RECEIVED BY TIC MAY 15 1978

To be presented at the First International
Symposium on Radiopharmacology,
Innsbruck, Austria, May 21 - 24, 1978

LBL-7407

CONF-780551--1

MASTER

EFFECT OF TRANSPORT IN DISTRIBUTION OF
RADIOIONS AND RADIOLABELED METABOLITES

Y. Yano

DONNER LABORATORY

December 22, 1977

Prepared for the U. S. Department of Energy
under Contract W-7405-ENG-48

LBL-7407

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

THE EFFECTS OF RADIATIONS AND RADIOLABELLED SUBSTANCES

BY J. H. D. JONES

1

1. INTRODUCTION

1.1. Introduction

1.1.1. Introduction

1.1.1.1. Introduction

The purpose of this paper is to review the effects of radiations and radiolabelled substances on the human body.

The purpose of this paper is to review the effects of radiations and radiolabelled substances on the human body.

The purpose of this paper is to review the effects of radiations and radiolabelled substances on the human body.

The purpose of this paper is to review the effects of radiations and radiolabelled substances on the human body.

The purpose of this paper is to review the effects of radiations and radiolabelled substances on the human body.

The purpose of this paper is to review the effects of radiations and radiolabelled substances on the human body.

The purpose of this paper is to review the effects of radiations and radiolabelled substances on the human body.

The purpose of this paper is to review the effects of radiations and radiolabelled substances on the human body.

The purpose of this paper is to review the effects of radiations and radiolabelled substances on the human body.

The purpose of this paper is to review the effects of radiations and radiolabelled substances on the human body.

I. INTRODUCTION

In recent years significant changes have occurred in the field of radio-pharmacology as it relates to diagnostic nuclear medicine which now places emphasis on the study of function and metabolism rather than on morphology. Two significant developments have resulted in the increased capability and interest in studying and quantitating metabolic function. First, improved multi-detector positron cameras can provide better resolution and transverse section reconstruction tomography with the capability of obtaining quantitative distribution data¹⁻⁴. Second, the development of radionuclide production and labeling chemistry provides positron emitting radioisotopes and radiolabeled metabolites such as 20 m carbon-11 or 10 m nitrogen-13 labeled amino acids or fatty acids and $^{13}\text{NH}_3$,⁵⁻¹² which are potentially useful for imaging and quantitating functional changes in pancreas, myocardium, brain and tumor tissues. Fluorine-18, 110 m half life, appears promising for studying regional myocardial or brain glucose metabolism as ^{18}F -deoxyglucose¹³⁻¹⁴. Other possibly useful positron emitters are gallium-68,^{11,12,15} zinc-62 amino acids¹⁶, iron-52, and rubidium-82 for myocardial perfusion¹⁷⁻¹⁹. Conventional gamma-emitters for nuclear medicine such as technetium-99m, gallium-67, indium-111, iodine-123 and thallium-201 are readily applicable to gamma-camera imaging but at present emission computed tomography is not easily accomplished with these isotopes,^{19a} although new concepts might lead to practical devices for single photon computed tomography.

This chapter will consider the important dimension of transport and how it relates to the biodistribution of radiolabeled ions and metabolites. Considerable insight into the transport process was obtained from the recent work of Christensen²⁰, and it is used as a basis for some of the material presented here. An understanding of the transport process could enable us to impose controlling agents such as hormones, ions, lipid soluble carriers, or complexing agents to obtain greater selectivity in uptake of the radiolabel as well as to study the transport process itself with

appropriate radiomarkers.

II. TRANSPORT

Many factors can influence the transport and thus the biodistribution of the radiopharmaceutical (radioions or radiometabolites). Some of the interrelated factors to be considered are: A) route of administration, B) carrier concentrations and complexed or ionic species, C) cell-membrane permeability (extracellular or intracellular accumulation) by a passive or active process and D) enzyme and hormonal stimulation or depression and the metabolic state, i.e. fasting, etc. An understanding of the transport process could provide selective and enhanced movement of the agent into cells of specific tissue such as bone, brain, kidneys, liver, marrow, myocardium, pancreas, prostate and tumors.

Some of the results of our recent work with radiolabeled metabolites such as amino acids, thioglucose and folic acid are presented to suggest some approaches to vary or control the biodistribution of radiolabeled compounds.

A. Route of Administration

1. Whether the radiopharmaceutical is administered orally, intravenously, interperitoneally or subcutaneously, it must first be absorbed into the plasma pool. From there it can go to the site of action, storage, metabolism or excretion²¹. The behavior of the substrate in-vivo would be dependent on its lipid solubility, i.e., ionized or unionized at the pH of the environment, and whether it was bound to plasma protein. Protein bound molecules and ionized molecules cannot readily permeate the cell membrane.

2. In a recent series of experiments we prepared ^{99m}Tc-labeled folic acid (^{99m}Tc-FA) by the electrolytic Sn(II) method and administered the ^{99m}Tc-FA by intravenous or intraperitoneal methods into normal mice and sacrificed the animals at 4h and 18h. The results are shown in Figure 1 and Table I respectively. There was a wide difference in uptake by the two series of mice. When given intravenously there was $22.4 \pm 9.6\%$ uptake in the liver and $1.27 \pm 0.36\%$ in the pancreas at 4h. On

the other hand when administered intraperitoneally there was $7.45 \pm 2.56\%$ uptake in the liver and $40.8 \pm 16.1\%$ in the pancreas. The substantial increase in pancreas/liver ratio to 5.36 from 0.07 by the intraperitoneal as compared to the intravenous method suggests a possible approach to enhancing pancreas uptake. The pancreas/liver ratio at 18h was 3.29 intraperitoneal compared to 0.05 intravenously. Many radio-pharmaceuticals such as $^{99m}\text{TcO}_4^-$ or ^{65}Zn -thioglucoase have essentially the same biological uptake by either i.p. or i.v. administration. However, in the case of ^{99m}Tc -FA in mice there was significant enhancement of pancreas uptake and depression of liver uptake by the intraperitoneal route. There appears to be a species variability in these results. In monkeys and dogs the uptake was primarily in gall bladder and intestinal tract as shown in Figures 2 and 3. This distribution was more apparent when administered intraperitoneally, while uptake in the liver was more pronounced when given intravenously. Further studies will be needed to determine the significance of these results to human studies.

B. Carrier Effects and Complexed or Ionic Tracers

The transport of metals in vivo as a cationic specie or as a complexed molecule is determined by the concentration of the metal ion, i.e., carrier added or "carrier free", the stability or binding constant for the complexing molecule and the site of deposition for the complexing agent.

Zn (II) is important in biochemical processes as evidenced by the ill-effects of zinc deficiency and the great amounts of zinc found in the normal body. Zinc is incorporated into many enzymes to activate and to maintain the structure of the enzyme system. As a metalloenzyme, Zn is known to accumulate in the islet cells of the pancreas and in the prostate.

Our studies with positron emitting ^{62}Zn and the effect of amino acid chelation on the biodistribution of radioactive Zn in animals has shown response to the complexing or chelating specie and to the carrier concentration (loading dose of Zn).¹⁶ Normal

rats were injected intravenously with ^{62}Zn amino acid chelates of alanine, cysteine, histidine, tryptophan, arginine and with $^{62}\text{ZnCl}_2$. The animals were sacrificed at 1.5 and 20h and the uptake determined in various tissues as shown in Table II.

The pancreas/liver concentration ratios were 0.86 for ^{62}Zn -histidine, 1.1 for ^{62}Zn -arginine, and 0.92 for $^{62}\text{ZnCl}_2$. The uptake in prostate was greatest for ^{62}Zn -histidine followed by ^{62}Zn -tryptophan and $^{62}\text{ZnCl}_2$ with prostate/muscle ratios of 5.7, 5.9 and 4.7 respectively.

Further studies were done with $^{65}\text{ZnCl}_2$ and ^{65}Zn -histidine to determine the effects of increased amounts of carrier Zn (II) and molar ratios of histidine to Zn (II) on the in vivo distribution of ^{65}Zn at 1.5 and 23h after intravenous injection in normal rats, Table III. At the 213 μg Zn (II)/kg level the ratio of pancreas/liver concentration was 1.44 at 1.5h compared to a pancreas/liver concentration ratio of 0.90 at the 30 μg Zn (II)/kg level or an increase of about 60% at the higher Zn (II) concentration. In the case of ^{65}Zn -histidine there was 1.54%/g uptake in the prostate at the 10/1 molar ratio of histidine to Zn compared to 1.29%/g at the lower 2/1 molar ratio or a prostate/muscle concentration ratio of 11.0 and 9.21 respectively.¹⁶ These results suggest that carrier Zn (II) saturate the liver binding sites. Thus, the liver uptake of ^{65}Zn is lower at the increased carrier levels of Zn (II) while the pancreas uptake is relatively unchanged. In the case of the increased molar ratio of histidine to Zn (II) which gave a 20% increase in the prostate to muscle concentration, there was a 23% decrease in the pancreas uptake. The results in Tables II and III are related to the stability constants of the Zn-amino acid chelates which are (log k): 9.5 alanine, 7.3 arginine, 18.6 cysteine, 12.9 histidine, and 9.3 tryptophan. The more stable Zn-histidine was transported to the prostate while the less stable Zn-arginine was taken up in the pancreas. This uptake was enhanced by a loading dose of Zn (II) which reduced the uptake in liver.

C. Membrane Permeability, Extracellular and Intracellular Concentrations

1. Alkali and Alkaline earth ions such as Na^+ , K^+ , Ca^{++} , and Mn^{++} are involved in passive and active transport across cell membranes. Plasma membrane of the cells are different than capillary membranes. Material that diffuses readily into extracellular interstitial space may penetrate cellular membranes very slowly.²² Substances that penetrate the capillary membrane but not the cellular membrane include inorganic ions and non-lipid soluble organic compounds. Cell membranes are highly selective permeability barriers which (1) regulate cell volume, maintain intracellular pH and ionic concentration in a narrow range favorable for enzyme activity, (2) extract and concentrate metabolic fuels and building blocks and extrude toxic substances and (3) generate ionic gradients for excitability of nerves and muscle.²³

In animal cells the concentration of K^+ is high on the inside of the cell and low on the outside. This situation is reversed in the case of Na^+ .²³⁻²⁵ In the case of Mn (II) the concentration is greater on the inside of the cell than on the outside of the cell, but in the case of Ca (II) the concentration is greater on the outside of the cell.²⁴

a. Metabolic energy is utilized by the sodium pump to maintain concentration gradients of Na^+ and K^+ .²²⁻²⁴ The sodium pump exchanged 3 moles of Na^+ for 2 moles of K^+ for 1 mole of ATP consumed.²⁰⁻²⁴ Membranes of cells which utilize a Na^+ -pump contain adenosine triphosphatase (ATPase).

Ion transport and ATP hydrolysis are closely coupled to conserve ATP energy. Hydrolysis of ATP does not occur unless Na^+ and K^+ are transported and Mg^{++} is present.²³⁻²⁶ The Na^+ requirement is specific but Rb^+ , Cs^+ , and NH_4^+ can be substituted for K^+ .²⁴ Thallium (Tl^+) can be substituted for K^+ since it accumulates in cells by the Na^+ -pump and as evidenced by the uptake of ^{201}Tl in the myocardium.^{27,28} Cardiotonic steroids such as digitoxigenin and ouabain are specific inhibitors of the Na^+/K^+ ATPase pump.²³ The sodium

pump is also involved in amino acid transport which will be discussed in a later section of the chapter. Nutritional deficiency of K^+ leads to elevated levels of cationic amino acids such as arginine and lysine.

b. Transport of Ca^{++} is also driven by hydrolysis of ATP in the presence of Mg^{++} , but the Ca^{++} -ATPase is different from the sodium pump ATPase.²³

Calcium homeostasis in the extracellular compartment is maintained by a balance in the kidneys, blood and bone, while Ca^{++} homeostasis intracellularly is maintained by constancy of Ca^{++} concentration in the serum.^{20,24,29} Calcium and phosphate ions are interrelated in metabolism, transport and turnover and they regulate each.

The pathophysiology of mitochondrial Ca^{++} accumulation indicates that (1) in tumor cells there is not only a different transport mechanism for Ca^{++} but also for adenine nucleotide which is characterized by low ATPase activity and very active Ca^{++} transport resulting in decreased phosphorylation and ATP formation; and (2) ischemic cell injury inhibits mitochondrial Ca^{++} transport which is a sensitive indicator of cellular ischemic injury.³⁰

2. An understanding of amino acid transport can be extremely useful in the utilization of ^{11}C and ^{13}N -labeled amino acids such as alanine, leucine, isoleucine, valine, tryptophan, 3,4-dihydroxyphenylalanine (DOPA) and glutamic or the decarboxylated glutamine to study the function of pancreas, heart or brain.

The distribution and relative uptake of amino acids in the myocardium has been studied by autoradiography and kinetic studies of the fate of ^{14}C -labeled compounds in vitro and in vivo.³¹⁻³⁶

Skeletal and myocardial muscle are the major sites for degrading the branched chain amino acids. These branched chain amino acids are the only essential amino acids which are oxidized mainly by muscles.³⁷ It is believed that these amino acids provide a carbon chain for intermediates in the tricarboxylic acid cycle and that amino groups released upon degradation of the branched chain amino acids are trans-

ferred to pyruvate derived from glycolysis to form alanine, and perhaps glutamine.³⁸ The fate of alanine and perhaps glutamine which are produced in the myocardium, is in hepatic gluconeogenesis.³²

The oxidation of the branched chain amino acids is stimulated by the presence of free fatty acids which inhibit the pyruvate dehydrogenase system in muscles. Octanoate can increase the rate of leucine uptake in the myocardium by a factor of 2. Oleate and palmitate are less effective but all three also reduce the uptake of alanine and pyruvate. This increase in incorporation of the branched chain amino acids by the presence of fatty acids appears to be a well established phenomenon in rat myocardial tissues. The oxidation of histidine, ornithine, glutamate, and threonine is not affected by adding fatty acids to the perfusate.³¹ It was also shown that fatty acids do not result in an increase in the amino acid pool but only the oxidation after transport into the cell.

The ability of cells to accumulate amino acids at a higher concentration than the extracellular environment is important in protein synthesis and it requires an energy-producing mechanism for transport against a concentration gradient.³⁹ Over 90% of free amino acids are in the cellular compartment where their concentrations are under endocrine and metabolic control and where each amino acid faces competition for transport from its analog. An amino acid analog at elevated concentration can cause diversion of transport energy for the maintenance of gradient for other amino acids as demonstrated in high systemic levels of phenylalanine in phenylketonuric patients which leads to depressed tissue levels of leucine, isoleucine, valine, and methionine in the brain.²⁰

The presence of glucose except at high concentration does not inhibit transport of amino acids. This suggests a separate mechanism for active transport of amino acids and carbohydrates.³⁹

Brain slices of rats accumulated both D and L tyrosine several times more than the surrounding medium while L-tryptophan but not the D-isomer was concentrated. However, the concentration of phenylalanine inhibited tyrosine accumulation.⁴⁰

D. Enzyme and Hormonal Stimulation or Depression and the Metabolic State (Fasting, etc.)

1. If the cells of an organism have essentially similar metabolic processes, how do they differentiate their functions? The answer to this question has at least two differing viewpoints. The first states that there are different transport relationships in which receptor sites developing on the surface of certain cells cause fixation of humoral agents which could accentuate or inhibit transport processes and allow particular solutes to gain access to those cells.²⁰ A second viewpoint states that the targets of hormone action are enzymes and enzymatic reactions.²⁰ The concept of endocrinologic effects on "barriers and access" has been proposed. Hormones act on cells by entering the surface of the cells to modify anatomic orientation in plasma membrane and in structural elements of the cell which then modify several enzymatic reactions simultaneously.⁴¹ Transport modification is a general method in which hormones modify the balance of cellular events.⁴²

The adenylyclase of target cells are often activated in specific response to the occupation of one or more hormonal recognition sites on the cell surface and the cyclic AMP released by these systems has an exceedingly wide range of metabolic actions. The effects of hormone via plasma membrane transmitted action which lead to a modification of transport are now widely recognized in endocrinology.²⁰

Calcium ion homeostasis is regulated by endocrine action in which the parathyroid hormone plays a major role. The parathyroid hormone regulates Ca^{++} resorption from kidney tubules and Ca^{++} resorption from bone which stimulates Ca^{++} uptake by cells of the bone.¹⁹ Cellular distribution of Ca^{++} is influenced by changes in cellular levels of 3, 5, cyclic AMP, Na^+ and HPO_4^- . Thyrocalcitonin blocks bone mobilization induced by cyclic AMP and appears to stimulate Ca^{++} exodus from bone.²⁰

2. Hormonal action effects transport into and out of cells and secretions across epithelial membranes. Sensitivity to stimulation by insulin might be a property of all Na^+ dependent transport systems both for sugars and amino acids.⁴³ In a recent experiment with ⁶⁵Zn-thioglucoase, we investigated the effect of porcine

growth hormone (pGH), somatostatin, insulin, glucose, and fasting state on the uptake of ^{65}Zn -thioglucoase in mice.⁴⁴ White mice weighing 15-20g were injected intravenously with 34 mg/kg (p.2 ml) of the preparation and sacrificed at 3 hours.

The uptake of zinc thioglucoase was determined in mice following the intraperitoneal injection of 0.5 ml 5% glucose, 0.04 units of aqueous insulin, and 4.4 units of porcine growth hormone (pGH) dissolved in 0.5 ml, 5% glucose immediately after the intravenous administration of the zinc thioglucoase. An additional group of animals was fasted for 24 hours prior to administration of zinc thioglucoase.

In an additional experiment two intraperitoneal injections of pGH were given, the first immediately after and the second 1 hour after the intravenous administration of ^{65}Zn -thioglucoase. The total dose of pGH was 5.0 international units. These results are shown in Table IV and V. The effect of pGH on the distribution of Zn-thioglucoase reflects increased uptake in pancreas and decreased uptake in liver to give a pancreas to liver ratio of 3.10 ± 0.30 in controls compared to 3.78 ± 0.33 for pGH treated mice from Table IV. Similar results were obtained for pGH-glucose but not for insulin + glucose as shown in Table V. The exact mechanism of growth hormone effect on membrane permeability and transport of glucose is unknown.⁴⁵ Some evidence implies that the transport-promoting activity of a single growth hormone injection depends on insulin.⁴⁶

E. Neoplasia and Transport

Amino acids are more intensely concentrated in neoplastic tissue,^{20,47} possibly due to some fundamental changes in membrane function at the basic level of energy transduction. The concept of neoplastic disease arising from membrane changes underlying the transport of nutrients was stated as a unifying hypothesis concerning the nature of malignant growth by Holley.⁴⁸ The selectivity of growth control could be by way of transport mechanisms at the cell surface membrane, which are in turn regulated by hormones or growth factors and permit higher concentrations of nutrients inside the cell.

F. Carriers for Radiopharmaceutical Binding to Membrane or Protein Sites

The use of bifunctional molecules for labeling and localizing radiopharmaceuticals was proposed by Goodwin in the use of ¹¹¹In with 1-(P-benzenediazonium)-ethylenediamine-N, N, N', N'-tetraacetic acid (azo- β -EDTA).⁴⁹ The covalent metal-binding molecule forms a link between the ¹¹¹In-EDTA chelate and the protein by means of a diazo bond.

Other studies have been made with radiolabeled liposomes and vesicles to obtain interaction with antibodies, antigens or peptide hormones.⁵⁰⁻⁵³ Vesicles or liposomes can be used to introduce radiolabels into specific targets by coupling other molecules to their surfaces which would act as membrane receptors. Artificial lipid vesicles were bound to human ¹²⁵I-antithyroglobulin and human ¹²⁵I-thyrotropin; the addition of a specific antibody or hormone to the vesicles might confer specificity in vivo.⁵² Whether liposomes and vesicles will be an important method for transport specificity to sites of tumors or function remains to be investigated.

III. CONCLUSION

An understanding of the transport process can help to provide a basis for the development of radiopharmacology for chemotherapy and diagnosis. To properly evaluate and utilize information obtainable by recent advances in instrumentation and radiopharmaceuticals for the study of function and biochemistry in vivo, it is necessary to consider the transport process in body fluids and intracellular space.

Recent studies using dynamic emission tomography for study of cerebral hemodynamics and a study of the quantitative potential of dynamic emission computed tomography,^{54,55} indicate that a greater understanding of metabolic and transport processes is possible with nuclear medicine procedures utilizing improved instrumentation and radiopharmaceuticals.

ACKNOWLEDGMENTS

My thanks and appreciation to Dr. Thomas F. Budinger for helpful discussions, support and encouragement in this work. Also to Dr. Harold L. Atkins for collaboration and permission to use material on the ^{65}Zn thioglucose work. My thanks also to Eugene P. Roth, and Brian R. Moyer for excellent technical assistance and to Gretchen Hermes for preparing the manuscript. This work was supported by the Biology and Medical Division, U. S. Department of Energy.

REFERENCES

1. Burnham, C.A., and Brownell, G.L., A multi-crystal positron camera, *IEEE Trans. Nucl. Sci.*, 19, 201, 1972.
2. Phelps, M.E., Hoffman, E.J., Mullani, N.A., et al., Transaxial emission reconstruction tomography: Coincidence detection of positron emitting radionuclides, in Non-Invasive Brain Imaging, Computed Tomography and Radionuclides, DeBlanc, H.J., and Sorenson, H.A., Eds., Society of Nuclear Medicine, New York, 1975, 87.
3. Derenzo, S.E., Zaklad, H., and Budinger, T.F., Analytical study of a high-resolution positron ring detector system for transaxial reconstruction tomography, *J. Nucl. Med.*, 16, 1166, 1975.
4. Chou, Z.H., Chan, J.K., and Erikson, L., Circular ring transverse axial positron camera for three-dimensional reconstruction of radionuclide distribution, *IEEE Trans. Nucl. Sci.*, 23, 613, 1976.
5. Washburn, L.C., Wieland, B.W., Sun, T.T., and Hayes, R.L., ^{11}C -labeled amino acids as agents for tumor and pancreas visualization, *J. of Labelled Compounds and Radiopharmaceuticals*, 13, No. 2, 203, 1976.
6. Vaalburg, W., Beerling-Van Der Molen, H.D., Reiffers, S., Preparation of carbon-11 labelled phenylalanine and phenylglycine by a new amino acid synthesis, *Int. J. Appl. Radiat. and Isotopes*, 27, 153, 1976.
- 7a. Weiss, E.S., Hoffman, E.J., Phelps, M.E., Welch, M.J., et al., External detection and visualization of myocardial ischemia with ^{11}C -substrates in vitro and in vivo, *Circulation Res.*, 39, 24, 1976.
- 7b. Hoffman, E.J., Phelps, M.E., Weiss, E.S., et al., Transaxial tomographic imaging of canine myocardium with ^{11}C -palmitic acid, *J. Nucl. Med.*, 18, 57, 1977.
8. Harper, P.V., Schwartz, J., Beck, R.N., et al., Clinical myocardial imaging with nitrogen-13 ammonia, *Radiology*, 108, 613, 1973.
9. Cohen, M.D., Spolter, L., MacDonald, N., et al., Sequential enzymatic synthesis and separation of ^{13}N -L-glutamic acid and ^{13}N -L-alanine, in Radiopharmaceuticals, Subramanian, G., Rhodes, B.A., Cooper, J.F., and Sodd, V.J., Eds., Society of Nuclear Medicine, New York, 1975, 184.
10. Gelbard, A.S., Christie, T.R., Clarke, L.P., Laughlin, J.S., Imaging of spontaneous tumors with ammonia and L-glutamine labeled with N-13, *J. Nucl. Med.*, 18, 718, 1977.
11. Hoop, B., Hnatowich, D.J., Brownell, G.L., et al., Techniques for positron scintigraphy of the brain, *J. Nucl. Med.*, 17, 473, 1976.
12. Tilbury, R.S., Dahl, J.R., Monahan, W.G., et al., ^{13}N -labeled ammonia for medical use. *Radiochim. Radioanal. Lett.*, 8, 317, 1971.
13. Gallagher, B.M., Ansari, A., Atkins, H., Christman, D.R., et al., Radiopharmaceuticals XXVII. ^{18}F -Labelled 2-deoxy-2-fluoro-D-glucose as a radiopharmaceutical for measuring regional myocardial glucose metabolism in vivo: Tissue distribution and imaging studies in animals, *J. Nucl. Med.*, 18, 990, 1977.

14. Clark, J.C., Goulding, R.W., et al., The preparation of fluorine-18 labeled compounds using recirculatory neon target, Radiochem. Radioanal. Letters, 14, 101, 1973.
15. Welch, M.J., Wagner, S.J., Preparation of positron-emitting radiopharmaceuticals, in Recent Advances in Nuclear Medicine, Lawrence, J.H., and Budinger, T.F., Eds., Grune and Stratton, New York, Vol 5, in press.
16. Yano, Y. and Budinger, T.F., Cyclotron-produced Zn-62: Its possible use in prostate and pancreas scanning as a Zn-62 amino acid chelate, J. Nucl. Med., 18, 815, 1977.
17. Budinger, T.F., Yano, Y., Hoop, B.: A comparison of ^{82}Rb and $^{13}\text{NH}_3$ for myocardial positron scintigraphy, J. Nucl. Med., 15, 429, 1975.
18. Hoop, B., Beh, R.A., Beller, G.A., et al., Myocardial positron scintigraphy with short live ^{82}Rb , IEEE Trans. Nucl. Sci., 23, 584, 1976.
19. Yano, Y., Chu, P., Budinger, T.F., et al., Rubidium-82 generators for imaging studies, J. Nucl. Med., 18, 46, 1977.
- 19a. Budinger, T.F., Cahoon, J.L., Derenzo, S.E., et al., Three-dimensional imaging of the myocardium with radionuclides, Radiology, 125, 433, 1977.
20. Christensen, H.M., Biological Transport, W.A., Benjamin, Inc., London, 1975, Chap. 12.
21. Cohn, V.H., Transmembrane movement of drug molecules; in Fundamentals of Drug Metabolism and Drug Disposition, LaDu, B.N., Mandel, H.G., and Way, E.L., Eds., Williams and Wilkins, Baltimore, 1972, Chap. 1.
22. Schanker, L.S., Drug absorption, in Fundamentals of Drug Metabolism and Drug Disposition, LaDu, B.N., Mandel, H.G., and Way, E.L., Eds., Williams and Williams, Baltimore, 1972, Chap. 2.
23. Stryer, L., Biochemistry, H. Freeman Co., San Francisco., 1975, 767.
24. Ochiai, Ei-ichiro, Bioinorganic Chemistry, Allyn and Bacon, Inc., Boston, 1977, Chap. 15.
25. Jorgensen, P.L., Isolation and characterization of the components of the sodium pump, Quart. Rev. Biophysics, 7, 239, 1974.
26. Skou, J.C., The $(\text{Na}^+ + \text{K}^+)$ activated enzyme system and its relationship to transport of sodium and potassium, Quart. Rev. Biophysics, 7, 401, 1974.
27. Passow, H., The red blood cell: Penetration, distribution and toxic actions of heavy metals, in Effects of Metals on Cells, Subcellular Elements and Macromolecules, Maniloff, J., Coleman, J.R., and Miller, M.W., Eds., Charles C. Thomas, Springfield, 1970, Chap. 16.
28. Atkins, H.L., Budinger, T.F., Lebowitz, E., et al., Thallium-201 for medical use. Part 3: Human distribution and physical imaging properties, J. Nucl. Med., 18, 133, 1977.

29. Rasmussen, H., Ionic and humoral control of calcium homeostasis, The Am. J. Med., 50, 567, 1971.
30. Mela, L., Mechanism and physiological significance of calcium transport across mammalian mitochondrial membranes, in Current Topics in Membrane and Transport, Bronner, F., and Kleinzeller, A., Eds., Academic Press, New York,
31. Buse, M.G., Biggers, J.F., Friderici, K.H., Buse, J.F., Oxidation of branched chain amino acids by isolated hearts and diaphragms of the rat, J. Biol. Chem., 247, 1972.
32. Mallette, L.E. Exton, J.H. and Park, C.R., Alanine is the main amino acid substrate of hepatic gluconeogenesis, J. Biol. Chem., 244, 5724, 1969.
33. Cohen, M.B. Spolter, L., MacDonald, N.S., et al., Radiopharmaceuticals and Labeled Compounds, Vienna, IAEA, 1973, 483.
34. DeBold, A.J., and Bencosone, S.A., In Recent Advances in Studies on Cardiac Structure and Metabolism, Vol. 8, University Park Press, Baltimore, 1975, 129.
35. Friedberg, F., Partition of I.V. administered amino acids in blood and tissues, J. Biol. Chem., 168, 411, 1947.
36. Busch, H., et al., The uptake of a variety of amino acids into nuclear proteins and other tissues. Cancer Res. 19, 1030, 1959.
37. McFarlane, I.G. and von Holt, C., Stimulation of oxidation of branched chain amino acids by fatty acids, Biochem. J., 111, 565-571, 1969.
38. Odessey, R., Khairallah, E.A., and Goldberg, A.L., Origin and possible significance of alanine production by skeletal muscle, J. Biol. Chem., 249, 7623, 1974.
39. Meister, A., Biochemistry of the Amino Acids, Vol. 2, Academic Press, New York, 1965, Chap. 6.
40. Chirgus, M.A., Greengard, P., and Udenfriend, S., Uptake of tyrosine by rat brain in vivo, J. Biol. Chem., 235, 2075, 1960.
41. Peters, R.A., Hormones and the cytoskeleton, Nature, 177, 426, 1956.
42. Noall, M.W., Riggs, T.R., Walker, L.M., and Christensen, H.N., Endocrine control of amino acid transfer, Science, 126, 1002, 1957.
43. Riggs, T.R., and McKirahan, K.J., Insulin stimulation of L-Alanine transport in rat diaphragm: Evidence that the hormone affects only one transport system, Fed. Proc., 30, 1116 abs, 1971.
44. Atkins, H.L., and Yano, Y. Pancrease uptake of zinc thioglucose, To be published.
45. Levine, R., Some mechanisms for hormonal effects on substrate transport, in Metabolic Pathways, Hokin, L.E., Ed., Academic Press, New York, 1972, Chap. 16.
46. Mahler, R.J., and Szabo, O., Insulin action upon insulin insensitive tissue following impaired degradation of the hormone, Proc. Soc. Exp. Biol. Med., 125, 879, 1967.

47. Busch, H., Davis, J.R., Honig, G.R., Anderson, D.C., et al, The uptake of a variety of amino acids into nuclear proteins of tumors and other tissue, *Cancer Research*, 19, 1030, 1959.
48. Holley, R.W., A unifying hypothesis concerning the nature of malignant growth, *Proc. Nat. Acad. Sci., U.S.A.*, 69, No. 10, 2840, 1972.
49. Goodwin, D.H., Sundberg, M.W., Diamanti, C.I., and Meares, C.F., ¹¹¹In-labeled radiopharmaceuticals and their clinical use, in *Radiopharmaceuticals*, Subramanian, G., Rhodes, B.A., Cooper, J.F., and Sodd, V.J., Eds., *The Society of Nuclear Medicine, Inc.*, New York, 1975, Chap. 9.
50. McDougall, I.R., Dunnick, J.K., and McNamee, M.G., Distribution and fate of synthetic lipid vesicles in the mouse. A combined radionuclide and spin label study, *Proc. Natl. Acad. Sci. U.S.A.*, 71, 3487, 1974.
51. McDougall, I.R., Dunnick, J.K., Goris, M.L., and Kriss, J.P., In vivo distribution of vesicles loaded with radiopharmaceuticals: A study of different routes of administration, *J. Nucl. Med.* 16, 488, 1975.
52. Caride, V.J., Taylor, W., Cramer, J.A., and Gottschalk, A., Evaluation of liposome-entrapped radioactive tracers as scanning agents. Part I: Organ distribution of liposome ^{99m}Tc-DTPA in mice, *J. Nucl. Med.*, 17, 1067, 1976.
53. Dunnick, J.K., Badger, R.S., Takeda, YI, and Kriss, J.P., Vescile interactions with antibody and peptide hormone: Role of vesicle composition, *J. Nucl. Med.*, 17, 1073, 1976.
54. Yamamoto, Y.L., Thompson, C.J., Meyer, E., et al., Dynamic positron emission tomography for study of cerebral hemodynamics in a cross-section of the head using positron-emitting ⁶⁸Ga-EDTA and ⁷⁷Kr, *J. Computer Assisted Tomography*, 1, 43, 1977.
55. Budinger, T.F., Derenzo, S.E., Greenberg, W.L., et al., Quantitative potentials of dynamic emission computed tomography, *J. Nuc. Med.*, in press.

Figure Captions

1. Distribution of ^{99m}Tc -Folic Acid in mice 4h after i.v. and i.p. administration. Percent injected dose/gram tissue plotted on log scale.
2. Gamma camera images of ^{99m}Tc -Folic Acid 19h after i.p. administration in a monkey, anterior views with and without $^{99m}\text{Tc}_2\text{S}_7$.
3. Gamma camera images of ^{99m}Tc -Folic Acid 2.5 and 20h after i.p. administration in a dog.

TABLE I
Tc-99m Folic Acid Distribution in Mice 18h
after i.v. and i.p. Administration

	10 μ g/kg % $\pm$ S. D.		% / g $\pm$ S. D.	
	i.v. (n=5)	i.p. (n=4)	i.v. (n=5)	i.p. (n=4)
Blood	---	---	1.35 $\pm$ 0.32	0.69 $\pm$ 0.08
Liver	12.87 $\pm$ 1.95	4.02 $\pm$ 1.70	9.44 $\pm$ 2.46	2.50 $\pm$ 0.43
Kidneys	3.89 $\pm$ 0.56	2.48 $\pm$ 0.33	12.37 $\pm$ 2.72	7.24 $\pm$ 1.06
Spleen	0.49 $\pm$ 0.08	0.48 $\pm$ 0.28	3.55 $\pm$ 0.50	3.66 $\pm$ 2.96
Gut & Stomach	4.75 $\pm$ 0.71	4.76 $\pm$ 0.41	1.77 $\pm$ 0.42	1.43 $\pm$ 0.14
Pancreas	0.07 $\pm$ 0.02	1.13 $\pm$ 0.56	0.49 $\pm$ 0.14	8.28 $\pm$ 3.97
Pancreas/ Liver	---	---	0.05 $\pm$ 0.02	3.29 $\pm$ 0.75
Total	29.37 $\pm$ 1.97	24.25 $\pm$ 3.12	---	---

XBL7712-4047

TABLE II

Uptake of ^{65}Zn -amino acid and chloride in normal rats, %/g. mean of 3-6 (range)

	1.5hr	2hr	1.5hr	2hr	1.5hr	2hr	1.5hr	2hr	1.5hr	2hr	1.5hr	2hr	1.5hr	2hr	1.5hr	2hr	1.5hr	2hr	1.5hr	2hr	1.5hr	2hr	1.5hr	2hr	1.5hr	2hr	1.5hr	2hr
Alanine	0.22	0.24	2.84	1.35	1.51	0.20	0.12	0.08-0.19	(0.02-0.56)	(0.22-1.90)	0.93	0.22	0.71	4.23	0.26	0.55	3.33	0.72	2.25	9.55	0.47	0.11	0.08	0.10	0.16	0.10	0.11	0.09-0.12
Cysteine	1.5hr	0.15-0.23	(2.03-2.35)	(1.92-2.94)	(1.14-1.50)	(1.49-1.69)	0.66	0.08	(0.07-0.09)	0.74	0.43	0.27	0.51	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19
20hr	0.10	0.10	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74
1.5hr	0.03-0.11	(0.79-1.07)	(0.68-0.86)	(0.66-0.82)	(0.27-0.51)	(0.81-1.47)	0.09	0.12	(0.09-0.12)	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19
20hr	0.19	0.19	1.40	1.30	1.23	1.07	1.19	0.21	(0.18-0.20)	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21
1.5hr	0.13-0.22	(2.03-2.15)	(2.07-2.41)	(1.07-1.32)	(1.00-2.14)	(0.10-0.53)	0.07	0.12	(0.02-0.12)	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
20hr	0.15	0.15	1.19	1.23	1.10	0.91	1.06	0.18	(0.12-0.25)	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Arginine	1.5hr	0.19	2.29	2.43	1.52	2.44	0.64	0.14	(0.13-0.25)	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14
20hr	0.12	0.12	1.03	0.83	0.33	0.66	0.93	0.16	(0.12-0.16)	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Chloride	1.5hr	0.35-0.59	(2.42-2.90)	(3.29-3.32)	(1.53-1.96)	(2.16-2.76)	0.57	0.19	(0.19-0.19)	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19
20hr	0.15	0.15	1.48	1.10	1.13	0.96	0.94	0.20	(0.17-0.26)	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20

Vano, V., Budinger, T.F., Cyclotron produced Zn-62: its possible use in prostate and pancreas scanning as a Zn-62 amino acid chelate, J. Nuc. Med., 18:815-821, 1977, The Society of Nuclear Medicine, New York.

XBL7712-4053

Table 1
³⁵S/³²S Optimal in Normal Rats
 mean of 3 animals (range)

Organ	³⁵ S at 1.5h		³⁵ S at 2h	
	Prep. A	Prep. B	Prep. A	Prep. B
Blood	0.17 (0.15-0.19)	0.23 (0.22-0.24)	0.63 (0.09-0.10)	0.10 (0.09-0.11)
Heart	0.95 (0.42-0.49)	0.37 (0.40-0.41)	0.43 (0.27-0.50)	0.44 (0.43-0.47)
Lungs	0.50 (0.50-0.60)	0.53 (0.52-0.55)	0.52 (0.50-0.60)	0.52 (0.45-0.56)
Liver	2.50 (2.07-3.10)	1.56 (1.53-1.59)	0.99 (0.78-0.83)	0.27 (0.04-0.71)
Kidneys	2.07 (1.60-2.60)	1.15 (1.66-1.19)	0.71 (0.67-0.78)	0.63 (0.61-0.64)
Spleen	1.13 (1.01-1.30)	0.81 (0.89-0.88)	0.14 (0.65-0.63)	0.65 (0.65-0.67)
Pancreas	2.25 (2.15-2.35)	2.21 (1.00-2.45)	0.54 (0.44-0.67)	0.54 (0.47-0.61)
Testis	0.51 (0.47-0.71)	0.21 (0.63-0.61)	0.03 (0.45-0.43)	0.03 (0.59-0.50)
Sem. Vesic.	0.16 (0.17-0.21)	0.27 (0.17-0.39)	0.25 (0.21-0.27)	0.30 (0.18-0.37)
³⁵ S femoral 24 hr	0.90	1.45	0.68	0.67
³⁵ S femoral 24 hr blood	3.12	3.03	9.11	8.00

Prep. B 30mg Zn/kg rat (A.S. wt. 300g)

Prep. B 213mg Zn/kg rat (A.S. wt. 300g)

Table 2
³⁵S/³²S Optimal in Normal Rats
 mean of 3 animals (range)

Organ	³⁵ S at 1.5h		³⁵ S at 2h	
	Prep. A	Prep. B	Prep. A	Prep. B
Blood	0.23 (0.22-0.24)	0.23 (0.22-0.24)	0.18 (0.12-0.16)	0.13 (0.12-0.15)
Heart	0.58 (0.51-0.62)	0.42 (0.47-0.70)	0.63 (0.59-0.78)	0.16 (0.62-0.70)
Lungs	0.80 (0.80-0.95)	0.65 (0.70-0.90)	0.72 (0.71-0.81)	0.71 (0.62-0.81)
Liver	2.80 (2.69-2.94)	2.81 (2.50-3.18)	1.88 (1.47-1.81)	1.00 (1.11-1.70)
Kidneys	2.81 (2.74-2.89)	2.81 (2.62-3.12)	0.98 (0.84-1.07)	1.01 (0.84-1.05)
Spleen	1.37 (1.25-1.50)	1.47 (1.27-1.56)	0.99 (0.93-1.09)	1.17 (1.05-1.19)
Pancreas	1.20 (1.10-1.23)	2.11 (2.16-2.47)	0.71 (0.53-0.76)	0.99 (0.42-1.00)
Testis	0.65 (0.58-0.71)	0.21 (0.63-0.67)	1.27 (0.26-1.00)	1.55 (1.21-1.87)
Sem. Vesic.	0.45 (0.41-0.50)	0.31 (0.28-0.42)	0.57 (0.42-0.74)	0.46 (0.36-0.55)
³⁵ S femoral 24 hr	1.13	0.86	0.51	0.69
³⁵ S femoral 24 hr blood	2.87	3.33	9.71	11.0

Prep. A 2.1 histidine: Zn
 Prep. B 10:1 histidine: Zn

1966, J. Radioanal. Chem. 10: 1-10. The authors are indebted to Dr. J. H. D. for his assistance in the preparation of the manuscript and to Dr. J. H. D. for his assistance in the preparation of the manuscript.

XBL 7712-4054

Table IV

Effect of Growth Hormone on ^{65}Zn -Thioglucose
Distribution in Mice at 3 hours

	<u>Control (n=8)</u>		<u>pGH, 2.5 I.U. x 2 (n=8)</u>	
	<u>% $\pm$ S.D.</u>	<u>%/g $\pm$ S.D.</u>	<u>% $\pm$ S.D.</u>	<u>%/g $\pm$ S.D.</u>
Pancreas	7.27 $\pm$ 0.98	37.76 $\pm$ 5.27	10.98 ^h $\pm$ 2.22	41.35 $\pm$ 5.93
Liver ^a	16.68 $\pm$ 1.16	12.58 $\pm$ 0.33	15.42 $\pm$ 1.83	10.89 ^h $\pm$ 0.65
Blood		2.51 $\pm$ 0.46		3.03 $\pm$ 0.95
Kidneys	3.11 $\pm$ 0.039	10.07 $\pm$ 1.23	3.31 $\pm$ 0.43	9.58 $\pm$ 1.41
Adrenals	0.040 $\pm$ 0.005	5.50 $\pm$ 1.21	0.056 ^a $\pm$ 0.016	5.98 $\pm$ 0.89
Intestines	29.80 $\pm$ 1.91	12.14 $\pm$ 1.89	25.25 ^h $\pm$ 1.52	9.24 ^a $\pm$ 1.02
Carcass	33.73 $\pm$ 2.59		37.17 ^a $\pm$ 2.14	
Total Body	92.96 $\pm$ 3.68		93.40 $\pm$ 4.34	
Pancreas/ ^c Liver		3.10 $\pm$ 0.30		3.78 ^a $\pm$ 0.33

^a $P = 0.01 - 0.001$

^b $P < 0.0005$

^c Only 7 animals in the control group. One animal was excluded because of a liver tumor.

Table V

⁶⁵Zn-Thioglucoase in Mice, 3 hrs. %/organ - S.D.

	Control A	Control B	24 hrs. fast	0.5 ml 5% D/W i. p.	40 m units insulin i. p.	10 ⁻⁷ M pGH i. p. + glucose
Pancreas	9.60 ± 1.63	7.30 ± 0.80	8.68 ± 1.80	7.57 ^a ± 0.58	9.55 ^b ± 1.55	11.44 ^b ± 1.70
Liver	16.35 ± 0.82	16.79 ± 0.74	17.56 ± 2.00	14.94 ± 0.86	15.08 ± 1.77	15.90 ± 2.59
Kidneys	3.11 ± 0.38	3.21 ± 0.11	2.91 ^b ± 0.34	2.77 ^b ± 0.32	2.65 ^b ± 0.35	2.80 ± 0.42
Intestines	34.04 ± 1.01	30.60 ± 1.38	36.88 ± 8.80	29.81 ± 1.38	29.31 ± 3.43	22.48 ^{a,b} ± 2.44
<u>Pancreas</u> <u>Liver</u>	3.77 ± 0.65	3.23 ± 0.15	3.84 ± 0.61	2.97 ± 0.33	3.33 ± 0.23	4.41 ± 1.48

^a P < 0.05 (Control A)^b P < 0.05 (Control B)

Tc-99m Folic Acid 4hr Mice I.V. vs I.P.

Fig. 1

XBL7712-4055

Monkey at 19h, I.P.
Anterior

XBB 7712-12687

Fig. 2

Dog I.P.

^{99m}Tc Folic Acid

XBB 7712-12688

Fig. 3