RADIOACTIVITY for PHARMACEUTICAL and ALLIED RESEARCH LABORATORIES

Edited by

ABRAHAM EDELMANN

Nuclear Science and Engineering Corporation
Pittsburgh, Pennsylvania

ACADEMIC PRESS • New York and London • 1960

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A Symposium sponsored by
Nuclear Science and Engineering Corporation,
held in Uniontown, Pennsylvania

ACADEMIC PRESS • New York and London
1960

ACADEMIC PRESS INC.
111 FIFTH AVENUE
NEW YORK 3, N. Y.

United Kingdom Edition
Published by
ACADEMIC PRESS INC. (LONDON) LTD.
17 OLD QUEEN STREET, LONDON S. W. 1

Library of Congress Catalog Card Number 60-9068

PRINTED IN THE UNITED STATES OF AMERICA

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FOREWORD

Advances in medical and biological research pose increasingly complex requirements for growth and progress upon the pharmaceutical and allied industries. Fortunately, an outstanding characteristic of the pharmaceutical and allied industries has always been the ability to adapt to new technological approaches. The growing use of radioisotopes for research, product development and process control, and radiation sterilization clearly demonstrates this ingenuity and foresight. The first use of ionizing radiation as an industrial processing tool was made by the pharmaceutical industry for product sterilization.

We are now on the frontier of an era of applied nucleonics where positive contributions to our welfare and economy extend to nearly every phase of industrial and social endeavor. No limits can clearly be set as to the eventual extent and value of the applications and contributions possible. Intensive efforts are, nevertheless, required to capitalize on this opportunity. The state of the art must be more widely understood, the pool of scientific technology must be enlarged, and, finally, a reservoir of trained manpower must be available for full exploitation of radiation potentials.

It is with these thoughts in mind that this symposium was held. A reader familiar with problem areas in the pharmaceutical and allied industries will find sufficient examples of radioisotope applications in his own field of interest to stimulate his imagination. The result of this can only be increased progress.

PAUL C. AEBERSOLD

Office of Isotopes Development
Atomic Energy Commission, Washington, D.C.
February, 1960

PREFACE

The era of radioactivity had its inception at the turn of the 20th century when the Curies isolated several naturally occurring radioactive elements. In 1934 the daughter of Pierre and Marie Curie, Irene, and her husband Frédéric Joliot-Curie, discovered that radioactivity could be induced in elements which were not naturally radioactive. This remarkable finding led to the production of many new radioisotopes.

While the physicists studied these new metastable elements, evidence gradually accumulated which indicated that the radiations emitted could do harm as well as good. Despite the harmful effects, which require certain necessary precautions, much has been gained by the discovery of radioactivity. The gains that have been made are often not understood or realized by the untrained eye, but have nevertheless influenced the lives of all of us. Its use as a source of energy has come about as a result of research and development largely in the last 10 to 15 years. In addition, its application in the life sciences is increasing in an almost autocatalytic manner since the beginning.

The physicist Hevesy was the first to use a naturally occurring isotope as a tracer element in a biological problem in 1923. Thirteen years later the same person used P^{32}, an artificially produced radioisotope, in tracer experiments. The initial work did more to demonstrate the limitations and pitfalls of the use of such isotopes than it did to add to our knowledge and understanding of biology. In more recent years the application of radioactivity has found a place in biology that has no equal in its rapid rate of development and its spheres of influence. It has particular value in several general areas: (1) as a diagnostic aid in clinical problems; (2) in its therapeutic applications including sterilization; and (3) as a research tool. Of these three, the most important application has been in research. The early research work was relatively simple, involving such elements as P^{32} and I^{131}. As time passed the experimental techniques have become more sophisticated, using elements more difficult to determine, such as C^{14} and H^3, and experimental instruments which are more sensitive and complex.

Many answers have resulted from the use of radioisotopes particularly in the metabolism of an organism's normal constituents as well as foreign substances, many of which can be classed as either foods or drugs. The high sensitivity of determination of radioisotopes, as well as the introduction of methods unavailable before the advent of radioactivity represent the major reasons for the new discoveries. Like all new discoveries, however, many new questions are produced by the answers obtained. This paradox is a characteristic of research, particularly in a rapidly developing field.

A. J. LEHMAN

Division of Pharmacology, Bureau of Biological and Physical Sciences
Food and Drug Administration, Washington, D.C
February, 1960

CONTENTS

Effects and Utilization of Ionizing Radiation: Radiobiology as a Research Tool

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The employment of radiation techniques in solving the problems of the pharmaceutical and related industries has been limited, in the main, to the production of mutations and the beginnings of investigations in radiation chemistry of plastics, petroleum products, etc. I shall direct this report to a brief discussion of the biological effects of radiation, and the potential application and significance of these phenomena.

While radiation effects on living organisms have been studied since the discovery of X-rays, it was only since 1940 that intensive experimentation and interest was developed. From the many experiments which have been performed, it is evident that any living organism can be killed by a sufficient amount of radiation. From a biological standpoint, therefore, death is one of the phenomena that can be elicited by irradiation. However, if a sublethal dose of radiation is administered, various biological phenomena occur. These phenomena and their time of onset will vary in different species, and as a result of different dosages and quality of radiation (1–5).

The lethal effect of radiation has resulted in the use of this form of energy for sterilization of products. Advantages may be derived from radiation sterilization if the product may be damaged by conventional methods, or when the sterilization in the final package will eliminate costly and cumbersome aseptic processing procedures. This aspect is discussed in greater detail in another section of this symposium.

Food sterilization and pasteurization by means of irradiation is being investigated by many laboratories, and there may be promising potential in this area.

Some of the physical-chemical actions of radiations offer means of altering the structure of compounds, and improving methods of polymerization and catalysis. For example, a radiation method has been developed recently which permits the grafting of a thin layer of impervious plastic to polyethylene. By this means, polyethylene bottles may be used to package such materials as contain oils and perfumes without distortion or loss of volatile oils and essences.

I mentioned earlier that radiation causes alteration of physiological mechanisms and, eventually, death in biological systems. The observable effects may be arbitrarily divided into the "direct" and "indirect" effects. The direct effects are those caused primarily by the transfer of energy from the rays themselves. It is known that ionizing radicals, usually oxidative in nature, are produced by direct action of the rays, as are also the so-called "hot" atoms, which for a very short time, a small fraction of a second, may have an electron transposed from one shell to another, thus producing a temporarily unstable, active atom. These, in brief, are the mechanisms thought to produce the effects directly. They exist for extremely short periods of time, but are probably responsible for the symptoms seen in mammals days or months later which are part of the group of "indirect" effects.

Another example of the direct effect is destruction of the gene, and fragmentation of chromosomes, which may result in the genetic death of the cell or of mutations.

There are several ways in which this phenomenon has been, or may prove useful to the pharmaceutical industry. As the mutation rate of microorganisms such as bacteria, neurospora, etc., is increased by irradiation, this technique has been used to obtain mutant strains which are more efficient in production of antibiotics, toxins, etc. Ionizing radiation such as X-rays, non-ionizing radiation such as ultraviolet, and neutrons, have been employed successfully for this purpose. New strains of bacteria, for use in biochemical syntheses have been produced in this manner.

A possible use is the irradiation of plant seeds of medicinal interest, in order to produce strains with increased yields of the desired products.

The "indirect" effects of irradiation include the symptoms of radiation sickness. The development of a therapeutic regimen for this syndrome offers a challenge with resultant benefits from successful resolution of the problems. Not only is such treatment applicable to nuclear warfare and its concomitant radiation, but also to the thousands of patients daily undergoing radiation therapy. The chief limitation of dosage in radiation therapy is the damaging effect of the rays on healthy tissue. If protection can be afforded normal tissues so that increased amounts of irradiation can be administered to tumorous tissue, then a notable increase in therapeutic efficiency will have been realized. In addition, such agents may offset any radiation fallout effects.

One of the effects of ionizing radiation is the production of cataracts. Indeed, experimentally induced cataracts in rats and rabbits may be produced in a matter of weeks with almost absolute certainty as to occurrence and timing. This experimental production of cataracts permits investigation and possible development of a treatment of cataracts in humans, and of means for preventing their formation.

I should like to discuss briefly, at this time, some of the "indirect" effects of irradiation, as knowledge of these may lead to further suggestions as to the value of irradiation studies or the use of radiation in pharmaceutical problems.

As mentioned earlier, enough radiation will lead to death, but death will not occur, necessarily, instantaneously. The time of death and the observable predeath symptoms will vary not only with the dosage but also with the species. For present purposes, let us assume that a composite mammal is being irradiated. If the dosage is on the order of several tens of thousands of roentgens, and the rate is high, then death will occur while the animal is still "under the beam," that is, while it is being irradiated. If the total dosage is between 25,000—50,000 r, and it is administered at a slower rate, then central nervous symptoms appear, especially in guinea pigs. Reflexes are lost and convulsions may occur. Death will follow in a matter of hours.

If the dosage is low enough so that the animals will survive this early period, symptoms which resemble those of shock appear in rabbits and dogs. The rat will not reveal this phase unless a fairly large dosage is given.

If this stage is survived, then the animals may die in 3 or 4 days; the symptoms resembling those of adrenal insufficiency.

Those surviving this period may die of bacteremia and its resultant fever in from 7 to 9 days. The bacteremia results from two deficiencies: (*1*) A loss of intestinal epithelium as a result of irradiation, thus permitting entry into the blood of intestinal flora. (*2*) The marked leucocyte depletion which is also a consequence of irradiation. If the animals can survive this period, tumors and cancers may appear in later life.

In any event, there is a life-shortening effect of irradiation which is manifested by the acceleration of aging. Young mice or rats, after sublethal exposures to irradiation may die at one year of age, for example, manifesting the appearance of a mouse or rat dead of old age.

What is the relation of these facts to the pharmaceutical industry? There is the obvious one of a radiation prophylactic or therapeutic product. The markets for such a product are, stockpiling in case of an "incident"; prophylactic preparations to be taken daily in case of potential exposure; use in radiation therapy to permit an increase in radiation dosage; to ameliorate the radiation effect of material deposited as a result of fallout.

An analysis of the problems indicate that this compound or mixture should have certain actions. Only relatively small doses of radiation need be the object of concern. Large doses, which would occur mainly in the event of nuclear warfare would be received by those close to the blast center, and would probably not be a factor since concussion, flying debris, burns, etc., would probably prove fatal before the irradiation effects would appear.

Irradiation effects may be reduced by means of a preventative. Such compounds have been described for some years, and will be discussed here by another speaker. They appear to offer a slight degree of protection against the direct effect. Investigators have not been as successful in protecting against the indirect effects.

The central nervous system damage is probably not pertinent in this regard, because the dosage required to produce these effects is so high. The adrenal deficiency symptoms might be treated successfully by means of adrenal steroids, sodium chloride, etc. There is evidence that the adrenals may be directly involved in the body's response to irradiation. Shielding the adrenals of rats during whole body irradiation will increase the LD_{50} dose (6). As might be expected the adrenalectomized animal is more radiosensitive than the intact animal (7). An interesting finding is that the adrenal secretions are necessary for survival the first 3 days after irradiation. The hormone requirements are very high just following irradiation, and decrease over the next 3 days to an approximately normal value (8, 9).

One of the earliest, if not the first, clinical uses of X-rays was in the treatment of sterility. Irradiation has been found to overcome this condition temporarily in a small percentage of cases. Results of experiments with whole body irradiation of rats, or the irradiation of the pituitary itself, were interpreted in that an increased production of adrenocorticotropin occurred at the expense of gonadotropin and thyrotropin production (10). Prior to this, the pituitary content of these hormones is released, so that there is a sudden, temporary increase in the blood levels of these substances. It might well be that in those cases of sterility which are the result of an insufficient amount of gonadotropins, the increased blood levels of gonadotropins result in the temporary hormonal relationship necessary for reproductive ability. It would appear, therefore, that the economy of the pituitary is directed toward the production of adrenocorticotropins resulting in the increased elaboration and release of adrenal corticoids which are apparently required for the survival of the irradiated animal.

In the rat, there is a period of diuresis which occurs during the first 24 hours following irradiation which appears to be due to a decreased secretion of antidiuretic principle from the posterior lobe of the pituitary (11–13). It has been suggested that teleologically speaking, this represents an attempt on the part of the animal to eliminate toxic substances which have resulted from the irradiation. Perhaps a diuretic is indicated in the therapy of radiation sickness.

As mentioned earlier, there is a leucopenia following irradiation Much work has been reported on this phenomenon, and current research is directed toward obtaining a principle from bone marrow and/or spleen which will stimulate the production of leucocytes. A use of the leucopenia phenomenon in pharmaceutical research and development is described in a later paper.

Perhaps the denuding of the intestinal wall referred to previously may be compensated by a product which will stimulate the proliferation of the intestinal epithelium or one which would afford a protective coating of the wall. Such protection might prevent or reduce bacterial penetration.

The acceleration of the onset of cancers in the irradiated animal may offer a means of decreasing the time required for studies of carcinogenicity of new compounds. This aspect would be worthy of investigation.

The aging phenomenon is already proving useful in that irradiation offers an experimental approach to problems of gerontology.

The blood of irradiated rats contains a substance which when injected into unirradiated rats and mice causes death (14, 15). Some of the symptoms of the radiation syndrome are seen in these animals. While further work is needed, perhaps the identification of this substance may lead not only to a better understanding of the mechanisms whereby the indirect effects are produced, but also to a therapeutic agent which may be effective against some, at least, of the noxious effects.

The concept of a single antidote or preventive to combat radiation sickness has, I think, been discarded except for the prevention of the direct effect. However, in those programs which have been established to screen compounds for antiradiation activity in mammals, the death of the animal is used as the end point. When the criterion is the relatively crude one of death, perhaps valuable information is being lost. It is conceivable that a compound may exert a protective action in that the leucopenia, for example, may be prevented, but the animals die because of one of the other radiation effects. It is believed that *(1)* a more sophisticated and thorough program should be developed to screen compounds and *(2)* compounds whose actions are directed towards one of the radiation phenomena be sought and tested.

I have attempted to mention a few of our thoughts concerning this subject. The reports which follow are examples of what has been accomplished to date. Much remains to be done and there is a large potential in this area.

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