

A physicist in biomedical investigation

There are many opportunities for collaborative efforts in physics and medicine; the radioimmunoassay, for example, was developed in one such effort.

Rosalyn S. Yalow



What was involved in my transition from nuclear physicist to biomedical investigator and recipient of a Nobel Prize in Physiology or Medicine in 1977 for the development and application of radioimmunoassay? What is radioimmunoassay? In what way did physics have a role in the development of the concepts and methodology of radioimmunoassay? How did it begin?

It was the Summer of 1947. School was out. Freed from my responsibilities of teaching physics at Hunter College (now Lehman College) in a special pre-engineering program for returning veterans, I was looking for something useful to occupy my time. (Hunter had no research facilities.) My husband, Aaron, was in Medical Physics at Montefiore Hospital in the Bronx. Through him I met Edith Quimby, a leading medical physicist at College of Physicians and Surgeons. I volunteered to work in her laboratory to gain research experience in the medical applications of radioisotopes. She took me to see "The Chief," Gioacchino Failla, Dean of American medical physicists. After talking to me for a while, he picked up the phone, dialed, and I heard him say "Bernie, if you want to set up a radioisotope service, I have someone here you must hire." Bernard Roswit, Chief of the Radiotherapy Service at the Bronx Veterans Administration Hospital and I appeared to have no choice; Dr. Failla had spoken. It was December 1947 when I joined the Bronx Veterans Administration Hospital, just two years after completing my PhD in Nuclear Physics at the University of Illinois under the guidance of Maurice and Gertrude Goldhaber. It

Bismuth-walled Geiger counter for detection of iodine-131 taken up by the thyroid. This counter was built in 1949, before the days of scintillation counters, and is still in use, as this recent photo of R. S. Yalow examining a patient shows. The bismuth walls permit much greater sensitivity to γ rays from I^{131} than the thin silver walls of the then available counters.

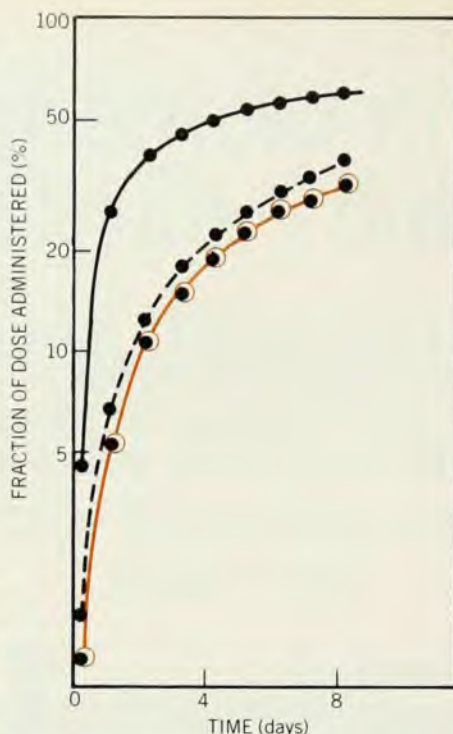
Figure 1

is of interest that several of us who received PhD's during that period later went into medical physics—perhaps due to the influence of Donald Kerst and Henry Quastler. My function at the hospital was to equip and develop a Radioisotope Service and to initiate a program of radioisotope-related research. This was shortly after the nuclear reactor in Oak Ridge began to supply radioisotopes in sufficient quantity and at low enough cost to make them readily available for therapy, diagnosis and biomedical investigation. In 1947 commercial instrumentation was not readily available and it was necessary to design, build and calibrate suitable radiation detection equipment such as that shown in figure 1. Then we had to plan experiments for the safe use of radioisotopes in humans. It soon became obvious to me that the most important applications of radioisotopic methodology would be in understanding human physiology and as an aid in clinical diagnosis. By 1950, I knew I was committed to biomedical investigation and finally resigned from Hunter.

A multidisciplinary approach is necessary to weave the tools and concepts of physics into medicine. Maximal effectiveness is achieved only when each member of an interdisciplinary team makes a commitment to at least on-the-job training in the discipline of the other(s). I was fortunate to be joined in my work by a very talented physician, Solomon Berson. We were close collaborators from 1950 until his death in 1972. I learned medicine and he showed a remarkable talent for physics and mathematics. We learned to talk the same hybrid language—a major factor in our success as a research team. Much of my recent work has been in collaboration with another physician, Eugene Straus, who joined my laboratory as a research associate in 1972.

Early studies

In the early 1950's our work was concerned with studies of the kinetics of iodide trapping in the thyroid gland and the subsequent release of thyroid hormone and its metabolism; with the use of the isotope dilution technique, first described by George von Hevesy (who received the Nobel Prize in Chemistry in 1943 for his work on the use of isotopes in the study of chemical processes), to determine the size of various body compartments; with investigations of the metabolism of proteins using radioiodine-labeled tracers. The common feature of these studies was the use of radioisotopes and of mathematical modeling.



Cumulative urinary excretion of radioiodide following intravenous administration of I^{131} -labeled albumin. The colored curve and data show the behavior of labeled albumin that was not irradiated. The black spots show the results for samples of albumin at various concentrations irradiated with 45 krad dose of x rays: top curve (black) 200 $\mu\text{g}/\text{ml}$ albumin concentrations; middle curve (dashed) 5000 $\mu\text{g}/\text{ml}$; bottom curve (indistinguishable from the control) 50 000 $\mu\text{g}/\text{ml}$. The more rapid excretion of radioiodide for the lower concentrations indicates that the radiation produced more damage than in more highly concentrated solutions. Figure 2

A digression into radiation chemistry was also required in order to understand the problems encountered in the preparation and storage of labeled proteins. We were analyzing, by compartmental modeling, the disappearance of I^{131} -labeled albumin from plasma following intravenous administration, and the appearance in the urine of radioiodide to permit us to determine the rates of degradation and synthesis of albumin. However, it was soon evident that widely differing rates that we often observed in the same clinical states occurred because of differences in the biologic behavior not of albumin per se but of different lots of I^{131} -albumin used as tracers. We considered that the variation among these lots might be a consequence of the indirect effects of radiation arising from the absorption of the β -particles from I^{131} during radioactive decay. Assuming complete absorption of this radiation, the absorbed dose in one week in a solution initially containing the usual concentration of 0.8 millicurie I^{131}/ml is about 45 kilorads. We were able to demonstrate that lots containing labeled albumin at a concentration of 200 microgram/ml and immediately exposed to 45 krad of external radiation could not be distin-

guished from an unirradiated preparation by physicochemical means. However such irradiated lots were recognized as different by the human body. The disappearance rate from plasma of the externally irradiated tracer was much more rapid than that of the unirradiated preparation. Furthermore the more rapid rate of disappearance was associated with the more rapid appearance of the degradation product, radioiodide, in the urine (figure 2). Increasing the concentration of protein in the solution protected the labeled albumin from irradiation damage since such damage is secondary in nature (it is due to the free radicals produced as a result of irradiation of the water), and can be minimized by the presence of protective scavengers or higher concentrations of protein. These studies provided the basis for preparing tracers labeled with iodine-131 suitable for studying the distribution and turnover of labeled serum proteins.

Diabetes

At this time, I. Arthur Mirsky, a distinguished diabetologist, suggested that diabetes in the adult might be due to abnormally rapid degradation of insulin by a liver enzyme he called "insulinase." This was a reasonable suggestion since it was known that the pancreas of the adult diabetic contains essentially normal amounts of insulin, and at the time, 25 years ago, it was generally agreed that all diabetes is due to a deficiency of circulating insulin. We therefore thought we could employ methods similar to those we had used for studying the distribution and turnover of serum proteins to the study of the turnover of the peptide hormone insulin. We administered I^{131} -labeled insulin intravenously to non-diabetic and diabetic subjects. We observed that radioactive insulin disappeared more slowly from the plasma of patients who had received insulin, either for the treatment of diabetes or as shock therapy for schizophrenia, than from the plasma of subjects never treated with insulin. We suspected that the retarded rate of insulin disappearance was due to binding of labeled insulin to antibodies that had developed in response to administration of exogenous insulin. However, classic immunologic techniques, which could detect concentrations no less than 10^{-6} M, were not adequate for the detection of antibodies that we presumed were of such low concentration as to be nonprecipitating. We therefore introduced radioisotopic methods of high sensitivity for detection of soluble antigen-antibody complexes. With these methods we were able to effect a 10^3 to 10^6 increase in sensitivity. The method we first used was quite simple—paper electrophoresis. The electrophoretic patterns of labeled insulin in the plasma of controls and insulin-treated subjects are very different (figure 3). During electrophoresis, labeled insulin in

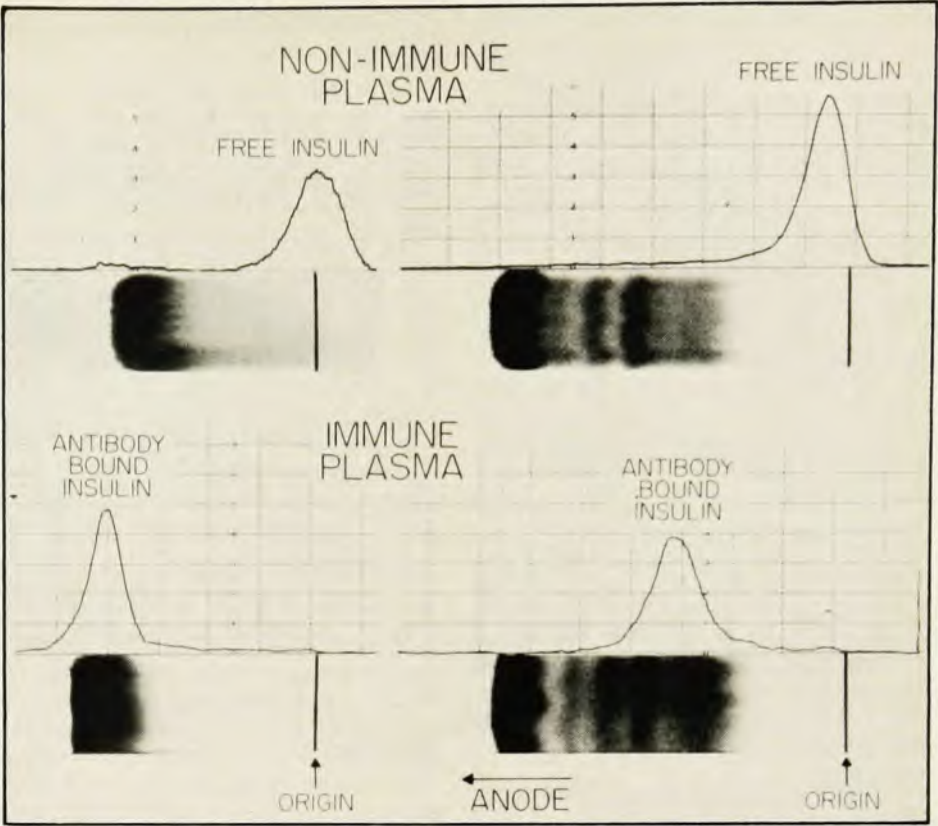
Rosalyn Yalow received the Nobel Prize in Physiology or Medicine in 1977 for her role in the development of the radioimmunoassay; she is with the Solomon A Berson Research Laboratory of the Veterans Administration Medical Center, Bronx, New York.

control plasma binds to the paper at the site of application. In the plasma of the insulin-treated patients, the labeled insulin is bound to and migrates with a plasma globulin with an electrophoretic mobility between that of the β and γ globulins. Using a variety of other physicochemical systems, we were able to demonstrate the ubiquitous presence of insulin-binding globulins, that is antibodies, in the plasma of all insulin-treated subjects.

We also demonstrated that the binding of labeled insulin to a fixed concentration of antibody is a quantitative function of the amount of insulin present. This observation was the basis for the radioimmunoassay of plasma insulin. However, investigations and analyses that lasted for several years, and which included studies on the quantitative aspects, especially the thermodynamics and chemical kinetics, of the reaction between insulin and antibody and the species specificity of the available antiserums, were required to translate the theoretical concepts of radioimmunoassay into experiments that led first to the measurement of plasma insulin in rabbits following administration of beef insulin and finally, in 1959, to the measurement of insulin in unextracted human plasma.

Before discussing radioimmunoassay, let me digress into a very interesting area in which the specificity of the reaction of antigen with antibody can be used as a tool for examining the three-dimensional configuration of peptides. The antigen-antibody reaction can be likened to a key-lock fit. We studied the inhibition of binding of labeled beef insulin to antisera from guinea pigs and different human subjects by insulins from three species—dog, pig and whale—whose amino acid sequences were reported to be identical by the laboratory of Frederick Sanger, who had received the Nobel Prize in Chemistry in 1956 for just this determination. Although it was one of the tenets of biochemistry that the amino acid sequence determines structural configuration, we noted striking differences, shown in figure 4, in the immunologic behavior of these insulins with some antisera but not with others. We considered it possible that structural differences might have been introduced during the extraction and purification of the insulins and therefore undertook to compare the behavior of endogenous circulating insulin with that of crystalline insulins purified from pancreatic extracts of the same species. We demonstrated superposability of endogenous dog plasma insulin on the dilution curve of crystalline dog insulin, as well as superposability of endogenous pig plasma insulin on the dilution curve of crystalline pig insulin. Dog and pig insulin are thus shown to be structurally different in spite of identical amino-acid sequences. Why?

The mystery seems to have been

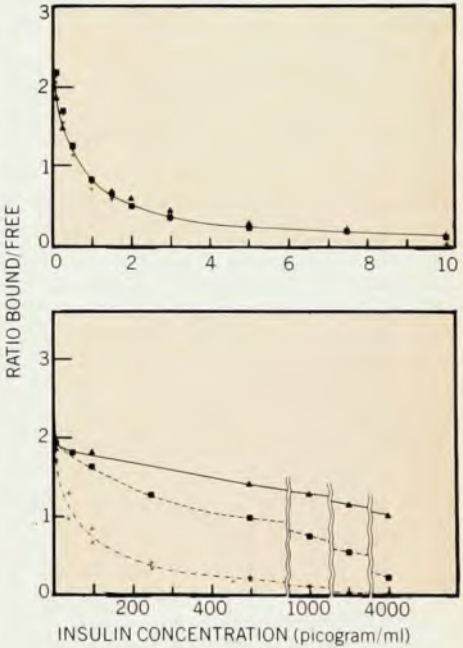


Electrophoresis patterns for plasma from subjects that had never been treated with insulin (top) and subject that had been treated with insulin (bottom). Insulin labeled with I^{131} was added to the plasma, and the mixtures were applied to paper strips for electrophoresis (right) or for hydrodynamic-flow chromatography combined with electrophoresis (left). Above the electrophoresis patterns we show scans of the radioactivity of the paper strip. The protein closest to the anode is albumin; the α , β , and γ globulins are progressively closer to the cathode. **Figure 3**

cleared by the discovery in 1969 by Donald F. Steiner and his associates of proinsulin, the precursor to insulin. Proinsulin is a 9000 dalton peptide in which the A and B chains of insulin are joined by a connecting peptide (C-peptide). Subsequent enzymatic removal of the C-peptide leaves the familiar insulin molecule. The configuration of the proinsulin molecule is determined at the time of its synthesis. Since it is now known that the amino-acid sequences of the C-peptides in dog and pig proinsulins are strikingly different, conformational differences between the two prohormones are not surprising. If the subsequent removal of the C-peptide leaves the folding of the molecules unaltered, then pig and dog insulins, in spite of the identity of primary structure, would remain distinguishable. Studies in which separated A and B chains of pig and dog insulins are connected by chemical means are necessary to determine which configuration is preferred without the constraint introduced by the C-peptide of proinsulin.

Radioimmunoassay

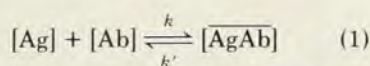
Let us return now to a consideration of radioimmunoassay. The assay is simple in principle. The concentration of the unknown unlabeled antigen is obtained by comparing its inhibitory effect on the binding of radioactively labeled antigen to specific antibody with the inhibitory



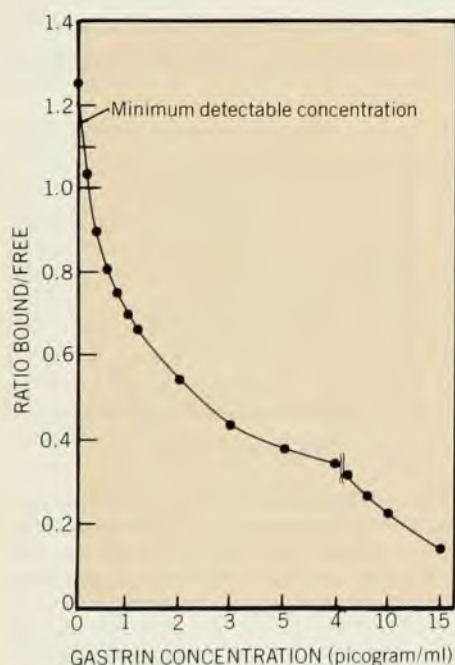
Cross reactivities of various unlabeled mammalian insulins versus beef insulin labeled with I^{131} in guinea pig (top) and human (bottom) anti-insulin serums. The guinea pig had been treated with pork insulin, the human (diabetic), with a mixture of beef and pork insulin. We show data for dog insulin (triangles), sperm-whale insulin (squares) and two lots of pig insulin (crosses). Note that the guinea-pig antiserum does not distinguish between insulin from the different species, while the human antiserum does. **Figure 4**

effect of known standards. Radioimmunoassay is an *in-vitro* test, that is, the ingredients—labeled antigen, specific antibody and standards or unknowns—are incubated in test tubes. After an appropriate reaction time, ranging from hours to days, depending on the association–dissociation rates for the particular reaction, the bound and free fractions of radioactive antigen are separated, a calibration curve is drawn and the concentration of the unknown sample is determined from the calibration curve (figure 5). The sensitivity is most remarkable, as little as 5×10^{-14} M of the peptide being measurable in some systems in the presence of billionfold higher concentrations of serum proteins. We can now measure concentrations of peptide hormones in a drop of blood—compared to the half-liter amounts required for bioassay procedures. The assay is also characterized by the specificity associated with antigen–antibody reactions and by comparative technical simplicity, especially now that radioimmunoassay kits and reagents are widely available commercially.

What factors in the reaction determine the sensitivity of a radioimmunoassay? Consider the biomolecular reaction between an antigen containing a single reactive site [Ag] and a single order of homogeneous combining-sites on antibody [Ab] to form an antigen–antibody complex [AgAb] and assume that labeled and unlabeled antigen behave identically. Then



At equilibrium, the concentrations of free



Standard curve for the radioimmunoassay of gastrin. Note that as little as 0.1 picogram gastrin/ml incubation mixture (a concentration of 5×10^{-14} M) is readily detectable. Figure 5

Ag, F , and of bound complex AgAb , B , are related by

$$B/F = K ([\text{Ab}_0] - B) \quad (2)$$

where the equilibrium constant for the reaction K is simply k/k' ; $[\text{Ab}_0]$ is the total molar concentration of antibody binding sites.

It is evident from equation 2 that when B is much less than $[\text{Ab}_0]$, B/F decreases only slightly for large changes in B ; thus if B increases 10-fold from 0.001 $[\text{Ab}_0]$ to 0.01 $[\text{Ab}_0]$ the change in B/F is less than 1%. For a sensitive assay, therefore, $[\text{Ab}_0]$ must be reduced by dilution so that $[\text{Ab}_0]$ is not much larger than B and since B , the bound antigen, must be less than the total antigen, it follows that $[\text{Ab}_0]$ should be near H , the minimal antigen concentration to be detected.

If we wish to start with B/F near 1 in the absence of added unlabeled antigen ("trace" conditions), then from equation 2

$$1 \leq K[\text{Ab}_0] \leq KH$$

and, therefore,

$$K \geq 1/H$$

Thus, there is an inherent sensitivity that can be achieved with any antiserum, which is dependent on the equilibrium constant, K , that characterizes the reaction of the predominating antibodies.

It is evident from this analysis that the limiting factors governing the sensitivity of a radioimmunoassay are the choice of specific antibody and the specific activity of the tracer (that is, the ratio between the radioactivity and chemical amount). The radioisotope of choice for high sensitivity assays is I^{125} with a 60-day half-life—the long half-lives of C^{14} or H^3 limit the attainable specific activity, and these labels are useful only in low sensitivity assays. Iodine-125 can substitute onto tyrosyl or histidyl residues of most peptides or can be incorporated using other chemical techniques into other substances of biologic interest. It is obvious that one can lower the chemical amount of tracer and maintain the same counting rate, and hence statistical accuracy, by incorporating more than one I^{125} atom per molecule. This has an inherent disadvantage. When a radioiodine atom undergoes decay, the molecule to which it is attached usually dissociates in what has been designated "decay catastrophe." If the molecule contains two or more radioactive atoms, decay of the first atom results in production of labeled fragments or free radioiodide. As a result the radioactivity is no longer associated only with unaltered molecules. These changes limit the shelf life of the labeled substance during storage and result in increased damage to it during incubation for immunoassay. Decay catastrophe is distinctly different from the chemical alterations induced by oxidizing or reducing radicals, which are, as we saw, produced when the water

molecules in solutions containing radioactive materials absorb ionizing radiation. In the latter case, damage may be decreased by reducing the concentration of radioactivity or using protective agents as radical scavengers. Since decay catastrophe results from self-damage within the molecule, no protective measures are effective.

Let us consider the stability of some very simple molecules and see the effect of more than one iodine-125 atom per molecule. The thyronines are amino acids with an inner benzene ring and an outer phenolic ring; radioiodine can easily be incorporated in the outer ring by exchange of existing stable iodine atoms with I^{125} or substitution of I^{125} for hydrogen. One can thus prepare radioiodothyronines with one or two radioactive atoms, depending on the substrate one starts with. The most stable of the radioiodothyronines are the ones with only a single iodine atom in the outer ring—diiodothyronine, T_2 , and triiodothyronine T_3 (figure 7). Next in order of stability are thyroxine, T_4 , and reverse T_3 , each of which has two iodine atoms in the outer ring. The least stable preparations are T_4 and reverse T_3 containing two I^{125} atoms in the outer ring. Once again the principles of physics are directly used in preparation of the radiolabeled tracer that is an essential ingredient for radioimmunoassays.

Medical applications

Having described the principle of the radioimmunoassay and some of the physics problems encountered and solved along the way, let us now consider its role in biomedical investigation and clinical medicine. The first application was to the measurement of plasma insulin in man. We soon learned that, contrary to Mirsky's hypothesis, the adult-onset diabetic does not have an absolute deficiency of insulin. He responds slowly but with a greater insulin output than does the non-diabetic to glucose, the classic stimulator of insulin secretion. Thus adult-onset diabetes is not due to an absolute deficiency of insulin but rather to some degree of insensitivity to the effects of insulin. This was but the first of new insights gained with the assay. The method has since been applied to the measurement of hundreds of substances of biologic interest—peptide hormones, steroid hormones, thyroid hormones, drugs, viruses, bacterial antigens, and countless others.

Let me just cite a few practical applications of public health interest. Many of you are familiar with the serious problems due to transfusion hepatitis, a liver infection due to contamination of transfused blood with a virus called hepatitis B antigen. Radioimmunoassay is the method of choice for testing Red Cross and other bank blood for this virus and for carriers of this virus, and its use has re-

sulted in dramatically reducing this form of hepatitis in the United States.

Another very important application is for mass screening for underactivity of the thyroid of the newborn. If the deficiency is not treated almost immediately after birth, before it becomes clinically evident, the resultant mental retardation cannot be reversed by later treatment. This disease occurs in 1 in 5000 births. Testing can be performed on a drop of blood from a heel prick of the new-born placed on filter paper. The test costs about \$1 per sample according to estimates from New England, where this screening program has been in use for several years. Treatment costs \$1 per year and it has already been demonstrated that treated children are equivalent in IQ to their siblings. What a small price to pay for healthy children—and radioimmunoassay made it possible!

It is possible to spend many hours citing the hundreds of applications of the assay and their relevance to biology and medicine. Instead, I want briefly to review the general role of physicists in medicine during the recent past and to gaze into my crystal ball and suggest potential new vistas.

As a trained nuclear physicist I have been concerned for more than 30 years with the application of radioisotopic methodology to analyze the fine structure of biologic systems. Most physicists in medicine who are organized in the American Association of Physicists in Medicine, a member organization of the American Institute of Physics, have had interests relating to imaging or to treat-

ment with radiant energy. From the time of Konrad Röntgen, through World War I when Marie Curie almost single-handedly mounted the entire Allied x-ray activity for the diagnosis of shrapnel wounds and other war injuries, until now with computerized tomography, physicists have been concerned with the use of x rays to image the human body and search for abnormalities related to disease processes. With the ready availability of radionuclides, the medical specialty of Nuclear Medicine developed, with particular emphasis on imaging the localization and dynamic changes in the distribution of administered radionuclides. Over the past few years tremendous strides have been made in the application of ultrasound to visualize human anatomy, normal and abnormal. New types of imaging are on the drawing boards—including the potential application of nuclear magnetic resonance.

Physicists have long been concerned with the problems of ensuring safety from the deleterious effects of radiation on normal tissue and with the therapeutic usefulness, primarily in the management of malignancies, of classical low- and high-energy x-ray machines, betatrons and linear accelerators and of treatment with natural and artificial radionuclides.

Thus one can consider most medical physicists to be radiation physicists. Let me consider another point of view—we, biomedical investigators, are asked "If we can put a man on the moon, why is there yet no cure for diabetes or cancer?" The answer is that the fundamental informa-

tion—the scientific breakthrough—is lacking. While we work and wait, are there other fields where physicists can serve?

Do we have the fundamental science necessary to make it possible for the blind to see, the deaf to hear, and the amputee and spinal-cord injury patient to make fuller use of replacement or existing limbs? Each of these processes is concerned with the transfer of information—and we are in the middle of a revolution in the transfer of information.

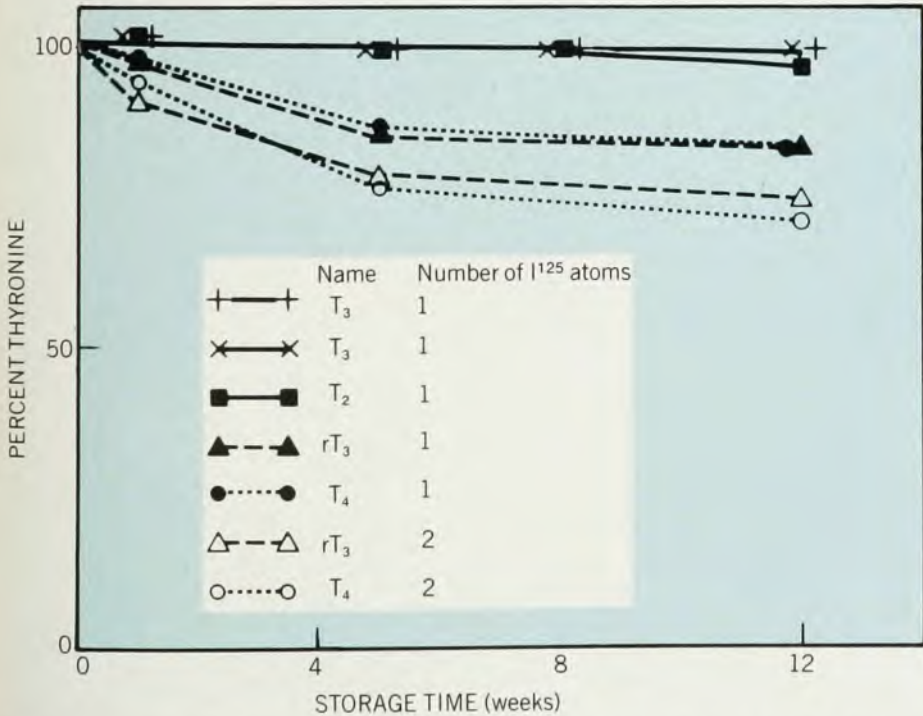
The blind can walk with seeing-eye dogs—but is it not time that sonar- or radar-equipped glasses or canes scan the surroundings and communicate the safe path? The blind can read with their fingers or be read to—but is it not time to scan ordinary books and newspapers and convert the printed page perhaps to sound and truly open the world of the written word to the blind? Is it perhaps even possible to effect artificial communication to the optical centers of the brain?

A critical aspect of basic science is its subsequent application to the needs of society. The fall-out of the Manhattan Project was nuclear medicine and nuclear power. The fall-out of the Radiation Laboratory at MIT was radar, which made possible a new era in transportation, for without radar we could not safely ride through the skies. If science permits a man to walk on the Moon, surely we can make more dynamic usage of the technology of the space age, microprocessors and microenergy sources, to restore as best we can the functions of the parts a man is missing. The six-million-dollar man comes to us from science fiction—but much of the fiction can be converted to reality with what we now know. Spurred by the needs of World War II, physicists served as leaders who sparked the transition from basic science to practical applicability. Will physicists again lead the way in converting basic phenomena into viable mechanisms that can make dreams come true? There are numerous opportunities for employment in the application of physics in medicine—but more importantly there are new frontiers for learning and contributing to the service of man. Let us hope that some among us will have the vision and wisdom to explore these frontiers.

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This article is based on a talk given during a Symposium on Physicists in Unusual or Alternative Settings at the joint meeting of The American Physical Society and the American Association of Physics Teachers in New York on 29 January 1979. Many of the technical aspects of the talk, including a bibliography, were published as part of the Nobel Lecture, and republished in Medical Physics 5, 247 (1978).

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Stability of radioiodothyronines stored in an organic solvent as a function of time after purification. The most stable preparations were those with a single iodine atom in the phenolic ring. There were no differences in the stabilities of the T₃'s prepared by different reactions. The least stable preparations have two ¹²⁵I atoms in the phenolic ring.