Landmarks and Landmines in the Early History of Radiopharmaceuticals

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This is the second article in a six-part series on new radiopharmaceuticals. Upon completion of this article, the nuclear medicine technologist will be able to identify many of the radiopharmaceuticals that were useful for imaging between 1960 and 1980 and discuss some of the highlights and disappointments that occurred in the field of radiopharmaceutical chemistry during this period.

Most radiopharmaceuticals have similar, routine, preparative and quality control methodologies, which obscure the excitement with which they were first developed and introduced. The author reminisces about these events of the 1960s and 1970s, describing highlights and disappointments that he deems noteworthy. The present can be better appreciated with this glimpse into an earlier period of radiopharmaceutical development.

A curious nuclear medicine technologist might very well be interested in the beginnings of radiopharmaceutical use in nuclear medicine. Knowledge of how the field got started, how it relates to the products and distribution system, and what the major milestones were along the way may provide a firmer basis for an understanding of radiopharmaceutical science as it exists today.

This is a contemplative exercise by an individual who has been actively engaged in the practice of radiopharmaceutical chemistry since 1961, spending over five years in research and development with a radiopharmaceutical manufacturer and twenty years in an active academic environment of nuclear medicine.

EARLY SOURCES OF RADIOPHARMACEUTICALS

After World War II, Congress provided an organization, the Atomic Energy Commission (AEC), to develop and control the peaceful uses of atomic energy. National laboratories in Oak Ridge, Tennessee and Los Alamos, New Mexico were created and the inorganic radiochemicals that they isolated from atomic fuel and from irradiation of stable nuclei were made available to biochemical researchers. Conversion to suitable products for animal or human use was the province of the researcher.

It was fortuitous that one of the earliest available radioactive substances was sodium iodide iodine-131 (^{131}I), purchasable in adequate quantities from the national laboratories. In its basic chemical form, it was suitable as a radiotracer in thyroid function studies and for therapy in thyroid cancer. It also had synthetic chemical characteristics which allowed it to be incorporated into other compounds of biological importance.

Credit must be given to Abbott Laboratories, Chicago, Illinois, for establishing a radiopharmaceutical laboratory at Oak Ridge, in the mid-1950s and making many radiopharmaceutical products available to medical researchers and practitioners. The products were prepared with the necessary quality control, and pharmaceutical grade materials were shipped to those having appropriate AEC licensure. The catalog displaying Abbott's products indicated that they made them in specific quantities only and shipped them on specific days of the week. Their ^{131}I diagnostic capsules had a unique appearance: The capsules looked as if they were completely empty because they were made with a trace of ^{131}I liquid solution, and the clear gelatin capsule was sealed with only a small amount of liquid.

About five years later, a competitor emerged in the radiopharmaceutical market when E. R. Squibb, New Brunswick, NJ began to sell most of the same radiopharmaceuticals that Abbott Laboratories provided, but in any desired quantity, on any day of the week, and precalibrated to any requested time. This change made it possible for nuclear medicine departments to schedule patients for studies on any day of the week, much as is done today.

PHYSIOLOGICAL BASIS FOR RADIOCHEMICAL LABELING

Very few radioactive inorganic ions have had significant use in nuclear medicine without further incorporation into other complex molecules. Those that come to mind are ^{131}I, mentioned above, strontium-85 (^{85}Sr), fluoride-18 (^{18}F), and thallium-201 (^{201}Tl), whose utility could have been predicted in advance, and technetium-99m (^{99m}Tc) pertechnetate, whose utility could not have been predicted since there was no prior availability of this chemical.

Bone-Seeking Radionuclides

Researchers' knowledge of bone composition led them to expect that calcium, strontium, fluoride, and phosphate would prove useful in bone studies. Due to the physical characteristics of the available radionuclides of these ions, ultimately only strontium nitrate and sodium fluoride proved useful in bone studies, despite some of their disadvantages. The half-life of ^{85}Sr is too long; the tracer takes a number of days to clear from the abdomen, which delays scanning. Nevertheless, the product did have a satisfactory shelf life, even though the absorbed radiation dose to the patient left much to be desired. This radiopharmaceutical was the best one for bone scanning for many years, although generator-produced strontium-87m (^{87m}Sr) was also used considerably for the same purpose.

Fluoride-18 would have been superior to ^{85}Sr for bone scanning, but its short half-life made it unavailable to all except those with nearby cyclotrons. When one pharmaceutical company, Medi-Physics, Arlington Heights, Illinois, finally made a very laudable effort to provide ^{18}F to the nuclear medicine community, despite large decay losses during shipment, their efforts were quickly overshadowed by the concomitant successful development of ^{99m}Tc-labeled polyphosphate and diphosphonate compounds in kit form. This vast product improvement became a boon to nuclear medicine, but also highlights the importance of minimizing the delays in bringing a new product to market.

Iodine Radionuclides

Radioisotopic labeling with iodine was successfully employed prior to 1960 for drugs whose tissue distribution or organ accumulation characteristics were already known. Radiopaque compounds used in radiology had iodine in their molecules and the exchange method of labeling with ^{131}I produced materials with which kidney studies could be performed. Hippuran®, Renografin®, Diodrast®, and Conray® were members of this class. Of these, only ^{131}I-Hippuran (orthoiodohippuric acid) and ^{125}I-Conray (iothalamate) are still in significant use.

Also in this class of known medically useful compounds having iodine in the molecule is the dye rose bengal. Labeling by the exchange process, which frequently meant heating with ^{131}I at the proper pH, produced the radiopharmaceutical ^{131}I-rose bengal, which is still available for liver function studies. Also available in earlier years were the thyroid hormones triiodothyronine and thyroxine, for human use and research; these are now used mainly for the preparation of in vitro kits.

The chemical reactivity of iodine has made a variety of nonisotopically labeled products available throughout the history of radiopharmaceuticals. Reactions of iodine which are especially important are those of substitution of the halide on the ortho and para positions of phenols and aryl amines, and of addition across unsaturated alkyl bonds. The phenolic group of the amino acid tyrosine, which is attached to the molecule in the para position, has the two ortho positions available for substitution. Most protein iodinations take place in this manner. In the early 1960s, there were products made from oleic acid and triolein by iodination of the unsaturated

bonds by the fatty acid; they had names such as iodinated oleic acid, although the very molecules that were so iodinated were really derivatives of stearic acid. Some of the fatty acids that are being investigated today in studies of fatty acid metabolism are prepared by attaching an iodinatable aryl group to positions along the alkyl chain of fatty acids.

Similarly, the plasma extender polyvinylpyrollidone (PVP) and inulin, a drug eliminated primarily by glomerular filtration, were initially labeled with ^{131}I by adding a ligand to these polymeric molecules; to PVP, an iodinatable aryl group was added; to inulin, an unsaturated allyl group was added.

Iodinated Albumin

Human serum albumin (HSA) labeled with ^{131}I was one of the early successful materials used in brain scanning. It had the disadvantageous imaging properties of ^{131}I, poor target-to-nontarget ratios, and produced relatively high radioactivity in the blood and brain, which required days to dissipate before scanning could proceed. Fortunately, this product was replaced by other radiopharmaceuticals more suited for brain scanning. The importance of this product, in addition to facilitating blood pool and blood volume determinations, was that two new products were prepared from it. By heating carefully to a temperature of 65°C–70°C, a slight but definite denaturation of the albumin could be produced. The resulting product became a colloidal solution, which upon injection was removed from the vascular system by the reticuloendothelial system. Upon heating to 70°C–80°C, the albumin would become denatured and coagulate into visible particles. With suitable stirring during the heating process, the particle size of the aggregates could be controlled to meet the limits suitable for pulmonary perfusion imaging. The resulting product, macroaggregated albumin (MAA), remained the mainstay for pulmonary scanning. It had a shelf life of one month and could always be kept in stock for emergencies and weekends.

Mercury Radionuclides

Prior to 1950, most of the effective diuretics used in humans were mercury-containing compounds, which were administered parenterally. In the early 1950s, the first orally administered diuretic, containing mercury, was approved for human use. This compound was named chlormerodrin, and it was an immediate success. With the availability of mercury-203 (^{203}Hg), it was just a matter of time before ^{203}Hg-chlormerodrin, an isotopically labeled drug, was described in the literature and made available by radiopharmaceutical manufacturers. It turned out to be useful for kidney studies, as anticipated, but also for brain scanning. It seemed that all new radioactive drugs were tested for utility in the brain scanning process and this one was successful.

After many months, another mercury radionuclide became available, ^{197}Hg, with a shorter half-life and lower energy gamma radiation than ^{203}Hg. This radionuclide was also used for synthesis of chlormerodrin and both products were available for the same purposes. After several clinical investigations designed to determine superiority, it became clear that the

[203]Hg product should be avoided for brain studies because of the unnecessarily high absorbed radiation dose to the kidneys. For delineation of structures from deep within the body, [203]Hg seemed preferable. But for kidney studies, [197]Hg-chlormerodrin was the preferred product. An expiration date of a few half-lives after the receipt date was considered adequate. This radiopharmaceutical remained in commercial manufacture and in official compendia long after more suitable products were available.

Another product that was labeled with [197]Hg and was favored in its day, was mercuryhydroxypropane (MHP). This relatively simple molecule was reported in the literature to have erythrocyte denaturation properties. When [197]Hg-MHP was incubated with human red cells, it caused these cells to be denatured and sequestered by the spleen. Interest in this spleen-scanning product diminished due to the suboptimal radiation properties of [197]Hg and the specific activity with which it was synthesized. With the minimum chemical quantity necessary for the denaturation effect to take place, passage of one half-life of the radionuclide was sufficient to lower the specific activity so that a toxic amount of MHP would have to be injected.

1-Selenomethionine-Se-75

Another nonisotopic label used in nuclear medicine studies was selenium-75 (^{75}Se); it was used as a replacement for sulfur in the amino acid methionine. This amino acid was administered to humans in a protocol that stimulated the pancreas to synthesize the protein for production of pancreatic hormone (needed for the digestion process). The manufacturing procedure is of interest because of the manner in which a natural process was used to make a radiopharmaceutical. Ordinary yeast was grown in a medium deficient in sulfur, but to which inorganic ^{75}Se salt was added as a replacement. The protein grown within the yeast cells contained ^{75}Se-methionine. The harvested cells were then heated with acid in a sealed glass vessel to digest the protein, and the liberated amino acids were separated on an ion exchange column. The fraction containing 1-methionine, now labeled with ^{75}Se in place of sulfur, was then processed as a pharmaceutical for pancreatic imaging. Although the pancreas generally incorporated ^{75}Se-1-selenomethionine with a high injected dose per gram of tissue (more than twice that of the adjacent liver), the larger total mass of the liver had a radioactivity level that obscured the pancreas. Thus, it was not diagnostically visible, except in individuals of slender build who have a minimal overlap of these organs.

GENERATORS

The importance of the radionuclide generator was known before 1960. The terminology frequently heard with respect to eluting the ^{99m}Tc generator was "milking the cow." Brucer wrote about these systems in one of his early vignettes, entitled, "Herd of Radioisotope Cows" (1), and ascribed the terminology to an earlier writer. Later, when it appeared that the development of an improvement to the generator in the form of a foolproof liquid/liquid extraction mechanism was

about to invade and revolutionize the preparation of ^{99m}Tc, the manufacturer of the apparatus started a sales program, which included a lapel button showing a cow on its back, with its feet up in the air, and the logo, "The cow is dead." It was not to be.

As early as 1961, there was already a well made generator, prepared by Brookhaven National Laboratory (BNL), Upton, New York. It contained strontium-90 (^{90}Sr) on an ion-exchange resin and produced yttrium-90 (^{90}Y) upon elution with a citrate buffer. To make the eluate useful, it was necessary to remove the citrate ion by oxidation with 30% hydrogen peroxide while heating it to dryness in a nitrogen stream. The dried yttrium oxide was then dissolved in an appropriate solvent and terminally filtered for clinical use in therapeutic nuclear medicine applications. Parent breakthrough measurement was performed by taking an aliquot of the eluate, diluting it with another solvent and passing it over a second ion-exchange column to remove all the yttrium and leave any strontium ions in the eluate.

This second eluate was brought to an exact volume in a volumetric flask; then, a small accurately measured aliquot was removed, placed on a planchette, dried, counted in a beta counting assembly, and compared to a range of known ^{90}Sr quantities. In this manner, the amount of ^{90}Sr per mCi of ^{90}Y could be determined. The life of this generator depended on the ^{90}Sr breakthrough.

Technetium-99m Generator

The usefulness of the ^{99m}Tc generator was demonstrated in the early 1960s at BNL. For approved clinical use, it was necessary to verify the absence of several radionuclidic impurities that originated in the fission sources from which the molybdenum-99 ([99]Mo) was obtained. This was a distinct drawback to widespread use. With the availability of reactor [99]Mo, these radionuclidic impurities were not possible, and such quality control was unnecessary. The relatively lower specific activity of reactor [99]Mo simply needed a larger bed of alumina to support the larger mass of parent radionuclide. Early eluting fluid was dilute hydrochloric acid, which had to be neutralized with consideration given to isotonicity and sterility. It was later determined that isotonic saline was adequate for separating the [99m]Tc.

At this point, Squibb Medotopes made a significant contribution by developing a sterile generator. Essentially, it was the same basic unit that exists today. It consisted of a tube, fitted with vial type openings at each end, which could be closed with rubber crimp seals. It also had a fritted glass support within the tube to retain the aluminum oxide. The unit, after loading with the generator essentials, was made sterile and housed in adequate lead shielding. The user was required to swab both ends of the generator through small holes at the top and bottom of the lead shield and then place the unit onto a tripod support. A sterile needle attached to tubing could be held upright and forced into the rubber septum of the bottom of the generator, through the hole at the bottom of the shield, as the generator was lowered onto the tripod. This was a little tricky to do without striking the

lead against the sterile needle. Strong hand and wrist muscles were necessary for this task, especially if the generator was stored behind an additional lead shield. Saline was introduced through the top hole of the shield.

This type of generator had a less than desirable characteristic: it required a relatively large volume of saline for effective removal of ^{99m}Tc. Typically, 30-ml volume vials were used and to obtain high concentrations of radioactivity for blood flow studies, it was necessary to perform fractional elutions, that is, to collect small fractions of the eluate at any one time, and use only those fractions with satisfactory radioconcentrations.

The eluate from these generators was used for brain imaging, for the preparation of sulfur colloid, and for albumin labeling, through the reducing effect of ferrous ascorbate. This reducing agent was subsequently found useful as a renal scanning agent with added DTPA.

Unusual problems were always there to be solved in the nuclear medicine laboratory, but there have been few events as vexing as the following. Generators were received ready to use on Monday mornings, but were calibrated for Friday, the last day of practical use. As an approximate example, a generator might produce 1 Ci of ^{99m}Tc on Monday, 800 mCi on Tuesdays, and so on. Periodically, a generator which was eluted successfully on Monday, was eluted on Tuesday in the usual manner, with an expected yield of ~800 mCi, but yielded only ~250 mCi. After checking all the numbers without finding any error, a second elution produced an additional 200 mCi. After calling the manufacturer to find out if there has been any defect reported (and of course, there were none), one would wait about 30 min, which is the time it took to make as many products as possible with the radioactivity available, and elute again. This time one was rewarded with another 300 mCi. After another hour or so, 150 mCi could be squeezed out. At this point, it did not matter anymore because one could manage for the rest of the day, and the situation did not occur again for the rest of the week. However, the situation would recur at unexpected weekly intervals with new generators, was not confined to one manufacturer of sterile generators, and would recur only following the first day's successful use. Manufacturers could not explain the situation and salespeople would refer to it glibly as "second day drop-off."

Ultimately, the cause proved to be lack of sufficient oxidation properties in the generator, which prevented ^{99m}Tc from being in the highest oxidation state (pertechnetate), which was the necessary form for saline elution to take place. Generator manufacturers then supplied saline for elution, with added hydrogen peroxide or sodium hypochlorite, and the problem disappeared. You could take some of this saline with hypochlorites, sprinkle some on your hands, rub your hands together, and they would smell as if you had been washing clothing in chlorine bleach. These eluates were useful for preparing the radiopharmaceuticals for the studies then being performed.

Following the solution of this problem, a new one appeared with the development of "kits" for newer radiopharmaceuti-

cals, in which stannous ion as a reducing agent was a key additive. The ^{99m}Tc from these generators was completely ineffective because of the oxidizing agent the kits contained. Brochures for these new products contained the warning that eluates containing oxidizing agents not be used for kit reconstitution. To the credit of New England Nuclear (NEN), North Billerica, Massachusetts, which subsequently became du Pont NEN, and is now Du Pont Pharma, its type of generator operated on a different principle, one which permitted sterile air to be held within the column of the generator between elutions and which provided an adequate level of oxidation. Other companies have since improved their generators, so this sequence of events did not occur again.

Instant Technetium

It must be understood that at this stage of the game, the use of a ^{99m}Tc generator, the preparation of the one or two kits available at the time, and the considerable amount of special work possible for preparing products known only by their description in the literature, required special chemistry expertise in hospital nuclear medicine departments. Large radiopharmaceutical manufacturers had not reconciled themselves to the decay losses involved in shipping already eluted ^{99m}Tc and its products through regular transportation channels, although there would have been great demand for them.

Throughout this time, Union Carbide Corporation, Sterling Forest, New York, had a private nuclear reactor and had been supplying numerous radionuclides to other manufacturers, just as the national laboratories did. Two of their employees, aware of nuclear medicine and its needs, recognized the utility of making ^{99m}Tc available to many medical institutions. They opened a company at another private reactor (IRL, Plainsboro, New Jersey), capable of producing ^{99}Mo. The new company, Cambridge Nuclear, Plainsboro, New Jersey, started its operations late in the evening and went through the night, shipping ^{99m}Tc made by liquid/liquid extraction, ^{99m}Tc sulfur colloid, and ^{99m}Tc-MAA to institutions along the eastern corridor, from Boston to Washington, DC, timed to arrive for early morning use. The company's operation made ^{99m}Tc comfortably available to many nuclear medicine departments. Cambridge Nuclear also developed xenon-133 (^{133}Xe) in multidose compressed gas cylinders, which were then in demand. Ultimately, this operation ceased and Cambridge Nuclear became Cadema, Middletown, New York. Since then, "instant ^{99m}Tc" has been continually available from other sources.

MAA and Microspheres

Making MAA with a ^{99m}Tc label for lung scanning was not easy in earlier years. Prior to the availability of the kits in use today, the most consistently adequate method for preparing it required mixing ^{99m}Tc sulfur colloid with a small quantity of serum albumin and heating the mixture to about 80°C with agitation. As the albumin became denatured by the heating process, it managed to enmesh the colloid, leaving only minimal amounts of colloid not included with the MAA. With practice, a consistently good product could be made, except

for one problem. Each batch made, required as part of its quality control, an examination under the microscope to be sure that the particle size of the aggregates was within limits for this usage. Since it was not really feasible to examine the entire batch under the microscope, it also required visual macro-examination to be sure there were no obvious large clumps of aggregates.

At about the same time, the 3M Corporation, Minneapolis, Minnesota, with its expertise in the field of microencapsulation, was induced to enter the field. It contributed a rather unique product, denatured human albumin microspheres (HAM) for kit labeling with ^{99m}Tc. The product contained aggregated albumin whose size was always limited to that of the starting microspheres, so that there was no concern about accidentally injecting unexpectedly large aggregates made by the extemporaneous heat aggregation method.

The kit vial was designed almost exactly like the column within the ^{99m}Tc generator; it was a double-ended vial with a sintered glass support about three-quarters of the way down the vial. The short side of the vial was filled with an ion-exchange resin designed to release a hydrogen ion when an aqueous liquid passed over it. With a quantity of ^{99m}Tc injected and the ion-exchange resin end up, the liquid picked up the hydrogen ion and the acidified ^{99m}Tc eluate was forced through the sintered glass frit to the other side. That side of the vial had the preformed microspheres and the ingredients in tablet form, which were necessary to prepare ^{99m}Tc sulfur colloid. After inverting the vial, it was inserted in a boiling water bath and heated as for the preparation of the colloid. When the heating stage was ended, much of the prepared sulfur colloid was in colloidal suspension, but also a large portion adhered to the outer surface of the microspheres, as could be identified by microscopic examination.

Through a needle inserted at the base, most of the unattached sulfur colloid could be removed through the fritted glass, leaving the labeled microspheres above, unable to pass through the sintered glass frit. The microspheres were then resuspended in saline containing a surface-active agent to facilitate resuspension. Unfortunately, an ultrasonic bath was necessary for their resuspension each time a dose had to be withdrawn from the top of the vial. Otherwise, there could be grape-like clusters of microspheres. An elegant cylindrical lead shield, open top and bottom for access to the rubber septa, was available to devotees of this product.

Stannous Ion Reduction of Technetium-99m

The development and use of stannous ion as a reducing agent reduced pertechnetate to valence states from which

chelation and complexation was possible with numerous materials, such as those which are in almost all of the kits in use today. It made a quick end to the complex procedure for producing microspheres and transformed the product to just a vial of microspheres and stannous ion.

CONCLUSION

An overall view of the changes that have occurred between 1960 and 1980 shows that, initially, radiopharmaceutical manufacturers were in a key position to supply nuclear medicine with its needs, ^{131}I products, ^{85}Sr, gold-198 colloid, iron-59, chromium-51, cobalt-57, etc. As the development of ^{99m}Tc generators proceeded, and sterile generators were supplied, the action shifted to the academic or hospital-based laboratories, which provided ^{99m}Tc-based products, supplanting less desirable radionuclides, at a time when kits were not available. However, the current capability with kits has restored new product development to the radiopharmaceutical manufacturers. They now find independent research is leading to new products that are quite profitable, and they are actively engaged in this research.

It is also important to note the changes in the radiopharmaceutical distribution system resulting from centralized nuclear pharmacies. They have taken on the burden of preparation and quality control of radiopharmaceuticals; this gives the nuclear medicine technologist more free time for other important duties.

If ten years from now, someone recalls the last 20 years of radiopharmaceutical changes, those changes might well include red and white blood cell labeling, the rubidium-82 generator, the superiority of stereospecific compound labeling, the success in labeling monoclonal antibodies and genetically engineered drugs, and perhaps, the convening of a task force to reconsider injected doses of radioactivity directly in terms of whole numbers or simple fractions of Becquerels, in place of the whole numbers of millicuries in use today.

ACKNOWLEDGMENT

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