

PHYSICAL TECHNIQUES IN BIOLOGICAL RESEARCH

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Volume II

PHYSICAL CHEMICAL TECHNIQUES

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PREFACE TO VOLUME I

These volumes have resulted from collaborative effort, in which a large number of physical techniques that have been found useful in biology are discussed briefly from both the theoretical and the practical viewpoint. No claim to encyclopedic range is made, but it is believed that from these volumes the reader can get a fairly comprehensive idea of the present place of the techniques of physics and of physical chemistry in biological research.

During the last two decades there has been unprecedented broadening of the scope of attack on fundamental problems of physiology. This is largely a consequence of the increasing use by biologists of modern physical techniques, some of which are of the most advanced types and characterized by precision and delicacy rarely employed in their application in physics. These refinements have not always resulted from the collaboration of a biologist and a physicist, as one might suppose. Fully as often the biologist has turned physicist and has himself adapted, or indeed sometimes developed, physical techniques suited to his needs. Thus, Martin and Synge developed adsorption experimental procedures into the enormously useful paper chromatography; Holter and Linderstrøm-Lang made from the diver of Descartes an apparatus delicate enough to obtain analytical data from single cells; while in the microtome electron microscopists have designed an engineering marvel that cuts slices about one order of magnitude above the range of monomolecular films.

Workers with any of the powerful new aids to biology from physics cannot avoid feeling strongly encouraged to continue to elaborate new methods and to broaden the range of biological problems to which the techniques are applicable. The present work has been conceived in the hope of accelerating such development and wider use. Every specialist in one of these techniques is constantly called upon to help his fellow biologist to decide whether, or how, a particular physical technique can serve a biological use more or less unlike that for which it was originally designed. It is also not an infrequent experience of a physicist or a physical chemist to have a biologist come to him with a difficulty which he hopes can be overcome if he can find the right physical approach. The success of such conferences depends to a large extent upon both participants having a fair knowledge of the details of the techniques and the biological uses to which they have already been put; and one of the aims of the present work is to serve such a need. For every such biologist who makes a vigorous effort to use new physical methods,

there must be many who have a vague feeling that their researches might proceed better if reoriented in a physical direction, but who hesitate to attempt this because of timidity. For such workers it is felt that the many examples of simple methods will be helpful. Finally, in a broad sense, it is hoped that this treatise will serve as a real orientation for biologists and for chemists and physicists who may be potential biologists. In these volumes each author, an expert in his field, has written in such a way that a biologist can see whether he may start to employ the technique, or whether the application to his particular biological problem demands collaboration with a physicist or a physical chemist. The latter, on the other hand, should be able to assess in realistic terms the possibility of fruitful and exciting application of his special training to the baffling problems of biology.

The arrangement of material has been determined primarily by biological considerations. Volumes I and II deal with theory and methods applied to relatively pure preparations of biological substances that are obtained from cells or other tissue elements. The optical approaches, so favored in biology, are in Volume I, while in Volume II are a wide variety of nonoptical techniques (the only exception is the chapter on X-ray diffraction which is in Volume II). Volume III deals with the application of physical techniques to cells and tissues.

The editors wish to express their appreciation to the authors for their contributions.

New York, N. Y.
June 30, 1955

GERALD OSTER
ARTHUR POLLISTER

PREFACE TO VOLUME II

The second volume in this series is concerned with those physical chemical techniques which have been most widely employed in the investigation of molecules of biological significance. In each chapter the author outlines the theoretical basis of the methods, describes the apparatus and manipulations employed, and illustrates the applications of the technique by examples of particular interest to biological chemists. Hence, each chapter is a comprehensive introduction to the field and, in conjunction with the extensive bibliography, should inform the reader of the scope of the technique and its potentialities for his particular research.

The Editors wish to express their warm appreciation to the authors for taking time from their active research careers to make these useful contributions to the field.

While this volume was in press, the whole field of the application of physical chemistry to biology suffered an irreparable loss in the untimely death of Professor Kurt G. Stern.

New York, N. Y.
September 14, 1956

GERALD OSTER
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CONTENTS

PREFACE TO VOLUME I	iii
PREFACE TO VOLUME II	v
CONTRIBUTORS TO VOLUME II	vi
1. Tracer Techniques: Stable and Radioactive Isotopes	
JACOB SACKS	
I. Introduction	2
II. Elements of Radioactivity	3
III. The Measurement of Radioactivity	9
IV. Radioautography	28
V. Safe Handling of Radioactive Isotopes	30
VI. The Measurement of the Heavy Stable Isotopes	34
VII. The Useful Tracer Isotopes and Their Properties	38
VIII. The Synthesis of Labeled Compounds	40
IX. The Scope of the Tracer Technique	43
X. Some Limitations of the Tracer Technique	49
References	55
2. The Measurement and Properties of Ionizing Radiation	
J. S. KIRBY-SMITH	
I. Introduction	58
II. Physical Background	59
III. Radiation Sources	69
IV. Dosimetry of Ionizing Radiations	81
V. Exposure of Biological Materials	102
VI. The Mechanism of Radiobiological Action	106
References	108
3. Sedimentation, Diffusion, and Viscosity	
A. G. OGSTON	
I. General Introduction	112
II. Sedimentation Velocity	115
III. Diffusion	137
IV. Viscosity	143
V. Sedimentation Equilibrium	147
VI. Interpretation of Dynamic and Equilibrium Measurements, in Terms of the Characteristics of Solute Particles	148
VII. Conclusions	152
References	153

4. Surface Film Techniques

ALEXANDRE ROTHEN

I. Films at the Air-Water Interphase.....	156
II. Interfacial Films.....	170
III. Transfer of Surface Films from a Liquid onto a Solid Surface.....	171
IV. Thickness of Surface Films.....	174
V. Surface Potential.....	183
VI. Chemical Reactivity of Surface Films.....	186
VII. Irradiation of Surface Films of Proteins.....	196
References.....	197

5. Adsorption and Chromatography

NORMAN APPLEZWEIG AND THOMAS F. CLEARY

I. Introduction.....	201
II. Methods.....	204
III. Applications.....	212
IV. Chromatography in Steroid Research.....	220
References.....	236

6. Electrophoresis and Ionophoresis

KURT G. STERN

I. Introduction.....	243
II. Microscopic Method (Cataphoresis).....	250
III. Moving Boundary Methods.....	255
IV. Other Electrophoresis Methods.....	290
V. Biological Applications of Electrophoresis.....	295
VI. Concluding Remarks.....	296
References.....	296

7. Electrical Potential Differences

K. S. SPIEGLER AND M. R. J. WYLLIE

I. Introduction.....	302
II. Basic Definitions and Units.....	302
III. Theory of Reversible Cells.....	304
IV. Junction Potentials.....	322
V. "Donnan" Equilibria and Potentials.....	335
VI. Membrane Potentials.....	339
VII. Phase Boundary Potentials between Liquids.....	352
VIII. Summary of Formulae for Electrical Potentials.....	357
IX. The Meaning and Electrometric Measurement of pH.....	357
X. The Measurement of Potential Differences.....	367
XI. Preparation and Use of Electrodes.....	372
References.....	390

8. Magnetic Methods

SCOTT BLOIS

I. Introduction.....	393
II. Elementary Magnetic Theory.....	394
III. The Diamagnetism of Atoms and Molecules.....	406
IV. Paramagnetic Atoms and Molecules.....	414
V. The Measurement of Magnetic Susceptibility.....	420
VI. Nuclear Magnetic Resonance Spectroscopy.....	433
VII. Magnetic Measurements on Biological Materials.....	437
References.....	439

9. X-Ray Diffraction and Scattering*

GERALD OSTER

I. Introduction.....	441
II. Apparatus.....	442
III. Methods.....	449
IV. Results for Some Bio-macromolecules.....	458
References.....	464

AUTHOR INDEX.....	467
SUBJECT INDEX.....	479

Chapter 1

TRACER TECHNIQUES: STABLE AND RADIOACTIVE ISOTOPES

Jacob Sacks

I. Introduction.....	2
II. Elements of Radioactivity.....	3
1. Types of Radioactive Decay.....	4
a. Alpha Decay.....	4
b. Beta Decay.....	5
c. Electron Capture.....	6
d. Gamma Rays in Radioactive Decay.....	7
e. Other Types of Radioactive Decay.....	8
III. The Measurement of Radioactivity.....	9
1. The Units.....	9
2. Measurement of Total Ionization.....	11
a. The Ionization Chamber.....	11
b. External Amplification.....	13
3. Counting Single Particles.....	13
a. Geiger-Müller Counters.....	14
b. Proportional Counters.....	16
c. Windowless Flow Counters.....	17
d. Gas Counting in the Proportional Region.....	18
4. Scaling Circuits and Mechanical Registers.....	19
5. The Statistics of Counting.....	21
6. Counter Geometry.....	23
a. True Geometry.....	23
b. Absorption.....	23
c. Self-absorption.....	24
d. Back Scatter.....	25
7. Scintillation Counters.....	26
IV. Radioautography.....	28
V. Safe Handling of Radioactive Isotopes.....	30
1. Personnel Protection.....	30
2. Prevention of Contamination.....	32
3. Disposal of Radioactive Wastes.....	33
VI. The Measurement of the Heavy Stable Isotopes.....	34
1. The Falling Drop Method for Deuterium Measurement.....	35
2. The Mass Spectrometer.....	36
VII. The Useful Tracer Isotopes and Their Properties.....	38
VIII. The Synthesis of Labeled Compounds.....	40
1. Discriminate Synthesis.....	41
2. Biosynthesis of Labeled Compounds.....	42

IX. The Scope of the Tracer Technique	43
1. Isotope Dilution Analysis	44
2. Transport of Ions Across Phase Boundaries	44
3. Intermediary Metabolism	45
4. Steady State Processes	46
5. Mineral Metabolism	48
X. Some Limitations of the Tracer Technique	49
1. Radiation Effects in Tracer Experiments	49
2. Chemical Exchange Reactions	50
3. Loss of the Isotopic Label from the Compound Administered	51
4. Criteria of Purity of Labeled Compounds Isolated	52
5. The Quantity of Tracer Substance Administered	52
6. Effect of Isotopic Mass on Reaction Rate	52
7. Multiple Fates of the Isotopic Label	53
References	55

I. Introduction

The fundamental property which makes possible the use of isotopes as tracers is that all the isotopes of any given element have identical chemical properties. This applies to the radioactive isotopes that have been created by the cyclotron or the nuclear reactor as well as to the heavy stable isotopes nature has provided for most of the elements. The distinctive physical property that makes it possible to determine the ratio of heavy stable to light isotope, or the quantity of radioactive isotope in a given sample, is independent of the chemical transformations through which the element has passed. The essence of the tracer technique, then, consists of an understanding of the principles and practice of the determination of the heavy stable or radioactive isotope which is being used in the particular experiment. This in turn depends upon an appreciation of the basic principles of atomic structure and radioactivity.

The atom consists of a *nucleus* composed of *protons* and *neutrons*, about which are rotating a number of planetary *electrons* in orbits of varying diameter. The nucleus contains practically all the mass of the atom, and has a diameter of the order of 10^{-12} cm. The outermost electron orbit has a diameter of the order of 10^{-8} cm. The number of protons, elementary positively charged particles, determines the *atomic number*, and the number of protons and neutrons together, the *mass number* of the element. The number of electrons in the electrically neutral atom is equal to the number of protons.

The *isotopes* of any given element have the same number of protons in the nucleus, and the same number of planetary electrons. They differ only in the number of neutrons; the mass numbers of the various isotopes are consequently different from each other. A single nuclear species is known as a *nuclide*. There are also *isobars*, nuclides with the same mass

number but different atomic number. For example, the radioactive isotope of potassium, K^{40} , is an isobar of both the most abundant argon isotope, A^{40} , and the most abundant calcium isotope, Ca^{40} .

Except for the most abundant isotope of hydrogen, the nucleus of which is the proton, and the very rare isotope of helium, He^3 , all stable nuclei contain at least as many neutrons as protons. In the light atoms of odd mass number, except for He^3 , there is one more neutron than proton. As atomic number increases, so does the ratio of neutrons to protons in the stable nucleus. The heaviest stable nucleus, that of bismuth, contains 126 neutrons and 83 protons, giving a ratio slightly greater than 1.5.

II. Elements of Radioactivity

The particles within the nucleus are held together by short-range forces of very great magnitude. The nature of these forces is not understood. The nucleus may be in one of several energy states. In the stable nucleus the particles occupy the lowest energy states available. In the unstable nucleus, the radioactive one, the particles occupy states of higher energy. In the process of the radioactive transformation the system goes from a more energetic to a less energetic state. The excess energy becomes available for imparting kinetic energy to one of the particles, and in alpha decay (see below) this is the mechanism by which the excess energy is dissipated. In other types of radioactive decay the available energy excess is dissipated by other means. The probability of the occurrence of this transition from a higher to a lower energy state in a given nucleus is not influenced by the decay of the other nuclei in the system, and this results in an exponential decay. This probability is expressed as the disintegration constant of the particular nuclide. The disintegration constant is stated in terms of the fraction of the total number of such nuclei present in which the decay will take place in unit time:

$$N = N_0 e^{-\lambda t}$$

where N_0 is the number of nuclei present at zero time, and λ is the disintegration constant. This disintegration constant, which is the distinguishing characteristic of the nuclide, is independent of all external conditions, such as the temperature and state of chemical combination of the element. The most convenient way of expressing the disintegration constant is in terms of the half-life of the particular nuclide, i.e., the time required for the number of nuclei present to decrease to half the original value. The half-life is obtained by setting N/N_0 equal to one-half in the equation above. This then becomes:

$$\frac{1}{2} = e^{-\lambda t}$$

The half-life is thus equal to $0.693/\lambda$.

1. TYPES OF RADIOACTIVE DECAY

a. *Alpha Decay*

This type of decay is seen frequently in natural radioactivity in the heavier elements, but only rarely is it encountered in the isotopes produced by the cyclotron or nuclear reactor. It consists of the emission of an alpha particle, the nucleus of the helium atom, with its double positive charge. The daughter nucleus thus has an atomic number less by 2, and mass number less by 4, than the parent nucleus. Most alpha particles are emitted with kinetic energies of the order of 5 million electron volts (Mev). An electron volt is the energy which a single electron acquires in being accelerated toward the positive plate of a condenser between the plates of which there is a potential difference of 1 volt.

The emission of the alpha particle may leave the nucleus with some excess of energy remaining distributed among the particles, i.e., in an *excited state*. The excess energy is emitted in the form of one or more *gamma ray photons* (see below), after which the nucleus is in the *ground state*. It may still be radioactive; in natural radioactivity there are decay chains in which several alpha particles are emitted in succession. There are a number of discrete energies with which the alpha particles from any one nuclide are emitted, but the range is generally quite narrow. For each of these energy levels the gamma ray emitted has an energy such that the sum total of alpha plus gamma energy is constant. The nucleus from which the emission takes place recoils in the opposite direction, with momentum equal to that of the alpha particle plus the gamma ray, in accordance with the laws of classical mechanics.

The alpha particle loses its energy by interaction with the electrons of the atoms in its path. This interaction may take the form of ejection of the electron from its orbit, thus creating an ion pair, or it may merely raise the electron to a higher energy level. In the former case, the average energy transfer amounts to about 35 electron volts (ev); in the latter, about 10 ev. The ion pair consists of the atom minus the electron and the electron. The electron may attach itself to a neutral atom, forming a heavy negative ion.

The electron so ejected is not necessarily from the outer, or valence shell, and the positive ion produced is therefore different from the ordinary cation of the chemist. The ion pairs tend to recombine rapidly unless they are produced in an electrical field which tends to separate them before there is time for recombination. The velocity of the alpha particle is low, on account of the high mass, and the tendency to form ion pairs is very great. The average alpha particle forms 30,000 to 50,000 ion pairs per centimeter of path in air at ordinary pressure, losing in this way 1 to 1.5

Mev of energy per centimeter of path. The range is therefore short, only a few centimeters in air. The path is straight, except that there may be an angulation toward the end. This angulation is known as *scattering*.

b. Beta Decay

(1) *Beta decay by negative electron emission.* The usual type of beta decay consists of the emission of a negative electron, which may be followed by the emission of one or more gamma ray photons. This type of beta decay is seen in natural radioactivity, among the fission products, and in the majority of isotopes produced by the nuclear reactor or the cyclotron, in nuclides which contain too high a ratio of neutrons to protons for stability. Electrons are not present as such in the nucleus. In a nucleus of this type, the electron is created by the conversion of a neutron into a proton and an electron, and is ejected at the instant of its creation. The process thus increases the atomic number by one. The mass number is unaltered, as the mass of the electron is negligibly small in comparison with that of the proton or neutron.

Unlike alpha particles, the beta particles are emitted with a continuous distribution of energies, up to a maximum characteristic of the nuclide. The most frequent energy level is about one-third the maximum. It must be assumed that the emission of a beta particle from the nucleus of a particular nuclide always results in the same energy loss, and that the recoil energy of the nucleus is constant. In order to account for the balance of energy between the observed range and the maximum, it has been necessary to postulate the existence of the *neutrino*, an uncharged particle of mass much less than that of the electron, as the carrier of the remaining energy and momentum. The properties which must be ascribed to the neutrino are such that it is difficult, if not impossible, to devise an experimental procedure capable of detecting it.

The beta particle may have a very high velocity, approaching within a fraction of a per cent of the velocity of light. Beta particles of high energy, over 1 Mev, are spoken of as "hard" beta particles, while those of low energy are called soft betas. The hard beta particles produce little ionization, perhaps 100 to 200 ion pairs per centimeter of path in air. Their range is therefore considerable, perhaps a meter or more in air. However, each interaction with an electron results in a proportionately much greater energy loss than an alpha particle undergoes in such interactions. Also, since the mass of the beta particle is of the same order as that of the electron with which it interacts, the beta particle is easily deflected. Toward the end of its path, the hard beta particle, its energy now reduced to the level of a soft one, is likely to undergo deflection at every interaction, so that the path is quite tortuous.

Absorption of beta particles in solids is a pseudo-exponential function. This is due to the wide range of energies present in the particles emitted from any radioactive source thereof, and the tortuous nature of the end of the path. Formulae relating range and energy have been developed, but the simplest way for the tracer experimenter to consider the problem is in terms of the weight of absorber needed for complete absorption. This is usually stated in terms of mg per cm² of aluminum. This empirical standard was chosen out of convenience because sheet aluminum of almost any desired thickness is readily available commercially. Since the absorption of beta particles is primarily a function of the mass of matter through which the particles pass, it makes relatively little difference what absorber material is chosen. However, some of the beta particles do interact with matter to convert their energy into x-ray photons. These are known as *bremstrahlung*. The x-ray photon produced may have any energy up to the maximum of that of the beta particle. The incidence of *bremstrahlung* increases with increasing atomic number of absorber. For this reason absorbers for beta rays are made of material with low atomic number. Lucite is one of the favorites for this purpose, because it can be machined into any desired shape with ease, and is available commercially in sheets of appropriate thickness. A sheet of Lucite $\frac{1}{2}$ -inch thick will absorb completely all beta particles except those of the highest energy.

(2) *Beta decay by positron emission.* Some of the isotopes produced by the cyclotron have too few neutrons in comparison with their charge for a stable nucleus. They would become stable if a proton were to change to a neutron. One process by which such a conversion takes place and a stable nucleus results, is conversion of a proton into a neutron and a positive electron, or *positron*. The positron, like the negative beta particle, does not exist within the nucleus but is ejected from the nucleus at the instant it is created. The positron has the same mass as the electron, and loses kinetic energy in the same way as does the negative beta particle, by interaction with electrons in its path. However, it is incapable of independent existence after it has lost all its kinetic energy. It then interacts with an ordinary negative electron, in what is known as the annihilation reaction. The result is the transformation of the two particles into two photons which are the equivalent of gamma rays. These annihilation radiations have an energy of 0.51 Mev, the equivalent of the rest mass of the electron. They move off in paths which are 180° apart, thus conserving momentum.

c. *Electron Capture*

The other process by which neutron-deficient isotopes can reach a stable condition is by having the nucleus capture one of the planetary

electrons, thus converting a proton into a neutron. This process is the formal equivalent of positron emission, in that it reduces the atomic number of the nuclide by one without affecting the mass number. There are some nuclides in which the two processes take place concurrently; a definite fraction undergoing one type of decay while another fraction undergoes the other type. The electron captured is usually from the innermost shell, the *K* shell, although there are some isotopes in which the electron may be captured from the second, or *L* shell, and even from the third, the *M* shell.

An example of the class of neutron-deficient isotopes which decays exclusively by *K*-electron capture is Fe^{56} , which becomes the stable Mn^{56} isotope. An example of the second class is Mn^{52} , of which 35% decays by positron emission and 65% by *K*-electron capture.

The capture of the electron by the nucleus leaves vacant one of the two places in the *K* shell, or one of the places in the *L* or *M* shell. This space is immediately filled by one of the electrons from an outer shell orbit. This process liberates energy, which appears as an x-ray photon. The x-ray given off is characteristic of the new nucleus. X-rays are identical in nature with gamma rays; the distinction is made solely on the basis of origin. If the photon arises in the nucleus, it is called a gamma ray; if it is of extra-nuclear origin, it is called an x-ray. The x-rays arising from electron capture are of much longer wavelength, i.e., lower energy, than the gamma rays which occur following alpha or beta decay.

d. Gamma Rays in Radioactive Decay

The difference in energy levels between the excited state which may result from the emission of an alpha or beta particle and the ground state, is emitted as one or a series of gamma ray photons. The gamma ray is electromagnetic radiation, of the same type as ordinary visible light, but of very much shorter wavelength and consequently of much higher energy. The gamma rays emitted in radioactive decay have energies of the order of 1 Mev, which corresponds to a wavelength of less than 1 angstrom unit, compared to the 4000-7000 Å of visible light. Gamma rays do not produce ionization directly, as alpha and beta particles do. Instead they interact with electrons in one of three ways depending on their energy and the atomic number of the absorber. The first, photoelectric absorption, consists of the transfer of the kinetic energy of the photon to an electron. The electron then loses energy by producing ionization in the manner described above for the beta particle. Photoelectric absorption is the usual method of energy transfer by gamma rays of relatively low energy. The second method, Compton scattering, consists of the transfer of part of the photon energy to an electron, which goes on to produce

ionization in the usual manner, while the photon, now of lower energy and longer wavelength, moves off at an angle to its original path. The electron also moves off at an angle from the original path of the photon. The sum of the electron and photon energies, and the angles that their paths make with the direction of the incident photon, are such that energy and momentum are conserved.

The third process by which gamma ray photons lose energy is by the transformation into a pair consisting of a negative electron and a positron. This type of interaction with matter takes place only with gamma rays of energy greater than 1.02 Mev, and to an appreciable extent only when the absorber material is of high atomic number. The process of pair production is unimportant in the isotopes of interest as tracers.

The gamma ray photon is electrically neutral, and the absorption by any given material is an exponential function of the absorber thickness. Gamma rays have very long paths in air or in materials of low atomic number, as the probability of interaction with an electron increases directly with the density of electrons in the path of the photon. Since the absorption is exponential, gamma rays cannot be said to be completely absorbed, but are merely attenuated in passing through matter. The half-value layer, the thickness of absorber which reduces the intensity of the emergent beam to half that of the incident one, is commonly used as a measure of gamma ray energy. In practice, the thickness of absorber used is such that the intensity of the emergent rays is reduced to negligible proportions.

Some isotopes which show beta decay emit soft beta particles and high energy gamma rays. In these cases it is sometimes easier to measure the gamma rays than the beta. This applies particularly to the use of I^{131} in the diagnosis of thyroid disease. The fraction of a tracer dose collected by the gland can be ascertained by the use of a properly calibrated counting device sensitive to gamma rays, placed at standard distance from the patient's thyroid. The beta particles from this isotope are rather soft, and those which do not lose all their energy within the gland are completely absorbed by the subcutaneous tissue and skin.

e. Other Types of Radioactive Decay

The types of decay outlined above describe all those which occur in the tracer isotopes of importance in biology. There are two other types of decay encountered in the isotopes produced by the cyclotron and the nuclear reactor, which should be mentioned for the sake of completeness. These are *isomeric transition* and *internal conversion*. Both these processes represent the liberation of the difference in energy between the excited state and the ground state of the nucleus. In isomeric transition, this excess energy is emitted as a gamma ray photon. The process differs from the ordi-