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Survey of Radiopharmaceuticals and Their Current Status

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Keyphrases □ Radiopharmaceuticals—survey and review of development, selection, availability, and mechanism of action as diagnostic and therapeutic agents □ Diagnostic radiopharmaceuticals—survey and review of development, selection, availability, and mechanism of action □ Scanning agents—survey and review of radiopharmaceuticals

The term radiopharmaceutical may be considered as encompassing *(a)* drugs that are specifically used by reason of their radioactivity for supplying fundamental information of the normal and pathological processes in mammalian systems and *(b)* compounds that are radioactive *per se* or may be converted *in situ* to radioactive substances whose radiation is then effective in destroying tissue. This presentation[1] will mainly examine scanning agents and other radioactive compounds that have proved to be useful diagnostic substances and therapeutic drugs that are capable of delivering intense ionizing radiation for tissue destruction. Usually, although not invariably, the latter group involves the eradication of malignant processes.

The nuclear chemistry associated with the formation, production, and purification of the radionuclides and the instrumentation and detection devices used to measure the emitted photons will be mentioned only briefly. Since this is a survey, what we shall discuss in a cursory fashion are the radiopharmaceuticals in current use, the methods for their development and selection, the mechanisms by which they function if known, and future trends in the formation of more effective drugs for both diagnostic and therapeutic uses. This article will serve as an overview of radiopharmaceuticals and will offer a useful beginning for an in-depth examination of certain radionuclides and those compounds that contain them.

The development of radiopharmaceuticals has largely evolved from the useful collaboration of the clinician with the nuclear physicist. As a consequence, the physical and preparative characteristics of the radionuclides have been of prime consideration, namely physical half-life, methods for production and separation, and type of emission including the percentage and energies of those particles in the decay processes. Development of such drugs on the basis of their chemical, biochemical, physiological, and pharmacological properties has certainly been of subordinate consideration. In fact, drug selection has been arrived at frequently in a pragmatic way, and only subsequently have efforts been expended to elucidate the underlying biochemical factors involved in the utility of a particular radiopharmaceutical drug. Examples of this development process are numerous in nuclear medicine. This emphasis on physical characteristics is readily understandable since isotopic half-life and emission characteristics are of prime importance in any diagnostic and therapeutic maneuver. Also, with few exceptions, the underlying biochemical and physiological differences between normal and pathological processes have not been fully understood, and there is little knowledge concerning drugs that specifically localize in various dis-

[1] A previous review article entitled "Radiopharmaceuticals," by W. Wolf and M. Tubis was published in *J. Pharm. Sci.*, 56, 1(1967).

ease processes. Such information is important and will continue to unfold, but clinicians have rightly not waited for such a complete understanding prior to using radiopharmaceuticals. However, these major deficiencies in our knowledge of drug distribution and related factors have limited the rational development of new radiopharmaceuticals. As the biochemistry of various diseases becomes clearer, radioactive drugs will be developed to utilize these physiological and metabolic differences between normal and pathological tissues as a means for diagnosis and treatment. For the purpose of this presentation, drugs in each category will be considered separately.

DIAGNOSTIC DRUGS

Diagnostic drugs may be divided into two categories. In the first are those that may be classed as *in vivo* drugs and must be administered to the patient to obtain diagnostic information. In the second are those substances that are used in *in vitro* procedures and no radioactivity is injected or ingested. Radioactive drugs for diagnostic purposes are in a higher state of development than are compounds suggested for therapeutic usage. The reasons for this difference are readily apparent when we consider what we are trying to achieve by each technique. In the case of *in vivo* diagnostics, we may wish to show either differences between normal or abnormal physiological processes or the delineation between normal and diseased tissues. In either case, we wish to visualize or determine by scanning and camera devices or to quantitate by external probes such differences. Ideally, the dosage of the radiopharmaceutical for such a purpose is miniscule, and the amount of radiation delivered by such drugs to the whole body or to a particular tissue or organ is inconsequential, even to the tissue being scanned. Therefore, the entire procedure is relatively innocuous from a radiation standpoint as well as other toxicological considerations. However, there have been instances where a radiopharmaceutical has an extremely long half-life for a particular tissue; under these conditions, radiation delivered to the organ in question may be sufficiently great so that procedures are required to minimize this radiation dose. But this is not the usual problem encountered in diagnosis, and because of the benign nature of the procedure as well as the minimal trauma to the patient, radiopharmaceuticals have achieved widespread use as *in vivo* diagnostic agents. It is, of course, apparent that *in vitro* procedures are even more attractive since the patient receives no radioactive drugs *per se*. It is a sample of the patient's body fluids or tissues that is removed and exposed to the radiopharmaceuticals under *in vitro* conditions.

The problem in the development of therapeutic compounds is totally different. In this case, the attempt is to utilize the radiation emitted by the drug to effect tissue destruction. For this reason, this procedure is usually confined to the eradication of malignant processes. Obviously, we are not attempting to destroy all tissues but only those responsible for the pathology. Consequently, a concentration differential between the diseased and normal tissues must be achieved that is orders of magnitude greater than would be needed for diagnostic purposes. This accounts for the limited usefulness of such tissue-destroying drugs since, generally, one cannot achieve the high selective localization of the radiopharmaceutical in the neoplastic lesion or other uneconomic tissue. This selectivity is important for therapy since the dose administered is of such an order of magnitude that it must be confined, for all practical purposes, to the target tissue. Even moderate to low percentages of the dose administered appearing in normal tissues may have a deleterious effect upon organ function. This requirement for therapeutic drugs is so stringent that the use of radiopharmaceuticals for therapy is greatly limited and not of major importance. Therefore, these drugs primarily are used for diagnostic purposes.

In the use of an *in vivo* drug, whether for diagnosis or therapy, one wants the drug in the system ideally only for the time required to complete the procedure. This applies whether one is considering anesthetics, analgesics, or radiopharmaceuticals. The question that presents itself is how a radiopharmaceutical can be localized and its time duration be limited in a particular target tissue. Before examining the various organs and tissues and the drugs currently in use that function effectively as *in vivo* diagnostic agents, let us consider in general the ways of assessing the duration of drugs in humans and, more specifically, these methods as applied to radiopharmaceuticals and factors that may affect this duration. If the drug were stable, we would simply determine its biological half-life, which is a measure of its duration in humans, by examining both excretion and metabolism. However, since a radioactive drug is constantly decomposing, we must also be concerned with its physical half-life as well as the products derived from it.

The nuclide produced as the result of the radioactive decay of another nuclide (referred to as the daughter and parent, respectively) must be considered when evaluating the parent and its products as a potential radiopharmaceutical. The ideal situation occurs when the daughter product is stable or has an extremely long half-life so that, when compared to the human lifespan, it can be considered stable. ^{99m}Tc falls into this category as a parent, since its daughter product ^{99}Tc has a $T_{1/2}$ of 2×10^5 years. Another acceptable condition is when the daughter product has a half-life shorter than, equal to, or only slightly longer than the parent and has photons of similar energy with the absence of β-rays and positron emission. The unacceptable occurs when the daughter is relatively long lived compared to the parent and has α-, β-, positron, or high-energy γ-rays.

To determine the effective half-life of such a drug, its physical decay scheme, including those of the daughter products, and its biological clearance or metabolism must be combined. The effective half-life, T_e, can therefore be expressed in terms of both its biological, T_b, and physical, T_p, half-lives as

shown in Eqs. 1a–1c:

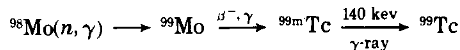
$$\frac{1}{T_e} = \frac{1}{T_b} + \frac{1}{T_p} \quad \text{(Eq. 1a)}$$

$$\frac{1}{T_e} = \frac{T_p + T_b}{T_b T_p} \quad \text{(Eq. 1b)}$$

$$\frac{1}{T_e} = \frac{T_b T_p}{T_p + T_b} \quad \text{(Eq. 1c)}$$

As instrumentation and, more specifically, the scintillation cameras, together with the requisite data processing methodology, have improved and the diagnostic procedure times have been progressively reduced, it has become highly desirable to match the effective half-life to this procedure time. If we have a drug with a particular biological half-life, we can strive to decrease T_e by incorporating a radionuclide with a shorter T_p. However, what is important is the time for optimum localization of the drug in the tissue to be visualized. Wagner's (1) criterion is that the ideal effective $T_{1/2}$ for a radionuclide totally retained by the mammalian system is equal to the product of 0.693 and the time at which scanning is to be performed. The matching of the scanning time with the drug's effective half-life is one reason for the development of shorter half-life radionuclides since the optimum localization time may then be sufficiently short. Certainly, the ability to administer larger quantities of radioactive compounds with a minimal radiation dose and a concomitant improvement in target visualization are important factors as well. When considering the use of isotopes with half-lives in the hour and minute range, it was necessary in the past that there be close proximity to reactors and cyclotrons. In many instances, this is still the case [*i.e.*, use of $^{15}O_2$ (2 min), use of ^{13}N (10 min), and use of ^{11}C-labeled carbon dioxide (20 min) in pulmonary studies]. However, the development of nuclide generators and the availability of nuclides from commercial sources have provided a convenient means for using short-lived materials at great distances from the source of production. ^{99m}Tc, probably the single most important nuclide in radiopharmacy, is an example of a radionuclide produced by such a generator system (Scheme I) (2). Other such nuclide generators may become of increasing importance in the future in nuclear medicine (3). These generator systems are presented in Table I.

Just as the optimum effective half-life should be matched to the scanning time, likewise very little energy, aside from the emission needed for detection or destruction, should be produced within or absorbed by the individual involved in the procedure. The desired γ-ray energies for use in external scanning or camera detection systems are roughly in the 150–400-kev range, with a possible optimum value of about 250 kev. Appreciably lower energies in the 30-kev range result in total absorption; in the 80–100-kev range, interaction with tissue occurs, resulting in the scattering of these photons and obscuring of the tissue visualization. Nuclides on the opposite end of the energy spectrum with γ-ray energies above 500 kev are extremely difficult to collimate and result in poor scan quality from the degraded resolution.



$(T_{1/2} = 67\text{ hr})$ $(T_{1/2} = 6\text{ hr})$

Scheme I—^{99m}Tc generator

Table I—Nuclide Generator Systems

Parent Isotope	Half-Life	Daughter Isotope	Half-Life
	γ-Emitters		
^{81}Rb	4.7 hr	^{81m}Kr	13 sec
^{68}Ge	280 days	^{68}Ga	68 min
^{113}Sn	118 days	^{113m}In	1.7 hr
^{132}Te	3.2 days	^{132}I	2.3 hr
^{87}Y	80 hr	^{87m}Sr	2.8 hr
^{99}Mo	67 hr	^{99m}Tc	6 hr
^{90}Sr	28 years	^{90}Y	64 hr
^{137}Cs	30 years	^{137m}Ba	2.6 min
^{44}Ti	10^3 years	^{44}Sc	3.9 hr
^{103}Pd	17 days	^{103m}Rh	57 min
^{77}Br	58 hr	^{77m}Se	18 sec
^{191}Os	16 days	^{191}Ir	4.9 sec
^{28}Mg	21 hr	^{109m}Al	2.3 min
^{140}Ba	12.8 days	^{140}La	40 hr
^{72}Se	8.4 days	^{72}As	26 hr
^{42}A	3.5 years	^{42}K	12.4 hr
	β-Emitters		
^{144}Ce	284 days	^{144}Pr	17.3 min

These physical decay characteristics are constants, and the given generalizations may be applied to all *in vivo* radiopharmaceuticals. The biological factors are, however, quite diverse, and the chemical composition and formulation are directly dependent upon such factors. In other words, understanding the biochemistry and physiology of both the normal and the abnormal or diseased tissue may permit the tailor-making of radiopharmaceuticals and their more selective localization in the abnormal structures. This would be the ideal.

It is certainly easier to visualize an elevated concentration of a radionuclide in a target organ than to observe the so-called "cold spots" areas of decreased isotopic concentrations in a matrix of high radioactive concentration. The problem, unfortunately, is the paucity of information regarding both biochemical and physiological processes which would permit the design of a radiopharmaceutical that could utilize such differences between normal and abnormal structures. In some instances, there is a great deal of information relating to normal function. But how the abnormal or diseased tissues differ in the first place frequently is unknown; and how this information, when it is known, may be used to develop a radiopharmaceutical may not be readily apparent.

Some important agents used for individual organs and the mechanisms for localization will now be discussed.

In Vivo *Radiopharmaceuticals*

Radiopharmaceuticals for Imaging—*Liver Agents*—The liver is one major organ for which scanning agents have been developed (4). These agents comprise two major categories: *(a)* those that are phagocytized by the Küpffer cells, and *(b)* those that are taken up by the polygonal cells and subsequently

excreted through the biliary system into the GI tract (Table II). Radiopharmaceuticals developed for the former are based on the fact that colloidal particles are phagocytized by the reticuloendothelial system; included in this system are the Küpffer cells of the liver (5). As will be apparent, this property of the reticuloendothelial cells is also utilized to scan the spleen and bone marrow. The uptake by these Küpffer cells is based primarily on particle size, a physical attribute of a particular material, and to a less extent upon any chemical property (6).

A number of colloidal agents have been considered and used at various times. Initially, clinicians used ^{131}I-labeled human serum albumin and colloidal ^{198}Au but now, with the availability of useful colloids containing the shorter T_p radionuclides ^{99m}Tc and ^{113m}In, the latter substances have become the drugs of choice, especially those containing ^{99m}Tc. Their use is dependent not only on half-life but also on suitable emission characteristics. In this regard, ^{99m}Tc is a particularly attractive radionuclide. Efforts have been directed toward more facile incorporation of these radioactive isotopes into colloidal particles or into substances from which colloidal particles may be generated (7, 8). In addition, attempts have been made to produce particles with a more uniform and predetermined particle size, as is essential in view of the short T_p of these isotopes.

Polygonal cells of the liver do absorb certain dyes and various organic and inorganic compounds in large amounts, and these are then excreted *via* the gallbladder into the GI system (9). This is the basis for the use of ^{131}I-rose bengal (sodium rose bengal I^{131}) and ^{131}I-sodium iodipamide (sodium iodipamide I^{131}). The facile incorporation of iodine into various organic structures had led to the widespread use of iodine-labeled radiopharmaceuticals. The physical characteristics of ^{131}I are not as suitable as other more current nuclides, but ^{123}I is highly desirable from a physical standpoint and its incorporation into organic structures may result in the more favorable competition of such compounds with ^{99m}Tc-labeled products. The key limitation has been the availability and cost of ^{123}I relative to other nuclides; but should compounds be developed having superior clinical properties, these restrictions will disappear (10). Other iodine isotopes have been considered and used in the past, but unfavorable half-life, decay pattern, and method of production have limited the use of ^{124}I, ^{125}I, ^{126}I, ^{130}I, ^{132}I, ^{133}I, and ^{135}I.

A most interesting technique which may have application to other organs in the future is the measure of liver metabolism by use of ^{75}Se-selenous acid (H_2SeO_3). In the liver, this compound is converted to the volatile gas $(CH_3)_2Se$, which is excreted by the lung. Hepatectomies or ligatures of the portal vein produce a significant diminution in the percentage of dimethyl selenide formed and excreted. It should be interesting to determine the effect of hepatomas on the metabolism of selenous acid. This procedure offers the opportunity of studying the metabolic function of an organ under *in vivo* conditions and is based upon the selection of drugs that are altered in specific ways by a single organ producing volatile products. It remains to be seen how general and widespread the applications of this technique will be in nuclear medicine.

Table II—Compounds for Liver Scanning and Function

Colloidal:
^{99m}Tc-sulfur colloid (Te_2S_7) (technetium Tc 99m sulfur colloid)
Human serum albumin (^{99m}Tc-labeled and ^{131}I-labeled)
^{113m}In-hydroxide gelatin
^{198}Au
^{99m}Tc-stannous phytate
Polygonal cells and biliary system excretors:
^{131}I-rose bengal (sodium rose bengal I 131)
^{131}I-sodium iodipamide (sodium iodipamide I 131)
^{99m}Tc(SCN)
^{99m}Tc-penicillamine and penicillamine–acetazolamide complex
^{99m}Tc-tetracycline
^{75}Se-selenous acid

Spleen and Bone Marrow Agents—Two other tissues that also have cells comprising the reticuloendothelial system are the spleen and bone marrow (Table III). Consequently, both tissues can be scanned by use of colloidal agents (11). As in the case of the liver, these drugs are concentrated by viable, normal tissue and the basis for using them as scanning agents is the possibility that they will be excluded from abnormal or pathological structures. The spleen also functions as a scavenger for damaged red blood cells, and it is on this basis that ^{51}Cr-labeled red blood cells, slightly damaged by heat treatment, are filtered out by the spleen and thereby serve as the means for scanning this organ (12). Once again, the normal functioning tissue will concentrate the radionuclide, and abnormal or pathological areas should show areas of decreased uptake. This procedure would also delineate any enlargement of this organ. ^{197}Hg-Hydroxy(2-hydroxypropyl)mercury[2] functions in a somewhat similar fashion (13). This drug, on intravenous administration, both labels and damages red blood cells; as a result, these cells are sequestered by the spleen. More recently, researchers have labeled red blood cells with ^{99m}Tc, which looks like a promising agent for scintigraphy of the spleen (14).

Bone marrow has been scanned by use of a colloid of ^{157}Dy-gluconate; 40–48% of the administered dose of this agent is concentrated by this tissue (8.1 hr $T_{1/2}$ ^{157}Dy-326 kev monoenergetic γ) (15). ^{111}In-Chloride has also been used for this same purpose (16). This cyclotron-produced radionuclide, with a half-life of 67 hr, has a γ-ray emission spectra that is ideal for use with gamma cameras and rectilinear scanners. These properties have resulted in increased consideration for the incorporation of ^{111}In into various compounds.

In addition to the reticuloendothelial structure, the bone marrow also contains cells with a hematopoietic function. This is the normal site for the production of erythrocytes; and since iron is an important component of red blood cells, erythrocyte incorporators such as ^{52}Fe and ^{59}Fe may serve as a means

[2] Merisoprol Hg 197.

Table III—Scanning Agents for the Spleen and Bone Marrow

Spleen scanning agents:
Colloidal agents
^{51}Cr-labeled red blood cells
^{197}Hg-hydroxy(2-hydroxypropyl)mercury (merisoprol Hg 197)
Bone marrow scanning agents:
Colloidal agents–reticuloendothelial system
Erythrocyte incorporators (^{52}Fe and ^{59}Fe)
^{157}Dy-gluconate
^{111}In-chloride

for delineating the functioning bone marrow elements (17).

Bone Scanning Agents—For the bone itself, several radionuclides have been used (18): strontium salts (as ^{85}Sr or ^{87m}Sr), gallium citrate (^{67}Ga and ^{68}Ga), ^{18}fluoride, barium salts (as ^{131}Ba, ^{135m}Ba, and ^{140}Ba), ^{47}calcium^{+2}, ^{99m}Tc-polyphosphates and ^{99m}Tc-diphosphonates, and ^{157}Dy-ethylenediaminetetraacetic acid chelate. Since the skeleton comprises 99% of the calcium of the body and its mineral composition is in a dynamic metabolic state, elements related chemically to calcium and other bone components have been used to visualize normal and diseased tissues. Two isotopes of calcium have been used, ^{45}Ca and ^{47}Ca. Also, in view of their chemical similarities to calcium, the other alkaline earth elements have been effective as bone scanning agents including two radionuclides of strontium and several of barium (19). Two of these, ^{135m}Ba with a single γ 268 kev ($T_{1/2}$ = 29 hr) and ^{131}Ba ($T_{1/2}$ = 11.6 days), have been proposed to be suitable for imaging with both the gamma camera and the rectilinear scanner. The bone uptake of ^{135m}Ba is very rapid; 60% of the dose is absorbed within 2 hr. Such isotopes are useful in the early detection of bone tumors, the visualization of fracture sites and osteoarthritic lesions, and the determination of metabolic bone diseases. These agents appear to have significant advantages over the strontium nuclides.

In addition to the alkaline earth elements, compounds of gallium have shown a propensity for bone (20). This observation has been the basis for the use of gallium citrate as ^{68}Ga and ^{67}Ga for the localization in this tissue, and these compounds are useful in delineating the extent of malignant processes (21). Recently, ^{157}Dy as its ethylenediaminetetraacetic acid chelate was reported to be an excellent bone scanning agent ($T_{1/2}$ = 8.1 hr, 100% electron capture with monoenergetic γ-ray 326 kev) (15). Approximately 50% of the injected dose appears in bone in 3 hr, with the remainder being excreted by the kidney. Based upon its physical and biological properties, this chelate is preferable with conventional gamma cameras and scanners compared to the other isotopes that have been considered heretofore. Not only have cations been used, but in the past ^{32}P-phosphate was found to appear even in the dense portions of the shaft of large bones. These findings led to the development of ^{99m}Tc-polyphosphate and various ^{99m}Tc-phosphonates as new drugs for skeletal imaging (22–25).

Fluoride, by reason of its facile ability to substitute in the hydroxyapatite crystals of bone, rapidly accumulates 10–20 times more in bone and skull than in the soft tissues of humans (26, 27). Tumors of the bone, with their increased metabolism, may attain 10 times the normal bone concentration. Consequently, even with a T_p of less than 2 hr, ^{18}F appears to be a useful isotope for this purpose. It has been applied in imaging radium-induced bone pathology and is especially useful in detecting active *versus* inactive lesions. This radionuclide also has been used recently in detecting lesions related to lymphoma metastases (28).

Kidney Agents—Kidney scanning agents take advantage of the biochemical and physiological properties of the renal system for their use in visualizing this structure (29). These agents include: organomercurials—^{197}Hg- and ^{203}Hg-chlormerodrin (chlormerodrin Hg 197 and Hg 203), ^{99m}Tc-iron–ascorbate complex, ^{125}I- and ^{131}I-sodium iodohippurate (sodium iodohippurate I 125 and I 131), and ^{99m}Tc-penthanil and other ^{99m}Tc-labeled agents which are rapidly excreted.

The development of mercury-containing drugs as kidney scanning agents arose from the initial use of certain mercurials as diuretics. It was observed that such compounds are rapidly taken up by the renal cortex and are also temporarily fixed by renal tubular cells. The thioactivated enzyme systems in this tissue, essential for renal tubular transport, are inactivated. As a consequence, these compounds labeled with ^{197}Hg have been useful in detecting the parenchymal structures (30). ^{203}Hg was used initially, but the high radiation dose delivered to the kidney by this nuclide favored the use of ^{197}Hg. Now more promising drugs are also used. Another compound which has been used is ^{131}I-sodium iodohippurate (31). It has utility because such compounds are urographic contrast agents and, in this particular case, the drug is rapidly excreted *via* the kidney. In this way, visualization of the kidney and the excretion process may be accomplished.

The need for improved renal imaging recently led to the synthesis and use of ^{123}I-sodium iodohippurate. ^{123}I has emission characteristics that are preferable to those of ^{131}I (32). Another drug which functions in a similar way is ^{99m}Tc-penthanil (diethylenetriaminepentaacetic acid) (33). It is rapidly cleared by the kidney *via* glomerular filtration and makes use of the desirable physical characteristics of ^{99m}Tc. Other ^{99m}Tc compounds have been prepared as possible renal imaging agents. These include a complex of ^{99m}Tc-penicillamine–acetazolamine (34), a ^{99m}Tc-iron–ascorbate complex (35), ^{99m}Tc-labeled meglumine diatrizoate (36), ^{99m}Tc-mannitol, and ^{99m}Tc-tetracycline (37).

Lung Agents—Particle size has been important to the development of lung scanning agents, and it will be useful to consider the rationale behind the use of such compounds (38). Radioactive colloidal particles, as already discussed, have been useful for visualizing the reticuloendothelial system. When particle size increases sufficiently, capillary trapping action re-

sults and the particular organ that acts as the filtering device may be visualized. This is the basis for the development of aggregated proteins and inorganic particulate materials, both labeled with those radionuclides having desirable physical properties. Such important characteristics are half-life and the energetics of the emitted radiation. Initially, ^{131}I-human serum albumin was macroaggregated. This compound has been used effectively in estimating regional pulmonary function, especially prior to preoperative lobectomy, and in locating nonfunctional and avascular regions (40). Subsequent research emphasized the preparation of materials with appreciably reduced pulmonary toxicity, greater uniformity in particle shape and size, autoclavability, and more suitable radionuclides from a standpoint of half-life and emission characteristics (Table IV). The desirable requirements have led to the preparation of metabolizable microspheres with greater uniformity (40–43) of human serum albumin containing ^{125}I, ^{99m}Tc, and ^{113m}In. The goal of uniform particle size was in part the basis for synthesizing ^{113m}In-, ^{111}In-, and ^{99m}Tc-labeled ferric hydroxide particles. Additionally, these can be autoclaved following their formation. The potential difficulty with these latter entities is that they are not completely biodegradable (44–49).

Radioactive gases also have been used to determine regions of decreased ventilation and perfusion (50). These agents include some very short-lived positron emitters: $^{15}O_2$ ($T_{1/2}$ = 2 min), ^{11}C (20 min), and ^{13}N (10 min) (51). In addition there are the noble gases, which are relatively inert biochemically and rapidly excreted *via* the pulmonary system. These include ^{85}Kr (10.3 years) and, more recently, ^{133}Xe (5.3 days), ^{135}Xe (9.2 hr), and ^{81m}Kr (13 sec) (52), which can be used to distinguish between pulmonary embolisms and chronic obstructive lung disease. Radioaerosols have been developed as inhalation scanning agents (50). ^{99m}Tc-labeled albumin, ^{99m}Tc-sulfur colloid, and ^{113m}In- and ^{111}In-hydroxides have been nebulized for this purpose. They have been used in studying obstructive pulmonary disease and are reported to have advantages over radioxenon diagnostic procedures in that they provide clinically useful information regarding precise sites of partial and complete bronchial obstruction. Their slow rate of removal has the distinct advantage of not requiring rapid imaging devices or procedures.

Circulatory Agents—Labeled proteins are also used in the measurement and/or visualization of the various circulatory systems (53–56). Other uses are the measurement of plasma volume, cardiac output, and cerebral blood flow (Table V). The labeling radionuclides for these proteins are ^{123}I, ^{125}I, ^{131}I, ^{99m}Tc, ^{113m}In, and ^{111}In (57). The latter two radionuclides are rapidly bound under *in vivo* conditions to plasma proteins (particularly transferrin) and in this way may be used for both circulation and blood scanning. Also, ^{129}Cs ($T_{1/2}$ = 32 hr) and ^{43}K ($T_{1/2}$ = 22.4 hr) have been suggested as suitable nuclides for myocardial uptake studies (58–60). These monovalent cations are rapidly accumulated by striated muscle and

Table IV—Lung Scanning Agents

Macroaggregated albumin and other proteins:
^{99m}Tc
^{131}I and other iodine isotopes
^{113m}In
Albumin microspheres:
^{99m}Tc
^{125}I
Ferric hydroxide aggregates:
^{113m}In
^{99m}Tc
^{68}Ga
Other macroaggregates:
Tc-sulfur colloid
Tc-labeled-glutaraldehyde
Radioactive gases:
^{11}C as CO and CO_2
$^{15}O_2$ as CO and CO_2
^{13}N
^{133}Xe and ^{135}Xe
^{81m}Kr and ^{85}Kr
Radioaerosols:
^{113m}In- and ^{111}In-hydroxides
^{99m}Tc-sulfur colloid (Tc_2S_7) (technetium Tc 99m sulfur colloid)
^{99m}Tc-albumin

myocardium. In an attempt to distinguish between normal and ischemic myocardium, ^{131}I-pyrazole was developed and shows a concentration gradient between normal and infarcted myocardium of 25 (61). Additional drugs which have been considered are ^{201}Tl, ^{13}N-ammonia, and ^{99m}Tc-tetracycline (62–64).

Several other tissues (Table V) that have been imaged by use of radiopharmaceuticals are the thyroid, pancreas, and parathyroid. Thyroid scanning was the initial basis for the development of radiopharmaceuticals (65). The propensity for the thyroid to sequester iodine and to incorporate it into hormones is well established. Therefore, thyroid scanning is on a firm biochemical basis, and uptake studies of iodine by this gland supply useful information to the clinician. Several iodine isotopes have been tried, including ^{131}I (8 days), ^{132}I (2.3 hr), ^{124}I (4.5 days), ^{125}I (60 days), ^{130}I (12.6 hr), and ^{123}I (13.3 hr). Of these, the two most widely used are ^{131}I and ^{125}I, due in large part to their availability, cost, and shelflife. From the standpoint of physical half-life and emission characteristics, ^{123}I is the more desirable γ-ray scanning agent. For diagnostic uses, it delivers only 1% of the radiation dose of ^{131}I and gives a clearer radiograph. A major limitation has been the difficulty to prepare ^{123}I free of undesirable impurities and in sufficient amounts. These obstacles now appear to be overcome with recent cyclotron techniques. This development presents the possibility of using this radionuclide as iodide itself for thyroid scanning and also to incorporate it into other carriers such as proteins for external visualization of certain tissues. Pertechnetate ($^{99m}TcO_4^-$) also has been used for thyroid scanning. It is trapped by the thyroid in a manner similar to iodide. However, iodide is preferable since the ratio of thyroid to nonthyroid tissue is appreciably greater for iodide vis-à-vis pertechnetate.

The pancreas incorporates certain amino acids with a high degree of localization. Since none of the elements in the structure of naturally occurring amino acids has suitable γ-emitters for diagnostic

Table V—Miscellaneous Scanning Agents

- Circulation and blood-pool scanning:
  - Labeled human serum albumin (^{99m}Tc, ^{131}I, and ^{125}I)
  - ^{113m}In-complexes
  - Radioactive gases (^{133}Xe and ^{81m}Kr)
  - ^{129}Cs
  - ^{99m}Tc-labeled red blood cells
- Myocardial agents:
  - ^{129}Cs salts
  - ^{43}K salts
  - ^{131}I-pyrazole
  - ^{201}Tl salts
  - ^{99m}Tc-tetracycline
  - ^{131}I-oleic acid
- Thyroid scanning:
  - Radioactive iodides (^{131}I, ^{125}I, and ^{123}I)
  - ^{99m}Tc-pertechnetate (sodium pertechnetate Tc 99m)
- Pancreas and parathyroid scanning:
  - ^{75}Se-Selenomethionine (selenomethionine Se 75)
  - ^{18}F-labeled amino acids
  - ^{11}C-amino acids

purposes, tagging of these amino acids has become an important goal. As a result of replacement of the sulfur in the essential amino acid, methionine, by selenium, an isostere is produced which functions sufficiently like the natural amino acid to be rapidly incorporated into the pancreas. ^{75}Se ($T_{1/2}$ = 127 days) is a radionuclide which has been used for this purpose. This same compound has also been reported as being useful for localizing parathyroid adenomas and lymphomas (66). Such fraudulent amino acids as ^{75}Se-methionine are incorporated facilely into tissues which are rapidly synthesizing proteins. It is for this purpose as well that the incorporation of ^{18}F into amino acids has been tried (67, 68). Also the formation of complexes of ^{99m}Tc with amino acids and peptides and the development of synthetic procedures for creating ^{11}C-amino acids have been undertaken. These compounds would also be useful in detecting processes, such as a malignancy, where extensive *de novo* protein synthesis is occurring. For example, L-aspartic acid has achieved elevated concentrations in tumor vis-à-vis surrounding normal tissues. As a result, ^{11}C-labeled amino acid may have direct clinical usefulness (69). However, it would be essential that the synthetic and clinical studies be very convenient to the cyclotron producing ^{11}C in view of its 20-min half-life (70).

Photoscanning of the adrenal gland and related tumors has also been undertaken. Radioiodinated compounds, which do show a predilection for this tissue, have served as the basis for such scanning procedures (71).

Brain Scanning Agents—In general, the basis on which brain scanning drugs have been developed stems largely from the difference in permeability between normal and abnormal brain to a wide variety of substances (72). Neoplastic brain is certainly not as selective or restrictive as normal brain of the compounds that are able to penetrate and concentrate within it. The so-called blood–brain barrier is either absent or in an appreciably altered form in tumor compared with normal brain. As one researcher remarked, most anything will achieve a higher concentration in brain tumor than in normal parenchyma

Table VI—Biological Basis of Localization

- Active transport:
  - Thyroid scanning with iodide or pertechnetate
  - Renal scanning with chlormerodrin
  - Liver scanning with rose bengal
  - Pancreatic scanning with selenomethionine
  - Thrombus scanning with fibrinogen or urokinase
  - Bone scanning with ^{18}F, ^{85}Sr, ^{87m}Sr, or Tc-polyphosphate
- Phagocytosis/pinocytosis:
  - Liver scanning with colloidal particles
  - Spleen scanning with colloidal particles
  - Bone marrow scanning with colloidal particles
  - Other (tumor, abscess, and lymphnodes) with colloid or labeled white blood cells
- Cell sequestration:
  - Spleen scanning with heat damaged ^{51}Se-labeled, ^{99m}Tc-labeled, or ^{197}Hg-hydroxy(2-hydroxypropyl)mercury (merisoprol)
- Capillary blockage:
  - Lung scanning with macroaggregate of albumin or ferric hydroxide labeled with ^{131}I, ^{113m}In, or ^{99m}Tc
  - Renal, liver, and brain scanning with the same aggregates injected intraarterially
  - Thrombophlebitis scanning with sulfur colloid or labeled white blood cells
- Simple diffusion:
  - Brain tumor scanning with pertechnetate or penthanil chelates
  - Tumor scanning with ^{67}Ga-citrate
- Compartmental localization:
  - Cardiac scanning with ^{99m}Tc-albumin, ^{99m}Tc-labeled, or ^{113m}In-transferrin
  - Placental and blood pool scanning with ^{99m}Tc-labeled or ^{113m}In-transferrin
  - Cisternography with ^{131}I-albumin, ^{169}Yb-penthanil, or ^{111}In-penthanil
  - Lymphatic scanning with colloidal ^{198}Au or ^{131}I-fats

of the brain and, as such, is a brain scanning agent. This is obviously not totally true since, in the scanning process of the central nervous system (CNS), one is visualizing more than just brain and any brain pathology. The scan is a composite of other tissues including blood, muscle, skull, and scalp. Consequently, other factors come into play in addition to the drug's exclusion by the normal brain; these factors may include persistently high blood concentrations, rapid accretion by skull or scalp, and elevated amounts in muscle compared with tumor.

Brain scanning agents that have been or are still being widely used in various diagnostic centers include: sodium pertechnetate ($^{99m}TcO_4^-$), ^{197}Hg-chlormerodrin [$NH_2CONHCH_2CH(OCH_3)CH_2HgCl$], labeled human serum albumin (^{131}I, ^{99m}Tc, ^{111}In, and ^{97}Ru), chelates of penthanil (^{64}Cu, ^{68}Ga, ^{113m}In, ^{99m}Tc, and ^{169}Yb), and miscellaneous substances [^{74}As (arsenate–arsenite), ^{204}Bi, ^{206}Bi, ^{131}I-polyvinylpyrrolidone, ^{111}In-bleomycin, and ^{67}Ga-citrate].

Sodium pertechnetate (Tc 99m) is in general use now as a brain scanning substance (73). This use results not so much from increased tumor specificity, but from its exclusion by normal brain, its reduced scanning time compared with longer half-life radionuclides, and the improved precision arising from the larger doses that may be administered. However, preliminary studies have indicated that cellular incorporation of ^{99m}Tc into brain tumors may occur. Binding of pertechnetate into albumin is not the *in*

vivo mechanism by which this agent delineates brain tumors, since *in vitro* incorporation of technetium into various blood proteins produced compounds that were completely unsatisfactory as cerebral imaging agents. Thus, the mechanism of how pertechnetate functions in delineating brain lesions still remains obscure.

The second agent ^{197}Hg-chlormerodrin is also a very effective brain scanning drug (74). This drug attaches itself rapidly and to a great extent (85%) to plasma proteins and, more specifically, to serum albumin. In view of the fact that the label is deposited on or within a wide variety of cerebral neoplasms, it is conceivable that protein binding *via* sulfhydryl groups is directly related to this incorporation. A pinocytotic mechanism may be involved by which the mercurated protein attaches itself to the pinocytotic vesicles (which are appreciably larger in human gliomas than in normal glial cells) and thereby is discharged into the intracytoplasmic compartment of the neoplastic cell. More extensive studies have been performed with radioiodinated human serum albumin. The findings support a pinocytotic mechanism or at least a pathway that preserves the unity of the protein with its tag during the intracellular localization of the drug. In addition to pertechnetate and proteins or drugs which become bound to proteins, a number of chelates containing radioactive nuclides have been used as scanning agents (75–78). Two widely used chelates are edetate (EDTA) and penthanil. As a result of the desirable physical properties of ^{99m}Tc, chelates such as penthanil have also been examined and found to have promise (79). ^{99m}Tc-Penthanil for brain scanning has advantages over pertechnetate because it does not accumulate in the choroid plexus, frontal sinuses, and salivary glands.

A few of the nuclides which have been tried in addition to ^{99m}Tc are ^{64}Cu, ^{68}Ga, ^{169}Yb ($T_{1/2}$ = 32 days), and ^{113m}In. ^{169}Yb has the advantage of a longer shelflife than the others, and its rapid excretion has permitted safe multimillicurie doses. As a result of these properties, it has also been considered as an agent for radiocisternography (80). More recently, however, ^{111}In-penthanil has become available and is considered to be superior to Yb-chelates for cisternography (81). The mechanism of how these chelates function is unclear. Studies using ^{64}Cu and ^{14}C-labeled edetate demonstrated that the two components do not remain as an entity under biological conditions[3]. Evidence has accumulated that different metals of the same chelate have significantly different biological properties, from the standpoints of renal excretion and of tumor localization. Thus, the mechanism of localization remains to be elaborated.

With regard to the miscellaneous agents, it has been observed that with ^{206}Bi as its nitrate, the radionuclide becomes bound to globulins and probably in this form enters the cytoplasm of the neoplastic cells (82). Certainly major amounts are attached to cytoplasmic proteins. This interesting property of bismuth compounds has led to the development of ^{206}Bi for diagnosis of brain tumors. In contrast, however, ^{74}As (as arsenate–arsenite) shows a negligible tendency to combine with proteins but has a high affinity for erythrocytes and undoubtedly is transported to the neoplasm in this form. From this information, it is apparent that much remains to be done to elucidate tumor cell localization and that this must be done for each chemical form of every nuclide.

Biological Basis of Localization—The mechanism of radionuclide localization is very important in enhancing differences between normal and pathological processes and in the design of more effective agents. In the foregoing, cursory consideration has been given to the mechanism of localization.

At this point a summary of the main biological mechanisms is pertinent. Six mechanisms are operative in the localization of radiopharmaceuticals in various organs and physiological compartments: active transport, phagocytosis and pinocytosis, cell sequestration, capillary blockade, simple diffusion, and compartmental localization (Table VI).

Active transport, from this standpoint, can be defined as the ability of a particular organ or tissue to concentrate selectively and to bind tightly a specific species within the pool of chemicals passing through the organ. In most instances, the blood serves as the transport vehicle, and it is the affinity of a particular organ for a certain type of reagent that produces this focal concentration known as active transport. The best known example utilizing this mechanism is the thyroid gland, as has been discussed, and its affinity for iodine in the form of iodide ion (I^-). Other tissues such as salivary glands, lactating mammary glands, gastric glands, and the choroid plexus also have the ability to concentrate iodide ions. In these glands, however, the radioiodide is simply concentrated for a short time and then excreted; in the thyroid gland, the iodide is bound covalently into the thyroid hormones. Therefore, the residence time of the iodide in the thyroid is significantly longer than in the organs that have the ability only to concentrate the anion and not to incorporate it chemically.

In the initial accumulation or trapping of radioiodide by the thyroid, the iodine first passes through the follicular cells of the thyroid gland and is concentrated within the follicles. This selective accumulation is an active metabolic process and, unless bound promptly, the trapped iodide quickly diffuses back into the blood.

Another example of active transport is the uptake of chlormerodrin [chloro(2-methoxy-3-ureidopropyl)-mercury] by the kidneys. As already noted, organic mercurial compounds form mercaptides with sulfhydryl-containing enzymes. These enzymes are found in abundance in the renal tubular cells, and both ^{203}Hg- and ^{197}Hg-chlormerodrin bind to the cytoplasmic components of the renal proximal tubular cells. They are eventually excreted into the tubular lumen and out through the remainder of the collecting system. Chlormerodrin has been shown to have the highest accumulation in the outer renal cortex, less in the medulla, and the lowest in the renal papillary tissue. The presence of the sulfhydryl en-

[3] A. H. Soloway and C. Carlson, unpublished data.

zymes and the subsequent formation of the sulfide linkage between these enzymes and the mercurial compounds lead to selective accumulation of these mercurials in the kidney and this accumulation leads to the active transport.

Other examples are the uptake of certain dyes by the liver and the uptake of fluoride, calcium, and barium by bone (83, 84). The foregoing are merely a few of the more prominent examples of the utilization of active transport in imaging radiopharmaceuticals.

Phagocytosis/pinocytosis is another biological mechanism of localization. Certain mononuclear cells that line the sinusoids of the liver and spleen and, to a lesser degree, the blood vessels of certain other regions of the body (bone marrow) have a special ability to remove particulate matter from the circulation by the process of phagocytosis. Phagocytosis can be described as the engulfment and eventual ingestion of foreign particles by cells. These cells constitute the reticuloendothelial system and the use of this system in liver, spleen, and bone marrow imaging was already discussed. After intravenous injection, particles in the millimicron to 1-μm range are phagocytized with great efficiency. Most inorganic particles remain in phagocytic cells almost indefinitely because they are not metabolized. Organic particles, such as bacteria, effete blood cells, and protein aggregates are metabolized after ingestion.

The distribution of radiocolloids within the reticuloendothelial system is dependent upon several physical factors including size of the colloid, chemical composition, and surface properties. In general, very small colloids in the millimicron range tend to concentrate primarily in liver and bone marrow; larger colloids, in the 0.1–1-μm range, tend to localize in the liver and spleen. Pinocytosis is a less specific process than phagocytosis, and the material need not be particulate to be ingested by the cell. This latter process has been implicated in the localization of proteins in tumor cells. Attachment of such structures to the surface of such cells followed by invagination is the mechanism by which such structures do achieve intracellular localization (85).

Cell sequestration is a third mechanism used in imaging. Chemical or thermal denaturation of radiolabeled erythrocytes results in their being actively sequestered by the spleen and is the mechanism by which ^{51}Cr, ^{99m}Tc, and ^{197}Hg-labeled red blood cells serve as a means for visualization of this organ.

A fourth mechanism for organ visualization and function measurement is *via* capillary blockade. This mechanism is utilized in assessing lung function and arterial organ perfusion. The smallest vessels in the vasculature are the capillaries, ranging from 7 to 10 μm in diameter. Thus, labeled macroaggregates greater than 10 μm in diameter are trapped in this capillary bed. When such particles are injected intravenously, the first arteriolar capillary bed contacted by the administered macroaggregates is that of the lung. It is such a blockade that gives rise to the feasibility of lung imaging. The lungs are not the only organs that may be visualized in this manner. By placing a catheter in the appropriate vessel, the macroaggregates can be selectively delivered to numerous organs. Studies aimed at determining regional blood flow and perfusion defects using the particle distribution technique have been performed in organs such as the kidneys, liver, brain, and myocardium. ^{131}I-Labeled macroaggregated human serum albumin, ^{99m}Tc-macroaggregated human serum albumin, and ^{113m}I- and ^{99m}Tc-ferric hydroxide macroaggregates all function by capillary blockade.

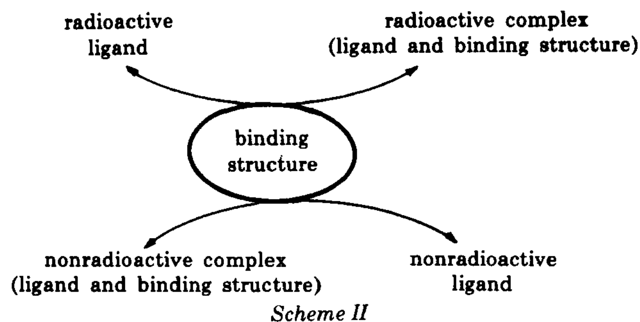
Simple diffusion is another mechanism applicable to the study of brain pathology. The diffusion of various organic and inorganic structures into those areas where the normal blood–brain barrier mechanism is no longer operative is the basis for the visualization of these pathological processes. These nuclides and their compounds were previously discussed.

Another mechanism of localization within the body is termed compartmental localization. The strict definition of this mechanism is the retention of the radiopharmaceutical in the administered tissue or body fluid space for a significant time. Thus, radioisotopic accumulation in the oral cavity, the GI tract, the vascular supply, the lymphatic system, and the cerebrospinal fluid compartment would be evidence for compartmental localization. Examples of drugs that function by this mechanism are ^{131}I-, ^{123}I-, ^{113m}In-, and ^{99m}Tc-labeled proteins for cardiac scanning, radioiodinated fats for lymphatic visualization, and ^{111}In and its compounds for cerebrospinal canal delineation (86).

Radiopharmaceuticals for Function—In addition to imaging radiopharmaceuticals, a number of drugs are injected for measuring physiological function and for determining the biochemical status of various organs. Such drugs may be classified as *in vivo* nonimaging radiopharmaceuticals. They are administered to the patient but are not used for structure visualization. Among these compounds are: *(a)* radiocobalt-labeled (^{60}Co and ^{61}Co) cyanocobalamin (vitamin B_{12}), used in the diagnosis of pernicious anemia and the malabsorption syndromes; *(b)* ^{55}Fe- and ^{59}Fe-citrate, used in a study of iron metabolism and ferrokinetics; *(c)* ^{51}Cr-labeled red blood cells, used for measuring erythrocyte mass, total circulating blood volume, red cell survival time, and GI blood loss; *(d)* ^{22}Na, ^{24}Na, ^{82}Br, and ^{35}S, used for the measurement of extracellular water; *(e)* radioactive sodium and ^{42}K, used for the determination of total exchangeable sodium and potassium; *(f)* radioiodinated human serum albumin and other labeled proteins, used for plasma volume measurement; *(g)* ^{45}Ca and ^{47}Ca, used in the assessment of calcium metabolism; and *(h)* radioiodinated fat and fatty acids, used for the study of fat absorption defects (87). The use of nonimaging drugs as radiopharmaceuticals was described in a previous review article (88).

In Vitro *Radiopharmaceuticals*

The diagnostic drugs considered so far must be administered directly to obtain meaningful information by scanning scintigraphy and excretion studies. In



Scheme II

all cases, a definitive radiation dose is delivered to the patient, even though this level may be of minimal importance now with the reduction in physical half-lives of a number of drugs. Ideally, one would prefer administering no radioactive material to a patient and yet obtain useful diagnostic data. The *in vitro* radiopharmaceutical drug meets this objective; as a consequence, use of this category of diagnostic agents is increasing at a rapid rate (87, 89–92). It offers as an ultimate goal the capability of measuring the levels of every hormone or other biochemical structure that is of importance in metabolism as well as determining the concentrations of exogenous compounds and their products which may be absorbed and/or generated. This procedure will be referred to here as competitive radioassay. The literature, however, is replete with a variety of terms that are very closely related and comprise the category of *in vitro* radiopharmaceuticals. Several of these will be used in this presentation, and they include radioimmunoassay, saturation analysis, radiostereoassay, competitive inhibition, and binding radioassay.

Essentially, this procedure involves the competition generally between a small molecule which can be considered a ligand and the radioactive form of this same ligand for some binding structure which has a demonstrable affinity for this ligand. The basis for this method of detecting analytically the ligand in biological fluids is that the radioactive and nonradioactive forms are readily exchanged and compete for those attaching sites on the binding structure. In this manner, the amount of unbound radioactive ligand is directly dependent upon the level of nonradioactive ligand in the fluid analyzed. This competitive binding may be visualized as in Scheme II.

Implicit in this assay method is a second important requirement, namely a capability for separating the complex from the ligand. In this way, it is totally feasible to measure the amount of radioactivity in each entity and to extrapolate to the concentration of the ligand in body fluids. From this brief description, it is apparent that no radioactivity is administered to the patient and that a sample of blood, urine, or other body fluid isolated from the patient may serve as the source for the nonradioactive ligand and sometimes even the binding structure. This technique is an extremely sensitive assay, comparable at least to and generally having greater sensitivity than photofluorometric and bioassay procedures.

In general, the ligand to be radioassayed is a small molecule such as a simple organic drug, its metabolites, a vitamin, hormone, or biochemical precursor. The radioactive form of the ligand should be totally comparable in its binding constant and mechanism of attachment to the binding structure as the nonradioactive ligand. For this reason, tritium may be used for hydrogen, ^{14}C for ^{12}C, ^{18}F for ^{19}F, ^{125}I or ^{131}I for ^{127}I, ^{32}P for ^{31}P, and ^{35}S for ^{32}S. Such ligands would behave identically to their nonradioactive counterparts. Alternatively, radioactive halogens could be incorporated into a ligand containing no halogen with the important proviso that this chemical modification should not modify the way the two ligands are complexed to the binding structure.

The third component in this triangle is the binding structure. Generally, this is a much larger component from a molecular weight basis than is the ligand. This structure may be a protein molecule such as an antibody, an enzyme, a plasma protein, or structural protein; it may also be a cell membrane or component thereof or other macromolecular entities such as certain resins, which show an affinity for the ligand regardless of whether it is specific or nonspecific. This three-component system comprises the essentials for a competitive binding assay procedure.

For the present purposes, with stress on the more widely used procedures, this subject of *in vitro* radiopharmaceuticals can be divided into two broad categories: *(a)* nonimmune systems, and *(b)* immune systems. Included in the former system are methods for measuring a variety of hormones and nonhormonal compounds. Among these are the hormones triiodothyronine, thyroxine, cortisone, cortisol, aldosterone, progesterone, estradiol, testosterone, ACTH, and vitamins and other important biochemicals such as vitamin B_{12}, folic acid, and cyclic AMP. The second category includes those procedures that may be called radioimmunoassay. Many of the same compounds measured by the nonimmune system have also been assayed by this method. These include triiodothyronine, aldosterone, estradiol, testosterone, ACTH, folic acid, and cyclic AMP. Additionally, a variety of other hormones and nonhormonal substances of biochemical importance have been determined, including human chorionic gonadotropin, follicle-stimulating hormone, oxytocin, thyrotropin, insulin, angiotensin, human growth hormone, glucagon, human luteinizing hormone, vasopressin, thyroglobulin, parathyroid hormone, bradykinin, calcitonin, and the prostaglandins (93). From this broad spectrum of compounds, it is apparent that there is a great scope for this assay procedure (94). It will permit ultimately a determination of their importance and the concentrations necessary for the maintenance of normal homeostatic conditions. An obvious corollary is that these techniques will furnish important diagnostic information as to the presence of pathological conditions. Such changes may actually precede a frank clinical manifestation. The structures considered to this point are those that are naturally occurring in mammalian systems. A variety of drugs, toxicants, and their metabolites can now be measured by radioimmunoassay. Examples are aspirin; morphine and its congeners (95); digitalis glyco-

sides such as digoxin, digitoxin, and digitoxigenin; and lysergide (LSD).

For the successful utilization of competitive radioassay by either method, a number of criteria must be considered and satisfactorily met. First, it is essential that the ligand to be assayed be prepared pure in both labeled and unlabeled forms. The unlabeled structure may serve as the starting material for synthesizing the radioactive ligand. For example, incorporating radioactive halogens into such a compound would yield the labeled ligand. Additionally, the unlabeled substance would serve as the standard for calibrating the radioassay procedure. A second requirement is that there must be a binding structure with sufficient specificity for the ligand so that other compounds will not displace it or interfere in any way with its complexation to the binding structure. This specific reagent may be a naturally occurring binding protein related in some respects to the biological activity of the ligand, an antibody prepared against this hapten, or an enzyme with specificity for this ligand. It is essential that there be no lack of specificity. For this reason, purification may pose difficulties since there may be similarities in physical and chemical properties between such structures and closely related biopolymers with much less avidity for the ligand. The degree of purification will depend upon the sensitivity, precision, and accuracy required by the assay. Similar purification problems may apply as well for antibodies and enzymes. Since these are proteins also, the whole gamut of protein purification technology may be utilized. Third, and of crucial importance, is the capability of separating the free ligands from the ligand-binding structure complex. Since many of these macromolecular structures are proteins or have a protein component, the techniques for separation are frequently those utilized in protein chemistry. Among those used are solvent partitions, chromatographic techniques, electrophoresis, dialysis, ultrafiltration and centrifugation, adsorptive methodologies, antigen–antibody precipitation, enzyme–substrate interaction, and various combinations of these.

To appreciate the potentialities as well as the current uses of competitive radioassay, it is pertinent to consider selected examples of the use of this technique and the advantages it offers for diagnosis. A more exhaustive treatment of *in vitro* diagnostics appears inappropriate and would negate the overview characteristics of this review.

One of the earliest and one of continuing importance is the use of *in vitro* procedures for thyroid function determination. Two small hormones produced by thyroid gland from iodide are thyroxine and triiodothyronine. They are released into the circulation and are largely transported by a specific glycoprotein, thyroxine-binding globulin to peripheral tissues where they mediate metabolic action and rate. Measurement of both of these hormones in free and bound states in the circulation provides important data as to thyroid function. The Murphy–Pattee method serves as a means for measuring total thyroxine in the serum. The technique involves the addition of known amounts of stable thyroxine to a system consisting of thyroxine-binding globulin and bound radioactive thyroxine (^{125}I or ^{131}I). As equilibrium is attained, some labeled thyroxine is displaced from the thyroxine-binding globulin binding sites by the stable ligand. By adding increasing amounts of this compound, increased amounts of radioactivity are displaced. A determination may then be made of the ratio between the free and protein-bound radioactive thyroxine. By plotting this ratio against the amount of nonradioactive thyroxine added, a standard curve is obtained. This curve may be utilized to measure the amount of endogenous thyroxine in serum. Thyroxine may be extracted from serum by use of ethanol; when this amount is added to the competitive radioassay system, labeled thyroxine is once again displaced. Use of the standard curve permits a direct readout of the concentration of endogenous serum thyroxine. This information is important in determining conditions where excessive amounts of thyroxine are secreted or where increased levels of thyroxine can be attributed to higher concentrations of thyroxine-binding globulin. The former is indicative of hyperthyroidism; the latter may result from greater estrogen levels, liver dysfunction, congenitally elevated thyroxine-binding globulin, or pregnancy. Lower serum thyroxine values may concomitantly be associated with hypothyroidism due to decreased thyroxine secretion or to a lower concentration of thyroxine-binding globulin. The latter may be a direct result of severe liver or kidney disease, androgen or steroid therapy, or congenital factors.

In addition to this measurement of bound thyroxine, free thyroxine concentrations have also been determined. Many of the methods used involve equilibrium dialysis. Dialysis of serum containing added radioactive thyroxine is performed, and the level of radioactive thyroxine in the dialysate is proportional to the concentration of unbound thyroxine in the serum. Abnormally high values of free thyroxine may be related to its increased secretion associated with hyperthyroidism or with patients experiencing sepsis or cardiac arrhythmias or following heparin therapy. Concomitantly, low values may be attributed to hypothyroidism. These diagnostic studies with thyroxine involve the use of nonimmune protein systems in which the radioactive and nonradioactive ligands compete for binding sites on the naturally occurring plasma protein carrier.

The second thyroid hormone, triiodothyronine, also plays an important role in normal human physiology and in patients with thyroid dysfunction. The development of analytical techniques utilizing *in vitro* radiopharmaceuticals has been of great importance in determining thyroid dysfunction. Difficulties have been encountered in the quantitation of this hormone, stemming in part from the fact that this compound has only one-sixtieth the concentration of thyroxine in serum and is not as tightly bound to thyroxine-binding globulin as thyroxine. The technique widely accepted as necessary for assessing thyroid function is the uptake test of ^{131}I- or

^{125}I-labeled triiodothyronine by cation-exchange resins. This *in vitro* test is carried out by the incubation of radioactive triiodothyronine with the patient's serum in the presence of the resin in sponge form. Effectively, the labeled triiodothyronine is being competed for by the resin and the thyroxine-binding globulin in the serum. Normal and abnormal ranges have been established for the amount of radioactive triiodothyronine bound by the resin. Excessive levels of thyroxine-binding globulin in the serum results in lower percentages bound to the resin; in a corresponding way, decreased amounts of thyroxine-binding globulin produce a higher percentage attached to the resin. Without a total consideration of the utilization of these data and those derived from thyroxine, it is sufficient to state that they assist materially in a determination of thyroid function. This triiodothyronine test illustrates another *in vitro* technique in which a naturally occurring protein and a resin are competing for the radioactive hormone. Both these tests for triiodothyronine and thyroxine fall into the category of nonimmune systems.

Of greater scope and potential are the immune system *in vitro* assays. For this presentation, two will be examined as examples of the potentiality of this analytical procedure. The first deals with the measurement of a naturally occurring protein hormone. In this respect, a consideration of the levels in serum of the pituitary protein, thyrotropin, seems pertinent in view of the previous examination of triiodothyronine and thyroxine. Thyrotropin concentration is most useful in diagnosing and determining the type of hypothyroidism, detecting early decreased thyroid reserve, and monitoring the effect of therapy upon function using exogenous thyroid hormones. From current studies, it is apparent that an elevated thyrotropin level may be the first and most subtle indicator of thyroid hormone deficiency. The development of radioimmunoassay for this hormone has provided a most sensitive test for thyroid bioactivity (96, 97). The labeled ligand, in this case, is ^{125}I- or ^{131}I-human thyrotropin. The chloramine-T method of radioiodination or other appropriate mild procedures which do not alter the native structural features of the protein molecule may be used for the synthesis of labeled thyrotropin. Separation of the protein bound and nonprotein bound radioactive iodine may be accomplished by column chromatography (Sephadex). The binding agent for the system is guinea pig or rabbit antihuman thyrotropin. Standard immunological procedures are followed for the production of high antibody titers, and their qualitative detection may be accomplished by immunoelectrophoresis, neutralization through antiserum dilutions, and radioimmunoassay. For actual quantitation, a system involving the competitive binding of labeled ligand, unlabeled ligand in serum, and the antibody preparation is used. In essence, there is a competition for the antibody by both the labeled and unlabeled ligands. Several methods have been employed for the separation of bound and free hormones and these have included ethanol–sodium chloride differential precipitation, chromatoelectrophoresis, and double-antibody precipitation using guinea pig antirabbit γ-globulin. From this brief description, it is apparent that the radioimmunoassay procedure in general has the capability of measuring every naturally occurring hormone or biochemical structure; with the widespread use of this technique, fluctuation in body levels of various components may be the first indicator of pathological changes.

Not only may normally occurring biochemicals be assayed but there is the feasibility of measuring concentrations of drugs, their metabolites, and other exogenous compounds as well in various body fluids. One of the present, more important uses of radioimmunoassay in monitoring effective drug therapy involves the use of digitalis glycosides (98). These drugs are prescribed in virtually all instances of heart failure, especially in the treatment of congestive heart disease and various arrhythmias. They increase the strength of contraction and thereby cardiac output. However, overdosage for the individual results in intoxication as shown by anorexia, nausea, vomiting, and diarrhea. These early symptoms lead ultimately to ventricular extrasystoles, hypokalemia, and cardiac conduction disturbances. The range between the effective dose and the toxic one is narrow and highly variable. In effect, what is essential is a determination of the correct dosage for the individual patient. Radioimmunoassay has become an important procedure in assisting the clinician to determine proper blood concentrations. The labeled ligand, in this case, depending upon the drug used, may be ^{3}H-labeled digoxin, ^{3}H-digitoxin, or ^{125}I-iodotyrosine methyl ester succinyl-3-*O*-digitoxigenin. The binding agent is rabbit antidigoxin antibody. This same antibody may be used for either digoxin or digitoxin in view of their structural commonality. A standard curve needs to be established for each drug with this same protein. Several techniques have been used for the separation of the free ligand from the protein-bound form of the drug. These include absorption chromatography with dextran-coated charcoal and double-antibody precipitation with goat antirabbit γ-globulin. By this procedure, the detection limit of these cardioactive drugs is in the nanogram range and even below. By a similar method, morphine, lysergide, and other exogenous compounds may also be assayed. From this broad range of drugs that can be measured, it is apparent that this procedure has great versatility.

THERAPEUTIC DRUGS

This second section relates to therapeutic radioactive drugs. Although of minor clinical importance by comparison, they do provide the radiotherapist with a useful tool for tissue destruction.

Therapeutic drugs may be divided into two separate categories: *(a)* drugs that are radioactive *per se* when administered, and *(b)* drugs that are nonradioactive but with *in situ* activation produce intense ionizing radiation. The major limitation to the use of therapeutic radiopharmaceuticals is that they must have a deleterious effect solely upon the uneconomic species to be destroyed. The corollary, of course, is

that normal tissues must be unaffected. To achieve total cellular destruction, doses on the order of 4000–5000 rads must be delivered to the target tissue and less than 1% of this dose may be tolerated by certain radiosensitive tissues without demonstrating alteration in function. This poses an extremely stringent requirement on the drug differentials between economic and uneconomic tissues.

Radioactive Therapeutic Agents—For the purpose of considering different radioactive therapeutic agents, these drugs can be divided into two categories: *(a)* those whose functions are to moderate and to modify abnormal metabolic function, and *(b)* those that will destroy malignant processes (Table VII). Since there are very limiting requirements for the drugs that can be used, both from a distributive pattern and the radiant energy emitted, first approaches involved the use of radionuclides demonstrating a frank biochemical basis for their specific localization.

Abnormal Tissue-Destroying Agents—The early use of ^{128}I-iodide ($T_{1/2}$ = 25 min) as an indicator of thyroid physiology foreshadowed the radiotherapy of the thyroid gland with radioactive nuclides of iodine. Initially, ^{130}I ($T_{1/2}$ = 12 hr) and then ^{131}I ($T_{1/2}$ = 8 days) were used. The latter nuclide supplanted ^{130}I as the isotope for the treatment of diffuse toxic goiter (Graves' disease). The normal dose administered is 5 ± 3 mCi, which deposits 8000 ± 2000 rads within the thyroid gland. More recently, it has been suggested that 1–2 mCi (2000 rads) may be adequate to moderate beneficially hyperthyroidism. The major undesirable long-term effect of radioiodide appears to be hypothyroidism and, in the use of ^{130}I, frank myxedema occurred in a significant number of patients.

The treatment of nodular goiters associated with hyperthyroidism (Plummer's disease) has been tried extensively. The response to ^{131}I is less satisfactory than that of diffuse goiter, and considerably larger doses (10–75 mCi) are required. Repeated doses are often needed, and worrisome nodules frequently persist. One important reason for the frequent therapeutic failure in toxic nodular goiter is the inability of the nodule to retain enough of the administered ^{131}I to accomplish the self-destruction.

Recently, considerable attention has been given to the possibility of treating thyrotoxicosis with ^{125}I in place of ^{131}I (99). The physical characteristics of ^{125}I theoretically allow one to deliver three to four times the dose to the area responsible for the hyperthyroidism (the colloid–follicle cell interphase) compared to the same amount of ^{131}I while reducing the dose to the cell nucleus. This reduction in dose to the cell nucleus could decrease the amount of hypothyroidism associated with the aftermath of radioiodine therapy. Initial clinical trials have been encouraging, but the relapse rate has been substantially higher than expected. The most promising results have been achieved by the administration of a mixed dose of ^{125}I and ^{131}I.

There are few contraindications to the use of ^{131}I in the therapy of hyperthyroidism. Most radiotherapists prefer not to give ^{131}I to patients under age 20 due to the potential danger of radiation-induced leukemia or carcinoma. Nevertheless, this is not a strict rule. However, pregnancy is considered to be the one absolute contraindicator to the use of radioiodine; the rapidly growing fetus is considered to be sensitive to small amounts of radiation. In addition to the increased possibility of congenital malformation, there is the fear of ablating the fetal thyroid. Since iodine is concentrated in breast milk and, therefore, can be ingested by the newborn, lactating women should not be given radioiodine therapy except in extenuating circumstances. Also, there is a close relationship between circulating thyroid hormones and coronary function; patients with recent myocardial infarction should not be given radioiodine therapy unless thyroid hormone levels are carefully monitored and offsetting drugs are given.

Table VII—Therapeutic Radiopharmaceuticals

Abnormal metabolism:
Hyperthyroidism (^{131}I and other iodine isotopes)
Polycythemia vera (^{32}P)
Malignant diseases:
Radioactive iodides (^{131}I and ^{125}I)
Radioactive colloids [^{198}Au, chromic phosphate (^{32}P), and ^{90}Y]
Implanted preparations (^{192}Ir and ^{182}Ta)

Radioactive Agents for Malignant Disease—A second major use of therapeutic radioactive drugs is in the management of malignant diseases (100). In view of the clinical usefulness of radioactive iodide in the treatment of hyperthyroidism, its utilization in the treatment of thyroid cancer would seem to be a normal extension. However, clinical utility has been quite limited. The more malignant and anaplastic the thyroid carcinoma is, the less ability it has to sequester iodide from the circulation. Therefore, the normal thyroid tissue receives the maximum radiation dose and not the malignancy. For this reason, radioiodine therapy for such tumors and their metastases has not achieved widespread clinical usefulness.

From this observation it is apparent that sufficient localization of the radioactive drug in the target tissue is essential for therapy. An example utilizing this requirement is the treatment of polycythemia vera, thrombocythemia vera, and myelogenous and lymphocytic leukemias by ^{32}P-labeled sodium phosphate. Initial dosages of 3–4 mCi have been used successfully. The basis for this therapy is the deposition of phosphate in bone marrow and the utilization of the emitted β-radiation to control the abnormal and unphysiological multiplication of formed elements of the hematopoietic system. This therapeutic maneuver has been shown to be of distinct value in the treatment of lympho- and myeloproliferative diseases.

Use of tumor-seeking carriers as a means of concentrating radionuclides in malignant cells has been tried for more effective localization of neoplasms, *e.g.*, ^{111}In-bleomycin (101). This same type of approach also has been considered for tumor therapy. The rationale is that the carrier structure has a proclivity for the neoplasm by reason of its chemical composition; incorporation of a radionuclide into the

compound may offer a means of preparing a tumor-seeking radioactive substance. This was the basis for the preparation of ^{3}H-labeled menadiol diphosphate[4]. This radiosensitizing compound was reported to achieve high selective concentrations in tumors, and tritium incorporation was considered as a means for selective eradication. Although this approach has not achieved general widespread use, the basic concept still remains attractive.

Another approach to therapy is the use of radioactive colloids as a means of delivering controlled amounts of radiation to discrete sites. The colloidal particle is selected for its insolubility and particle size and, consequently, it functions not as a pharmacological agent but as an inert internal radiation source. Ideally, the radiotherapeutic drug for these purposes should be a pure β-emitter whose energy would be totally absorbed within the tissue to be destroyed. The radiant energy of suitable β sources would not be confined to very limited areas as would be expected for α sources, nor would the energy be distributed over large areas, including normal structures, as would be expected for energetic γ-nuclides. Radiocolloids that have been prepared and used are ^{198}Au, ^{32}P-chromic phosphate, ^{90}Y, and ^{177}Lu. They have been used clinically largely for two purposes: *(a)* intracavitary irradiation, and *(b)* interstitial therapy. Intracavitary radiocolloid therapy has been used for the irradiation of pleural and peritoneal cavities where invasive tumorous processes have interfered with normal fluid absorption and secretion. This form of treatment is an accepted one for the palliation of such malignant effusions. It is to be recommended for patients who have failed to respond to chemotherapy or conventional radiation therapy. There is no specific tissue selectivity afforded by this procedure; that is, normal tissues in this cavity would likewise be irradiated.

Infusion of radiocolloids into tumors directly has also been used. This interstitial administration of radioisotopes offers the advantage of delivering high dosages of radiation to the neoplasm and relatively little radiation to normal structures. An important proviso for this selectivity is based upon the radiation characteristics of the nuclide; those that are weak γ-, β-, and α-emitters are certainly preferable. The key problem for useful therapy is the uniform distribution of this colloidal suspension throughout the neoplasm. Unfortunately, the tumor process is usually not discrete but is an infiltrating one; consequently, the intratumor distribution pattern can be extremely variable. Another important problem encountered with such radiocolloids is their possible transport to the reticuloendothelial system. In this way, damaging radiation may be delivered to normal tissues such as bone marrow.

In addition to the use of radiocolloids for malignant tissue destruction, external and implanted radioactive sources have been utilized. Since the concern here is with radiopharmaceuticals, only implanted sources will be considered. These may be subdivided into two categories: *(a)* removable implants, and *(b)* permanent implants. With the removable implants, the half-lives of the encapsulated radioactive sources are such that permanent insertion is impossible. The function of this implant is to deliver the desired calculated radiation dose to the specific area; once this has been achieved the source is removed. The key advantage of this technique is good control of the radiation dosage to the discrete site. However, dose distribution cannot be readily altered after the implant has once been made. These sources are used principally in the therapy of accessible tumors where long-term palliation and even a complete cure may be feasible. Intraoral lesions and superficial cancers of the skin, breast, and neck may be subjected to this form of therapy. The following radionuclides have been used for such implants: ^{60}Co ($T_{1/2}$ = 5.3 years), ^{137}Cs ($T_{1/2}$ = 30 years), ^{182}Ta ($T_{1/2}$ = 118 days), ^{192}Ir ($T_{1/2}$ = 74 days), ^{198}Au ($T_{1/2}$ = 2.7 days), and ^{226}Ra ($T_{1/2}$ = 1600 years).

Three of these nuclides (^{182}Ta, ^{192}Ir, and ^{198}Au) have also been used in permanent implants. Other radionuclides that have also been used for such purposes are ^{125}I ($T_{1/2}$ = 60 days), ^{131}Cs ($T_{1/2}$ = 9.7 days), ^{133}Xe ($T_{1/2}$ = 5.3 days), and ^{222}Rn ($T_{1/2}$ = 3.8 days). This approach generally makes use of shorter half-life nuclides. The advantage of this method vis-à-vis temporary implants is that the procedure is simple and safer with less trauma; moreover, unresectable, deep neoplasms may be treated in this way at a surgical exploration without the concern for prolonged destructive radiation being delivered to those vital normal structures that may be in close proximity.

Radioactive-Induced Therapeutic Agents—The major limitation presented by radioactive therapeutic drugs is the fact that there must be a high differential between the target tissue and normal tissues, especially those that are radiosensitive. The ability to induce ionizing radiation in a discrete target tissue possibly remote from radiosensitive structures and in a highly selective way is the basis for attempts to utilize neutron capture radiation as a tissue-destructive maneuver. Although this technique has not achieved clinical utility, it does have desirable potential. The rationale for this therapy rests upon the capability of certain nonradioactive nuclides to absorb thermal neutrons and then emit intense ionizing radiation which may be largely confined to specific loci. Thermal neutrons and these nuclides are relatively innocuous alone, but their interaction produces a nuclear reaction capable of destroying cells. The key requirement is that the absorbing isotopes have large cross-section capture values for such slow neutrons compared with those values for normal tissue constituents (Table VIII) (102). The transuranic nuclides shown are radioactive *per se* and therefore possess some of the main drawbacks inherent with the radioactive therapeutic drugs.

The major research effort in developing this procedure clinically has involved the use of boron compounds (103). The basis for this is that the particles

[4] Synkavite

Table VIII—Thermal Neutrons and Potential Absorbers

Cross-Section Values for Thermal Neutrons Tissue Constituents (values in barns)			
H	0.32	N	1.7
Na	0.45	P	0.15
K	2.0	O	0.001
Mg	0.07	S	0.5
Ca	0.42	Cl	32.5
C	0.0045	Fe	2.43
Potential Therapeutic Neutron Absorbers			
^{6}Li	930	^{157}Gd	200,000
^{10}B	3990	^{164}Dy	2620
^{113}Cd	19,500	^{199}Hg	2500
^{149}Sn	46,000	^{235}U	687
^{155}Eu	14,000	^{242}Am	8000

emitted upon the capture reaction are large by nuclear standards ($_2{}^4$He and $_3{}^7$Li) and are, therefore, restricted to those cells and their immediate neighbors that contain the neutron absorber. This technique has been largely concerned with the destruction of malignancies, but it also has been considered as a means for pituitary ablation. The major factor that has prevented the use of neutron capture therapy as a viable cancer therapeutic procedure has been the inability to produce boron compounds that will be largely restricted to the neoplastic area and not be distributed to normal structures contiguous to it. Thus, the important problem with these potential therapeutic agents, as with the radioactive therapeutics, is the need for adequate selective localization in the cancerous tissue. However, the requirements are not as stringent as for the radioactive therapeutics since the ionization radiation is only produced when the two components interact. In this manner, for example, high concentrations of the nuclide in normal liver, kidney, or bone marrow do not preclude the use of this technique for the treatment of brain tumors. These other tissues will not be exposed to neutron levels that will impair their physiological functions by neutron capture. This two-component technique for producing ionization radiation is certainly attractive. When compounds are developed that do show preferential localization in neoplasms, this type of procedure will have clinical utility.

NEW DIRECTIONS IN RADIOPHARMACEUTICAL DEVELOPMENT

There are multifaceted approaches in the development of new radiopharmaceutical diagnostics. These stem from the fact that nuclides with widely divergent half-lives may now be readily prepared by generators, reactors, and cyclotrons. The problems of preparing and using ultrashort-lived radioactive isotopes have been met (Table IX), and their increasing use, especially in examining the dynamics of pulmonary and cardiac function, can be expected. Some agents that are clinically useful for organ imaging are presented in Table X.

Another main development will be the continuing effort to produce agents with a biochemical rationale for their specific localization in certain target tissues, whether they be malignant or have other abnormal features. The present availability of nuclides with

Table IX—Directions in Radiopharmaceutical Development

Ultrashort-lived nuclides:
^{11}C, ^{13}N, ^{15}O, ^{81m}Kr, and ^{144}Pr
Short-lived nuclides:
^{18}F, ^{68}Ga, and ^{99m}Tc
Intermediate-lived nuclides:
^{67}Ga, ^{97}Ru, ^{111}In, ^{131}Ba, ^{135m}Ba, ^{169}Yb, and ^{201}Tl
Therapeutic agents

suitable emission characteristics and desirable half-lives will permit the emphasis being placed upon the tissue-localizing moieties into which the isotopes may be incorporated. Frequently, the basis for the selection of these localizing structures is their chemotherapeutic use. However, in many instances, knowledge as to the degree of localization in a specific target tissue is unknown; even when this information is available, the time course for this concentration, especially compared with other adjacent and normal tissues, remains undetermined. This is readily understandable because such information may not be pertinent to the compound's therapeutic utility, but it certainly is germane if the compound is being used as a scanning agent. Utilization of the bleomycin antibiotics is an example of the types of agents to which radionuclides may be attached. These compounds are rapidly incorporated into tumor cells and apparently to a high extent. They have shown very desirable cancer chemotherapeutic effects *per se;* on this basis, the incorporation of ^{111}In and ^{99m}Tc into the antibiotic has produced agents with desirable scanning features. Thus, there needs to be an assessment of the distributive properties and time course for a particular compound prior to any consideration of radionuclide incorporation. With the facile methods available now for binding radioisotopes to various compounds, incorporation is no longer a problem. Much, however, remains to be done in the development of discrete target-oriented drugs to which these radionuclides may be attached.

A major advance will be attained once immunochemistry is refined to the point of complete purification of tumor and organ antibodies. Then radiopharmacy will have reached a new plateau. The ready incorporation of ^{123}I, ^{99m}Tc, or ^{111}In into such proteins may produce the ultimate in organ and tumor visualization. The basis for this view is that the incorporation of these nuclides can now be performed in such a manner as to prevent major conformational changes in the protein. Certainly this requirement is a *sine qua non* for the attainment of such highly selective *in vivo* radiopharmaceuticals. The integrity of such proteins and proof of their lack of conformational alteration can be readily assessed by their viability in antigen–antibody reactions and their failure to cross-react with similar structural proteins. From these comments, it is apparent that important advances in immunochemistry may be a necessary prelude to major improvements in the development of *in vivo* radiodiagnostics.

The increasing use of *in vitro* radiopharmaceuticals both for the measurement of hormones and

Table X—Some Clinically Useful Organ Imaging Agents

Organ	Radiopharmaceutical	Mechanism of Localization	Administered Dose, mCi	Comments
Brain	$^{99m}TcO_4^-$ (pertechnetate)	Simple diffusion	10–20	In universal use
	^{99m}Tc-tin-penthanil	Simple diffusion	10–20	Choroid plexus blocking with ClO_4^- not necessary
	^{197}Hg- or ^{203}Hg-chlormerodrin	Diffusion and protein binding	0.5–1.0	Increased tumor uptake over TcO_4^- offset by physical decay characteristics and dosimetry
	^{131}I-human serum albumin	Diffusion and pinocytosis	0.1–0.5	24–48-hr wait for diffusion into tumor
Cerebrospinal fluid	^{111}In-penthanil	Compartmental localization	0.5	Current "agent of choice"
	^{169}Yb-penthanil	Compartmental localization	1	In use when ^{111}In is not routinely available
	^{131}I-human serum albumin	Compartmental localization	0.1–0.2	Was first and only agent for many years; now being replaced by penthanil chelates
Salivary glands	$^{99m}TcO_4^-$	Active transport	10–15	
Thyroid	^{131}I	Active transport	0.2–0.5	Most widely used radionuclide for thyroid diagnosis
	^{123}I	Active transport	0.1–0.2	Dosimetry 50–100× less than ^{131}I but expensive and not generally available
	$^{99m}TcO_4^-$	Active transport	1–2	Useful in certain types of thyroid disease as an imaging agent
Lung	^{99m}Tc-macroaggregated albumin	Capillary blockade	2–4	Instant one-step commercial kits just introduced; an excellent agent
	^{99m}Tc-albumin microspheres	Capillary blockade	2–4	Particle-size range a big factor in favor of this product; expensive, moderately complicated preparation procedure
	^{99m}Tc-ferric hydroxide aggregates	Capillary blockade	2–4	Kits are simple and rapid to prepare; question concerning clearance of iron particles still lingers
	^{131}I-macroaggregated albumin	Capillary blockade	0.2–0.4	Was most widely used agent, but physical properties of ^{131}I are a detriment; will be totally replaced by a ^{99m}Tc agent
	^{113m}In-ferric hydroxide aggregates	Capillary blockade	1–3	First iron hydroxide aggregate used; not used much due to decline in the use of ^{113m}In
	^{133}Xe, ^{127}Xe	Compartmental localization	10–25	^{133}Xe widely used for ventilation studies; physical characteristics of ^{127}Xe are excellent but as yet it is not available
	$^{11}CO_2$, $^{15}CO_2$	Compartmental localization	0.1–1	Requires "on-site" cyclotron and positron camera for good imaging
Liver	^{99m}Tc-sulfur colloid	Phagocytosis	2–4	Most widely used liver scanning agent at present
	^{99m}Tc-stannous phytate	Phagocytosis	2–4	Newest agent; forms colloidal particles *in vivo*
	^{113m}In-gelatin colloid	Phagocytosis	1–3	Used extensively from 1967–1971; not used much now
	^{198}Au-colloid	Phagocytosis	0.2	Almost completely replaced by Tc-sulfur colloid
	^{131}I-microaggregates	Phagocytosis	0.4	Short $T_{1/2}$ in liver; never achieved widespread clinical use
	^{131}I-rose bengal	Active transport	0.4	Used for determining patency of biliary tree
Spleen	^{99m}Tc-sulfur colloid	Phagocytosis	2–4	Gives a liver–spleen scan
	^{113m}In-gelatin colloid	Phagocytosis	1–3	Not used much at present
	^{99m}Tc-labeled red blood cells	Cell sequestration	2–4	Just introduced; spleen specific agent
	^{51}Cr-labeled red blood cells	Cell sequestration	0.3	Standard spleen scanning agent; should be replaced shortly by Tc-labeled red blood cells
	^{197}Hg-hydroxy(2-hydroxypropyl)mercury-labeled red blood cells	Cell sequestration	0.3–0.5	Used clinically in the 1960's but dosimetry always a problem
Kidney	^{99m}Tc-tin-penthanil	Compartmental localization	1–10	Excellent imaging and GFR agent; rapidly leaves kidneys and requires camera
	^{99m}Tc-iron ascorbate	Compartmental localization and active transport	1–10	Good agent; binds to the tubules for several hours

Table X—(*Continued*)

Organ	Radiopharmaceutical	Mechanism of Localization	Administered Dose, mCi	Comments
	^{99m}Tc-Sn-tetracycline	Compartmental localization	1–10	New agent, not in general use; gives twice as much activity in kidneys
	^{99m}Tc-Sn-glucoheptanate	Compartmental localization	1–10	New agent, currently in clinical trials
	^{99m}Tc-Sn-casedein	Compartmental localization	1–10	Small clinical study showed some clinical utility
	^{131}I-hippurate	Compartmental and active transport	0.2–0.4	Most widely used function and imaging agent
	^{197}Hg-chlormerodrin	Compartmental localization and protein binding	0.1–0.3	Still used in many centers despite high dose to kidneys
	^{113m}In-iron ascorbate or penthanil	Compartmental localization and active transport	0.1–1	Has been replaced by the Tc-counterparts
Bone	^{99m}Tc-Sn-polyphosphate	Exchange diffusion	15	Excellent bone imaging agent when proper polyphosphate is used
	^{99m}Tc-Sn-pyrophosphate	Exchange diffusion	15	Good bone scanning agent; lower kidney and bladder activity
	^{99m}Tc-Sn-EHDP	Chemisorption	15	Organic compound; also very good for skeletal imaging
	^{18}F	Exchange diffusion	2–4	Excellent physiological properties, but high cost and physical decay characteristics of ^{18}F are distinct disadvantages
	^{87m}Sr, ^{85}Sr	Exchange diffusion	2, 0.2	Being rapidly replaced by one of the three Tc-labeled products
Adrenal	^{131}I-cholesterol	Compartmental localization and active transport	1–2	New agent; only agent available for adrenal imaging; far from ideal physiologically but nevertheless useful presently
Bone marrow	^{99m}Tc-sulfur colloid	Phagocytosis	6–8	Used clinically but of limited value
	^{113m}In-gelatin colloid	Phagocytosis	3–4	Not used much any more
	^{198}Au-colloid	Phagocytosis	1–2	Not used at all any more
	^{52}Fe-citrate	Active transport	0.1–0.3	Just available; a positron emitter
	^{111}In-chloride	Active transport	3	Recently introduced; has already achieved moderate utility
Blood pool (static)	^{99m}Tc-human serum albumin	Compartmental localization	2–4	Used for pericardial effusion and placentography
	^{113m}In-chloride	Compartmental localization	1–3	Preferred by many for placentography due to decrease in bladder activity
	^{99m}Tc-labeled red blood cells	Compartmental localization	2–4	Just introduced; main use appears to be application in isotope angiocardiography
	^{131}I-human serum albumin	Compartmental localization	0.1–0.3	Seldom used
	^{123}I-human serum albumin	Compartmental localization	1–2	Expensive and presently not available
	^{51}Cr-labeled red blood cells	Compartmental localization		Still used in certain clinics but rapidly being replaced by Tc or In agents
Myocardium	^{43}K-chloride	Active transport	1–2	Concentrates in normal myocardium; not in widespread use
	^{129}Cs-chloride	Active transport	0.5–1	Similar to ^{43}K
	^{201}Tl-nitrate	Active transport	1–2	Appears to have physiological and physical decay parameters superior to ^{43}K or ^{129}Cs
	^{13}N-ammonium	Active transport	10	Currently in clinical trials; short-lived positron emitter
	^{99m}Tc-tetracycline	Diffusion and protein binding	10–20	Currently in clinical trials; localizes in the infarct
Tumor	^{67}Ga-citrate		3	In general use but of limited utility
	^{111}In-chloride		3	Not quite as good as ^{67}Ga in animal and human models; forms ^{111}In-transferrin
	^{111}In-bleomycin		3	Newly introduced; not clear if it converts to the transferrin complex *in vivo*
	^{99m}Tc-bleomycin		5–10	Recent clinical trials look encouraging
	^{99m}Tc-tetracycline		10	In clinical trials; preliminary results are encouraging

(*continued*)

Table X—(*Continued*)

Organ	Radiopharmaceutical	Mechanism of Localization	Administered Dose, mCi	Comments
Pancreas	^{75}Se-methionine	Active transport	0.2–0.3	In general use for many years; not a very satisfactory agent; active search is on to find a replacement

other biochemicals and for the analysis of drug concentrations in body fluids can be readily predicted. The former will be used both in the prevention and early diagnosis of disease; the latter will be used in the correlation of disease amelioration with drug concentrations. The great differences and vagaries among patients and even for the individual depending upon physiological status would make it very desirable to determine drug concentrations on a continuing basis. Here, once again, the field of immunology is of direct importance since the ability of purifying antihapten antibodies will assist materially in the development and use of highly specific radioimmunoassay procedures.

The therapeutic area has an even greater dependence upon immunology for the development of therapeutic radionuclides. Radiopharmaceuticals for such purposes have certainly lagged and although this is readily understandable, the need is there and is of great importance. However, until discrete localizing agents are obtained, whether they are antibodies, antibiotics, or other structures, successful radiotherapy by *in vivo* radiopharmaceuticals will remain simply a desired goal. Expected advances in immunochemistry will certainly foreshadow important developments in the field of radiopharmaceuticals.

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