, see main article) that the ORNL group labelled with iodine-131 (8-day half-life) were administered to normal rats also injected with thallium-201, which has a half-life of 72 hours. The same mixture was administered to a second group of rats made hypertensive by sodium chloride (a high-salt diet). (Thallium-201 is commonly used for the clinical evaluation of coronary heart disease and is primarily distributed through the heart muscle as a function of blood flow.) Thus, if the autoradiographic studies show a homogeneous distribution of thallium-201, the blood supply to all regions of the heart muscle is normal.

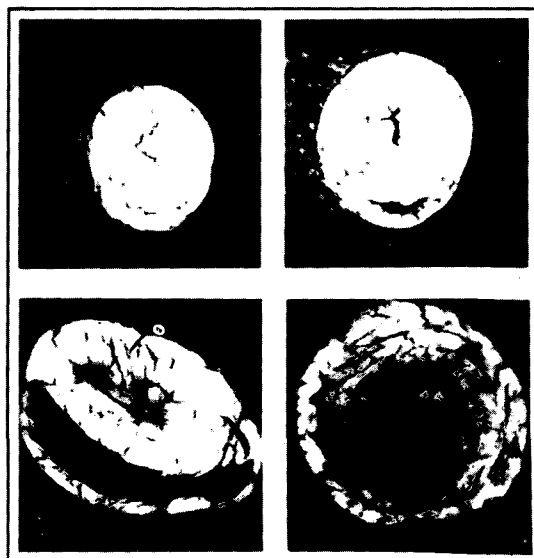
The first autoradiographs indicated normal regional blood flow in the hearts of rats of both the control group and the hypertensive group because of the homogeneous distribution of thallium-201. After allowing 30 days for all of the thallium-201 to decay (10 half-lives later), Dr Brill's group could determine the distribution of the fatty acid labelled with iodine-131 by examining the later autoradiographs of the same tissue slices.

The group found that (1) in the normal rats both thallium-201 and the fatty acid labelled with iodine-131 are evenly distributed, and (2) in the hypertensive rats thallium-201 has an even distribution but the fatty acid labelled with iodine-131 clearly shows an uneven distribution. The thallium results show that regional blood flow is normal in the hypertensive rat hearts, indicating that fatty acid delivery to the heart was not impaired. However, the heterogeneous distribution of fatty acid indicates that hyperten-

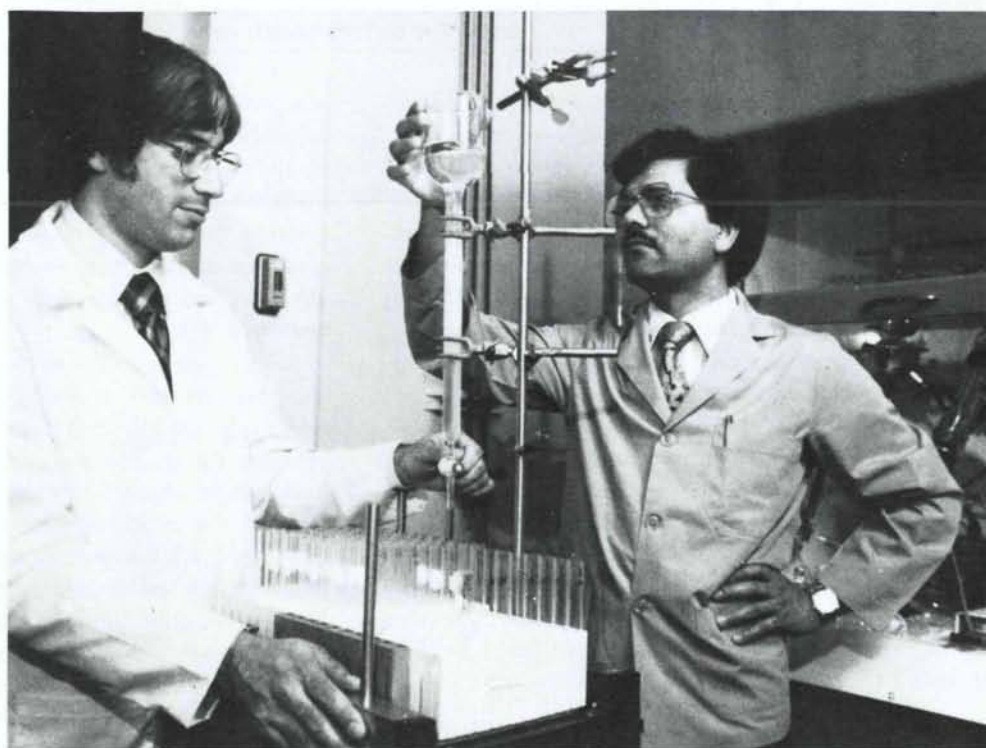
sive heart disease may have altered the ability of portions of the heart to concentrate and/or metabolize fatty acids.

These observations are very important because they suggest that a metabolic change may occur in severe hypertension before any differences in blood flow (ischemia) can be detected. Therefore, because agents such as thallium-201, which are widely used to detect and evaluate coronary artery disease, can indicate only differences in blood flow, they may not be effective in evaluating aberrations in regional fatty-acid uptake and/or metabolism that may occur in heart disease. On the other hand, the combination of fatty acids labelled with iodine-131 and SPECT can potentially evaluate hypertensive disease and assess the effects of drug therapy.

Autoradiographic images of thin slices of heart muscle from rats show how two radiolabelled agents are distributed in normal (left column) and hypertensive cases (right column). (Credit: ORNL)



Work on development of substrates used for radio-labelling. (Credit: ORNL)



Besides determining the extent of damage and the effectiveness of therapy, a third, and probably the most important, major application of these agents will be to evaluate changes in regional metabolism of myocardial fatty acids that occur in the absence of coronary artery disease. In this application, the flow of blood in the hearts of test animals is normal, as verified by thallium-201 studies. But certain other diseases of the heart, such as cardiomyopathies and hypertensive heart disease, may be detected by fatty-acid agents because these diseases can cause the heart to change the mechanism of uptake or rate of metabolizing fatty acids. This application may offer a unique opportunity to evaluate the early stages of heart disease prior to severe ischemia or when blockage of the coronary arteries is absent. (See accompanying box.)

Alternative for clinical application

The tellurium fatty acids labelled with iodine-123 are very difficult to prepare, so their availability for experimental studies is unfortunately limited to very few institutions. More recent investigations by the ORNL group have focused on a third generation of structurally modified fatty acids in which methyl-branching instead of tellurium was introduced to inhibit metabolism of fatty acids by the heart. Although this strategy is not as effective as using tellurium fatty acids, it offers a better alternative for clinical application.

Methyl-branching — attaching an alkyl carbon-hydrogen (CH_3) group — can be used to inhibit metabolism because of the well-established sequence of reactions involved in the initial stages of beta-oxidation in the heart muscle for energy production. The term "beta-oxidation" is used because the chemical bond

between the second (alpha) and third (beta) carbon atoms is broken to release energy. This sequence is initiated by enzymatic attachment of an oxygen to the beta-carbon. Because the methyl-branched fatty acid cannot be oxidized in the first cycle of beta-oxidation, the resulting fragment of the agent may be more slowly transported or perhaps bound to an enzyme component. As a result, it may be trapped; it will probably be released more slowly from the heart muscle.

To prepare the methyl-branched fatty acids, Mark Goodman, an organic chemist, has developed innovative multistep organic syntheses. A 16-step sequence was required to prepare the "substrate", which was used to introduce the iodine-123 isotope. Our model agent, called BMIPP, is the methyl-branched, fatty-acid analogue of an unbranched fatty acid that is used at several institutions in Europe for the evaluation of heart disease.* The unbranched analogue, however, shows considerably more rapid washout from the heart than the branched analogue. Because this fatty-acid analogue is retained in the heart muscle longer than the straight chain version, the agent may be more suitable for SPECT analysis as a probe of regional fatty-acid uptake.

Just recently, the group also has prepared an analogue of BMIPP that showed nearly irreversible myocardial retention in animal studies. This analogue, called DMIPP, thus represents the group's first fatty-acid analogue that does not contain tellurium as the structural modification showing this unique retention property.** The first clinical studies with iodine-123 DMIPP recently have been initiated at the Institute for Clinical

* BMIPP is 15-(p-[^{123}I]iodophenyl)-3-R,S-methyl-pentadecanoic acid.

** DMIPP is 15-(p-iodophenyl)-3,3-dimethylpentadecanoic acid.

and Experimental Nuclear Medicine in Bonn, Federal Republic of Germany, and the Department of Nuclear Medicine in Vienna, Austria. Because of the long myocardial retention, excellent SPECT images have been obtained and studies are continuing to determine the usefulness of this agent for the evaluation of aberrations of fatty-acid uptake that may occur in different types of heart disease in comparison with other agents.

A second type of mono methyl-branched fatty acid (BMIVN) and dimethyl-branched fatty acid (DMIVN) in which the iodine-123 has been stabilized by the vinyl iodide approach also has been developed and evaluated at ORNL. These methyl-branched analogues also show greater retention than the unbranched analogue. Both DMIVN and DMIPP are under intensive evaluation in ORNL's programme and in conjunction with investigators in medical co-operative programmes who have special expertise to study selected aspects of the properties of these two agents.

Other types of radiopharmaceuticals

Organic cations are another radiopharmaceutical that the ORNL group is developing for heart imaging. Studies involve the separation and evaluation of positively charged (cationic) phosphorus and related compounds in which a radioisotope is attached.*

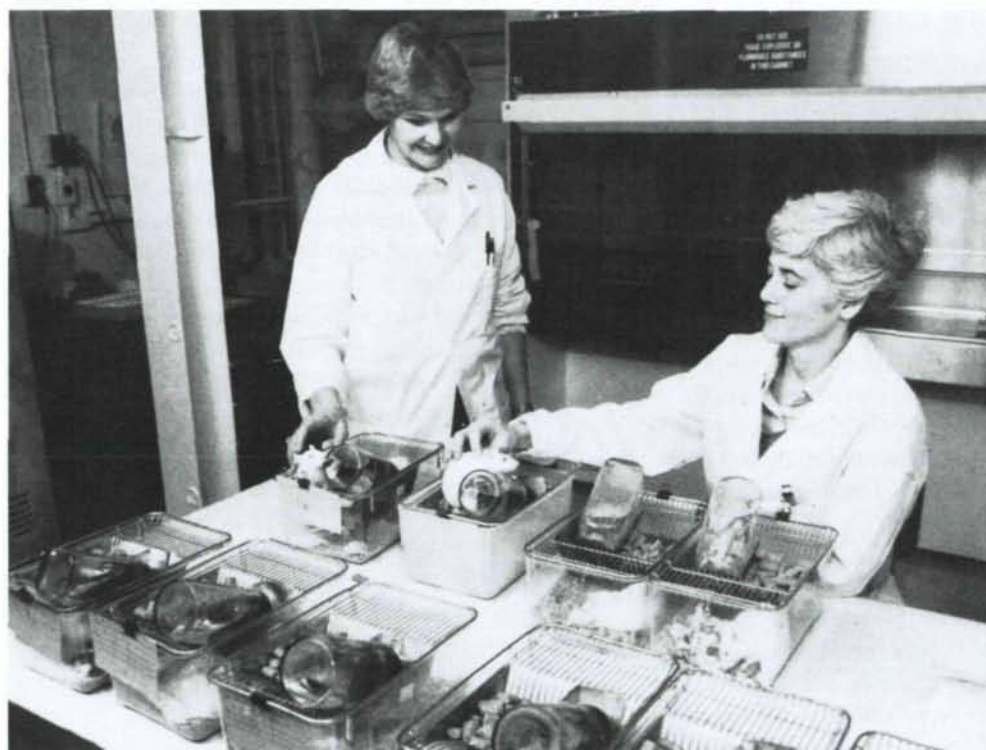
P.C. Srivastava, a medicinal chemist working in the ORNL Nuclear Medicine Group, has developed methods of preparing these phosphonium cations. One of these cations, in which iodine-123 has been stabilized as a vinyl iodide, shows high heart uptake in dogs and

rats. This model agent also shows significant hepatobiliary localization in dogs — that is, it is taken up by the liver and excreted in the bile through the gall-bladder into the small intestine. To investigate the relationship between heart selectivity and hepatobiliary clearance of these types of agents, a variety of structurally modified phosphonium agents were prepared and evaluated in rats.

Because the heart lies so close to the liver and because phosphonium cation analogues show excellent heart selectivity and low liver uptake, these agents could be valuable for diagnosing certain heart diseases. High liver uptake obscures a clear delineation of the lower part of the heart muscle and therefore should be minimized to allow good imaging of this region. ORNL phosphonium cation agents have been designed and tailored to exhibit the most desirable biological properties for imaging the lower part of the heart muscle.

In experiments with rats injected with structurally modified phosphonium cations, the group observed high heart uptake and moderate liver uptake in the early images. It also found that the background radioactivity

*Interest in these agents was stimulated by cell culture (*in vitro*) studies by biophysicists that have shown that the uptake of model phosphonium cations from the growth medium by living cells depends upon the transmembrane potential. Thus, cells that have a slightly negative charge in their interior environment show uptake of the positively charged cations. Model phosphonium cations, such as tetraphenylphosphonium bromide, also show high myocardial uptake in experimental animals. Thus these agents show promise in evaluating the types of heart disease that cause aberrations in myocardial cell membrane potential.



Experiments on rats are performed to evaluate new radiopharmaceuticals. (Credit: ORNL)

Improved radionuclide generators

Significant progress has been made over the years in developing an improved radionuclide generator for detecting heart defects in children and adults. A generator developed by ORNL now is in clinical use at several institutions in Europe.*

Radionuclide generators are widely used in clinical nuclear medicine to produce short-lived imaging agents, which are desirable because they expose patients to only a very low level of potentially hazardous radioactivity. A radionuclide generator consists of a small column containing an adsorbent material (such as activated carbon) onto which is placed a "parent" radioisotope, such as osmium-191. The parent decays to a "daughter" radioisotope, such as iridium-191m, which is a different element with properties that make it a useful tracer for clinical diagnostics. Because the parent and daughter are different elements, they have different chemical properties, which can be exploited by selecting the proper adsorbent for their separation.

The most efficient radionuclide generator uses an adsorbent that tightly binds the parent isotope yet allows the daughter to be removed easily when an aqueous solution is passed through the generator. The process of removing the daughter is known as elution, or "milking" the generator; the generator itself is referred to as a radioactive "cow". The parent radioisotope generally is produced in either a reactor or a particle accelerator (cyclotron). Usually the parent is undesirable for use in humans, however, because of its long physical half-life and high radioactive emissions. Such properties can increase the absorbed radiation dose to the patient and lead to poor images. Elution of the parent, also known as "breakthrough", must be completely avoided or, at worst, minimized. Thus, the generator design is based upon the ability of the adsorbent to bind the parent tightly and to allow easy elution of the daughter radioisotope.

For some years, ORNL's Nuclear Medicine Group has been exploring improved designs for the osmium-iridium generator. Interest in the development of an improved osmium-191/iridium-191m generator was stimulated by Dr Salvador Treves at Boston's Children's Hospital in Massachusetts, USA, who pioneered the clinical use of iridium-191m with an earlier generator design. Iridium-191m is an attractive radioisotope for diagnostic applications for several reasons. Unlike fatty acids, iridium stays in the blood and is not extracted by the heart muscle. The agent thus can be used for "blood pool" imaging, in which the radioactive blood is monitored as it flows through the heart chambers and lungs. This type of monitoring is needed to evaluate heart function and arterial blood flow in adults and also to detect a shunt, an abnormal hole between the chambers of the heart.

Because the iridium-191m daughter has an extremely short physical half-life — 4.96 seconds — it is also ideal for pediatric patients. The radiation dose is very low, and repeat studies can

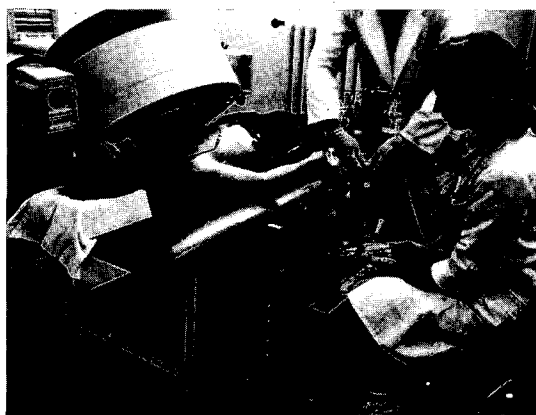
be performed within a short time. A further advantage of the iridium generator is that the parent has a relatively long half-life (15 days). Thus, the generator could have a potentially useful "shelf-life" of two to four weeks, providing that the problems of parent "breakthrough" and decreasing yields of iridium-191m can be overcome.

Extending the "shelf-life"

In collaboration with Claude Brihaye, a scientist from Liège, Belgium, who was at ORNL in 1983 on sabbatical, 40 different adsorbents using three different oxidation states of osmium-191 were evaluated to find out how to extend the useful life of the iridium-191m generator. These studies, which involved more than 1000 different measurements, consisted of mixing the various osmium species with the individual adsorbents and then measuring the uptake of the radioactive osmium on the adsorbents after centrifugation. If the osmium shows good uptake on an adsorbent, that adsorbent has the desirable property of tightly binding the osmium. Also studied was the chemical problem of separating a small amount of iridium from osmium and of preventing the osmium from contaminating the iridium product (such contamination would increase the radiation hazard to the patient). The key to this development was the selection of the best adsorbent. It was found that a special heat-treated activated carbon adsorbent exhibited excellent properties. The useful "shelf-life" of this new generator thus has been increased to several weeks and may offer for the first time the routine availability of an ultra-short-lived, generator-derived radionuclide.

The generator now is being used in adult patients in Europe, and it is expected that it will be approved in a matter of months for use in the United States to detect heart problems in children and adults.

This patient at the Institute for Clinical and Experimental Nuclear Medicine in Bonn was injected with iridium-191m obtained from the generator system shown here to evaluate heart function. (Credit: F.F. Knapp)



* Clinical use is in conjunction with ORNL collaborators Claude Brihaye and Marcel Guillaume at Sart Tilman University in Liège, Belgium, and with Dr Sven N. Reske and Dr Hans J. Biersack at the Institute for Clinical and Experimental Nuclear Medicine in Bonn, Federal Republic of Germany.

is low, indicating rapid clearance of the agent from the blood. This rapid clearance is an important property because the goal is to visualize (image) the radioactivity concentrated within the heart muscle with little or no interference from radioactivity in the blood pooled within the heart chambers. In the later images, the heart still could be clearly seen, despite a slight increase of radioactivity within the gastrointestinal tract caused by the excretion of part of the agent through the liver.

In experiments with rats injected with a differently modified cation, the group noted from the early and late

images the dramatic effects that structural modification can have on an agent's biodistribution properties. In this analogue, the heart uptake in the early image is clearly decreased, and radioactivity in the liver (hepatobiliary area) is high. More importantly, in the later image the group observed the transit of radioactivity into the intestinal tract. In short, the first analogue is clearly superior to the second one for imaging the lower portion of the heart muscle.

The effects of these structural changes on the tissue specificity and biodistribution properties of these agents

is an important aspect of the ORNL programme. A comprehensive and systematic evaluation of a series of modified agents (structure-activity study) is required to develop agents with the most desirable biological properties. After several structurally modified agents have been prepared and evaluated in rats, the effects of structural groups on biodistribution properties can be accurately assessed, and these data can be used to design an agent with optimal properties. Such an agent may be studied further and eventually evaluated in clinical trials for possible use in human patients.

Clinical tests

Although the ORNL nuclear medicine programme receives funding from the US Department of Energy's Office of Health and Environmental Research and the National Institutes of Health to pursue new ideas and concepts for the development of improved radiopharmaceuticals, the eventual goal is making these new agents available for clinical testing. As an organization, ORNL is not licensed to distribute radiopharmaceuticals, but it is actively associated with a variety of outstanding clinical and research organizations. It supplies collaborators in these organizations with agents developed in the programme for further, more extensive preclinical testing or clinical evaluation.

When the agents reach the point of being considered for clinical evaluation, the clinical investigators are responsible for obtaining approval from their human-use committee to use these agents on a limited basis in clinical trials. Such approval depends upon the results of chemical toxicity tests and calculations of the expected absorbed radiation dose to the patient. New agents must be known to be non-toxic and to expose the patient to only minimal radiation at the expected dose levels before they can be used in humans. The chronology of events leading from the development of a concept to use in patients is a very long path: Steps along the way include design of an agent, chemical synthesis, radiochemical synthesis, biological evaluation, imaging studies, toxicity testing, and determination of radiation dosimetry. As in the development of therapeutic drugs, only a few new radiopharmaceuticals will reach clinical testing. However, test results from even early steps can guide investigators in designing other radiopharmaceuticals.

Several of the heart-imaging agents that have been developed at ORNL are now being or will soon be used clinically on human patients. A new generator system has been developed that now is in clinical use in Europe, and the DMIPP fatty acid labelled with iodine-123 also is under clinical testing there. This fatty acid, considered a key agent, represents the culmination of several years of intensive investigation at ORNL to develop new agents to detect heart disease.

