000-3167-64

AEC Contract Nos. AT(30-1)-4039 and AT(11-1)-3167

Phase I Radiation and Radioisotope Studies with
Laboratory and Domestic Animals

Phase III Studies on Neural and Behavioral Response
to Radiation

Phase IV Biological Effects of Implanted Nuclear
Energy Sources for Artificial Heart Devices

Three Year Summary Progress Report

July 1, 1970 to May 15, 1973

Submitted by

C. L. Comar

Department of Physical Biology

New York State Veterinary College

Cornell University, Ithaca, New York

NOTICE

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Atomic Energy Commission, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights.

This information should not be released to other persons, quoted or extracted for publication, or otherwise disseminated, without permission of the Principal Investigator.

MASTER

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency Thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

Three Year Summary Progress Report

Contents	Page
Introduction	1
Phase I - Radiation and Radioisotope Studies with Laboratory and Domestic Animals	4
I. Radiation Effects on Reproduction and Embryogenesis	4
II. Membrane Transport of Alkaline Earths and Phosphate: The Involvement of Vitamin D and the Calcium Binding Protein	13
III. Strontium Metabolism	31
IV. Environmental Problems	39
V. Secretion of Milk Constituents	51
VI. Veterinary Nuclear Medicine	56
Phase III - Neural and Behavioral Response to Radiation	61
I. Behavioral Assay of Radioprotective Agents	61
II. Factors Affecting Assay of Performance Decrement (ETI) Following Massive Radiation Doses	65
III. Taste Stimuli: Quality Coding Time	70
Phase IV - Biological Effects of Implanted Nuclear Energy Sources for Artificial Heart Devices	72
Graduate Training	75
Federal Support for the Research Program of the Department of Physical Biology, New York State Veterinary College, Cornell University, Ithaca, New York (July 1, 1973)	76

INTRODUCTION

The program supported by this contract with the Division of Biomedical and Environmental Research is a coordinated research effort focused on the biological implications for man of exposure to radiation, on environmental contamination, and on some specialized peaceful uses of radiation and radioisotopes. The approach has been multidisciplinary in nature in order to be meaningful and has required competence in subject areas as diverse as agricultural production and distribution, nutrition, food technology, and energy production in addition to competence in the basic sciences.

The emphasis in this contract has been on the identification, short-term solution, and the long-term approach to practical problems. Over the years, however, fundamental studies have developed which have far-reaching implications as well as providing insight into the practical problems of immediate interest.

As an example, the studies of the metabolism of bone-seeking fission products has led to the study of the relationship of Vitamin D to calcium absorption and the discovery of the intestinal calcium-binding protein. It is expected that this understanding will have application in dealing with many problems of nutrition and disease in addition to opening the way towards exploitation of the role of protein-carriers in general.

Collection of data on the secretion of ^{131}I into milk has not only enabled us to develop models for predicting the human radionuclide commitment after radionuclide contamination of the environment, but has increased our basic understanding of nutrient transfer across the mammary gland.

It is to be noted that perhaps the most important societal problem we face today stems from the need to balance environmental contamination against society's needs. We have more experience with radiation than with any other substance or agent that man exposes himself to in regard to evaluation of actual effects of low levels predictable for populations, benefit-risk assessments, and the application of cost-effectiveness analyses; this experience has grown out of the type of study carried on under the present contract. We need badly to apply such approaches to non-radioactive pollutants and to bring the general public to a realization of the understandings we do have.

Our research effort is divided into three major areas: Phase I, Radiation and Radioisotope Studies with Laboratory and Domestic Animals; Phase III, Neural and Behavioral Response to Radiation; Phase IV, Biological Effects of Implanted Nuclear Energy Sources for Artificial Heart Devices. All our studies, however, are closely integrated and many of our professionals are engaged in several of the project studies. This provides for a flow of ideas among our staff and a coordination of effort. In addition, there is a considerable interaction of our staff with members of other departments and colleges in research and teaching. This leads to an interchange of ideas with subsequent benefit to the direction and completeness of our research program.

This is a 3 year Summary Progress Report. For the convenience of the reader we have organized it as follows for each main subject area.

1. Introduction - a brief statement of background.
2. Objectives - a statement of each major objective as originally conceived.
3. Main Research Accomplishments - for each objective, a statement of the extent of accomplishment including a list of publications

during the three years, relevant and in support of the extent of accomplishment.

4. List of graduate students and post-doctoral fellows.
5. Status - an assessment of the state of knowledge in the subject area and an opinion of the most needed investigations for the near future.

It should be emphasized that details are not presented in this 3 year summary - space would not permit; rather the attempt has been to state qualitatively the essence of our findings. For further detail and substance reference should be made to: (a) previous progress reports, (b) the progress report accompanying this document and covering the year July 1, 1972 to May 15, 1973, and (c) the published papers themselves.

this did not accompany the 3 year progress report.

PHASE I
RADIATION AND RADIOISOTOPE STUDIES
WITH LABORATORY AND DOMESTIC ANIMALS

I. Radiation Effects on Reproduction and Embryogenesis. A. Casarett

1. Introduction

The human population is presently, and will continue to be, exposed to ionizing radiation from medical and industrial usage, from background sources, and from fallout and nuclear accidents. Of particular scientific and public interest is the effect of this radiation on reproduction and embryogenesis--both from irradiation of parents and from irradiation of early fertilized ova. There has been considerable concern over danger from total body incorporation of tritium and from the incorporation into bone of radioactive strontium.

Numerous reports have described effects such as abnormal embryo development or decreased litter size after exposure of experimental animals to ionizing radiation. Little is known, however, of the sequence of events which lead to these endpoints--of the relative importance of decrease in number of ova or sperm, impaired fertilizing ability, aberrant embryo development and implantation failure.

The experiments which have been done over the past three years and those which are proposed for the future are directed at understanding and quantifying certain effects of radiation on reproduction and embryogenesis which occur during preimplantation development. Our system for the culture of preimplantation mouse embryos in vitro has proven to be a successful method for study of this problem.

2. Objectives

The long term objective of these experiments has been to characterize the response of the preimplantation mammalian embryo to ionizing radiation delivered to the embryo itself or to parental generations. More specifically, it has been, first, to study the morphologic development of preimplantation embryos which have been exposed to ionizing radiation, and to identify the radiosensitive events or stages in the developmental process. Secondly, it has been to study the effect of maternal and paternal irradiation on embryo development to blastula stage. Thirdly, it has been to compare the effects from external radiation with those from radionuclides incorporated in or near the reproductive tissues ^{or} ~~in~~ embryos. Fourthly, it has been to correlate radiation-induced morphologic abnormalities or developmental delay in the preimplantation period (as observed in vitro) with failure of implantation or subsequent development.

Minor objectives have been to establish suitable systems for culture of preimplantation embryos and for subsequent implantation into foster mothers; to determine the G_1 , S and G_2 times of the mitotic cycle of two- and four-cell embryos in order to characterize periods of interphase sensitivity; to justify the use of the culture system by comparing the radiation response of embryos developed in vitro with those developed in vivo; to compare the effects of acute and chronic irradiation on preimplantation response; and to establish a technique for fusion of embryos in order to characterize the contribution of irradiated blastomeres to the development of the zygote.

3. Main Research Accomplishments

The experiments in this project are of two general types:

- a) Fertilized embryos are irradiated in vitro and their development

to blastula is followed. Radiation is given at selected stages of embryo development from one-cell to blastula, acutely from external sources (X- or gamma-radiation) or chronically either from external sources (gamma-radiation) or from radionuclides (^{90}Sr - ^{90}Y , ^3H) incorporated into the culture medium. Observations of cleavage times and morphologic development are made at intervals throughout the four-day culture period. Radiosensitivity is generally determined by survival to blastocyst stage, with other endpoints used as appropriate. Other embryos are exposed in vivo to the same treatments, and blastocyst development is determined.

b) Radiation is given to parents for one or more generations prior to mating. The embryos are then studied from two-cell to blastula in the culture system, with particular emphasis on the development from morula to blastula (since cavitation requires genomic expression). Parents are irradiated with external acute radiation, by continuous exposure to tritium incorporated into drinking water, or by a single injection of ^{90}Sr - ^{90}Y . Radionuclide distribution, organ dose, gonadal effects and sperm parameters are measured in certain experiments to supplement studies of effects on embryos.

In conjunction with these major studies, new techniques have been developed for embryo culture, for embryo fusion and for non-surgical transfer of cultured blastocysts into foster mothers.

Our results to date include the following:

a) The LD_{50} values for acute exposure to x-rays of embryos which develop from two-cell to blastula in vitro are as follows: one-cell (3 hours post-coitus) 175 rads; one-cell (13 hours post-coitus) 255 rads; two-cell, 390 rads; four-cell, 570 rads; morula, 325 rads. These

values were obtained with embryos from random-bred Swiss Albino mice, cultured in Brinster medium.

b) Comparable LD₅₀ values for embryos irradiated and developed to blastula in vivo are 150 rads, 200 rads, 420 rads, 640 rads and 340 rads. It is apparent that there is little difference in the response to acute radiation of the embryos in vivo and in vitro, as measured by this system.

c) The LD₅₀ values for chronic exposure of embryos which develop and are irradiated from two-cell to blastula in vitro are as follows: cobalt-60 (external), 100 rads/day, 259 rads total effective dose; tritium oxide in culture medium, 450 µCi/ml, giving a radiation dose of 115 rads/day, 298 rads total effective dose; ⁹⁰Sr-⁹⁰Y in culture medium, 0.94 µCi/ml, equivalent to 52 rads/day, 135 rads total effective dose. Effective doses are based on radiation exposure during the 62-hour time in vitro from start of culture at late two-cell to beginning of blastocyst expansion. A biphasic dose-response curve was obtained in all of the in vitro chronic radiation experiments, indicating either an unusually high sensitivity of a fraction of the population (5-20%), or a sequential, multiple event phenomenon in the radiation response. All of these experiments were done with embryos from random-bred Swiss Albino mice, cultured in modified Whitten medium.

d) Comparable LD₅₀ values for chronic exposure of embryos which develop and are irradiated from two-cell to blastula in vivo are: cobalt-60 (external), 220 rads/day, 414 rads total effective dose; tritium oxide, concentration in body water 900 µCi/ml, equivalent to 230 rads/day, 433 rads total effective dose. Effective doses are based on radiation exposure during the 45-hour time in vivo from start of

irradiation at late two-cell to beginning of blastocyst expansion.

e) The survival curves for beta radiation from tritium and for chronic gamma radiation from cobalt-60 were compared by a weighted regression analysis for exposures both in vivo and in vitro. There was no significant difference between the response to the two types of irradiation. It is, therefore, concluded that the RBE for tritium, relative to cobalt-60, is one for this experimental system.

f) A system has recently been developed for culture of embryos from the pronuclear one-cell stage, using DuFrain medium, a modification of Whitten medium. With this system, the LD₅₀ values for pronuclear irradiation of embryos from B 10SJ F₁ females mated with random-bred Swiss Albino males are 1135 rads and 110 rads for development to two-cell and expanded blastocyst, respectively.

g) Based on serial observations of embryos developing in vitro, it was determined that the major effect of chronic irradiation during preimplantation development from two-cell to blastula is failure of development from morula to blastula. An experiment with tritium confirmed this observation for in vivo development. Preliminary results of an ongoing experiment in which embryos are given a single, acute x-ray exposure at the two-cell stage suggest that the same effect occurs with acute irradiation. Irradiation at the pronuclear one-cell stage has indicated that blastocoel formation is a radiosensitive process. It is suggested that this marked radiosensitivity is related to the need for expression of the genome of the zygote.

h) Delay in cleavage from the four- to eight-cell stage is produced by x-irradiation given one to three hours before expected cleavage. In

preliminary experiments, delays of 1.4, 1.8, 2.0 and 2.9 hours were found after radiation doses of 25, 50, 100 and 300 rads, respectively. Cleavage times were also determined for the division from two- to four-cell stages, for embryos irradiated at the pronuclear stage. A linear increase in cleavage time was found for doses up to 600 rads, with a delay of 0.8 minutes per rad.

i) Tritium oxide was incorporated into drinking water of C57BL/6J mice for two generations. Drinking water concentrations of 10 and 25 $\mu\text{Ci/ml}$ resulted in average body water concentrations of 7.1 and 17.8 $\mu\text{Ci/ml}$, respectively. Males of the first generation had a 23% and 56% reduction in testis weight and a 18% and 89% reduction in numbers of motile sperm for the 10 and 25 $\mu\text{Ci/ml}$ levels, respectively. Males of the second generation had a 25% and a 58% reduction in testis weight and a 60% and 89% reduction in numbers of motile sperm at the same dosage levels. Females of the first generation had an ovarian weight reduction of 20% and 47% at the 10 and 25 $\mu\text{Ci/ml}$ levels, respectively; the second generation females had reductions of 87% and 91%. The second generation females were not fertile. Males of the first generation mated with non-irradiated Swiss Albino females fertilized 24% (10 $\mu\text{Ci/ml}$ level) and 47% (25 $\mu\text{Ci/ml}$ level) fewer ova; second generation males fertilized 26% (10 $\mu\text{Ci/ml}$ level) and 36% (25 $\mu\text{Ci/ml}$ level) fewer. Of the fertilized two-cell embryos from these matings which were cultured, there was a decrease of 7% (10 $\mu\text{Ci/ml}$) and 30% (25 $\mu\text{Ci/ml}$) in development to blastula of embryos sired by first generation males and a decrease of 3% (10 $\mu\text{Ci/ml}$) and 31% (25 $\mu\text{Ci/ml}$) of embryos sired by second generation males.

j) An intraperitoneal injection of 28 μCi of ^{90}Sr - ^{90}Y to young adult male mice gave a total dose (over a 28-day period) of 142 rads. More than half of this dose was received in the first 5 days after injection,

and was from radionuclides localized in soft tissue in the vicinity of the scrotum. This dose resulted in a maturation depletion of the testes which is characteristic of an acute radiation exposure. Depressed sperm counts at 5 and 6 weeks after injection were associated with decreased mating success. The proportion of two-cell embryos which developed to blastula was reduced when mating occurred between 10 and 72 days after injection.

k) Techniques for embryo fusion have been established, but attempts to standardize a chromosome karyotyping procedure (using fusion of embryos from CBA/H and CBA/H-T₆T₆ mice) have not been successful. Coat color markers will be used for future fusion experiments.

l) Non-surgical transfer of cultured blastocysts into recipient foster mothers (by injection through the cervix) has resulted in viable offspring. The efficiency of this system relative to standard surgical transfer techniques is not known as yet.

Kirkpatrick, J. and Casarett, A. (1970) Radiation Response of the Preimplantation Mouse Embryo In Vitro. Radiation Res. 43: 231.

Casarett, A. and Kirkpatrick, J. (1970) Radiation-Induced Cleavage Delay of the Preimplantation Mouse Embryo In Vitro. Radiation Res. (Abstract)

Morgan, R. and Casarett, A. (1972) Effects of Tritium Oxide Upon Development of the Preimplantation Mouse Embryo. Radiation Res. 51: 503.

Parker, E. and Casarett, A. (1972) Effects of ⁹⁰Sr-⁹⁰Y on Reproduction in the Male Mouse. Radiation Res. 51: 503.

Casarett, A. and Kirkpatrick, J. Differential Sensitivity of Preimplantation Stages of Mouse Embryogenesis In Vivo and In Vitro (in preparation).

DuFrain, R. and Casarett, A. (1973) Response of the Pronuclear Mouse Embryo to X-irradiation In Vitro. Rad. Res. (Abstract).

Casarett, A. and Morgan, R. (1973) The Relative Biological Effectiveness of Tritium on Preimplantation Mouse Embryos. Health Physics (Abstract).

DuFrain, R. and Casarett, A. A System for the Culture of One-Cell Mouse Embryos from Random-Bred Swiss Albino Mice (in preparation).

4. Graduate Training

A major part of this project has been the work of predoctoral graduate students. Their thesis titles are as follows:

Kirkpatrick, J. (1971) Radiation Response of the Preimplantation Mouse Embryo In Vitro.

Parker, E. (1972) The Distribution, Dose and Effect of ^{90}Sr - ^{90}Y in the Male Mouse Following Intraperitoneal Injection of Equilibrium Mixtures.

Morgan, R. (1973) Effect of Tritium Oxide on Preimplantation Mouse Embryos.

Jacobsen, F. (1973 - in preparation) Effects of ^{90}Sr - ^{90}Y on Male Reproduction and Embryo Development in Mice.

DuFrain, R. (1974 - in preparation) Radiosensitivity of Early Preimplantation Mouse Embryos.

5. Status

Our experience has indicated that there are several important and relevant problems that can be approached effectively with our embryo culture system. Of particular concern, recently, is the effect on embryos of small doses of radiation, such as might be received from medical diagnosis or from environmental release of radionuclides. The large populations necessary for measuring low dose effects can be easily obtained and evaluated with our culture system. The system can also detect preimplantation loss due to certain parental mutations, and is, therefore, of value in studies of population "fitness" as influenced by radiation.

After a series of technical modifications, our experimental system is now standardized. We plan first to repeat certain of the acute x-irradiation experiments to verify previous results of relative stage sensitivity and to more fully characterize the morphologic changes which occur. We will also expand the acute and chronic studies of one- and two-cell embryos to include lower radiation doses and exposure at other stages of the cell cycle.

With a minor modification of the culture media, it is possible to induce hatching, outgrowth and attachment of embryos in vitro with a high level of success. This procedure will be examined as a possible supplement to our implantation techniques. We will correlated preimplantation changes with implantation success and subsequent development.

Reproductive "fitness" will be evaluated by studies of preimplantation development of embryos sired by males irradiated for successive generations. These studies are intended to detect dominant lethal and recessive detrimental mutations (which result in preimplantation loss) which occur both in premitotic spermatogonia and post-meiotic spermatids and spermatozoa.

A short exploratory experiment will examine the use of the embryo fusion technique to study development of embryos containing both irradiated and non-irradiated blastomeres. Other short experiments will deal with radiation-induced cleavage delay, duration of the G_1 , S, and G_2 stages of interphase, and techniques for measuring sperm parameters in male mice.

II. Membrane Transport of Alkaline Earths and Phosphate: The Involvement of Vitamin D and the Calcium Binding Protein. R. H. Wasserman, A. N. Taylor, R. A. Corradino.

1. Introduction

This work grew out of early studies on the metabolism of strontium and of calcium which is known to govern strontium behavior. The well known effect of Vitamin D in increasing calcium absorption was used as a tool to modify the efficiency of the calcium transport system. As a result of these studies the Calcium Binding Protein (CaBP), which is induced by Vitamin D, was discovered in intestinal mucosa by R. H. Wasserman and A. N. Taylor. The work described in this section has been supported by NIH funds as well as under the present AEC contract.

2. Objectives

The primary and broad objective has been to gain as complete an understanding as possible of the mechanisms of alkaline earth absorption from the intestine. The main thrust has been to elucidate the functional role of CaBP in the alkaline earth transport process. Some of the specific objectives include: (a) purification and characterization of intestinal CaBP, (b) study of distribution and localization of the protein, (c) study of factors that control CaBP formation and rate of Ca absorption, (d) interrelationships with other Vitamin D dependent changes especially at the molecular level, (e) involvement of Vitamin D in phosphate absorption, (f) effects of Vitamin D on other organs functional in alkaline earth transport.

3. Main Research Accomplishments

a. Isolation of Calcium Binding Proteins (CaBPs). The chick CaBP was isolated by a three step procedure, including ammonium sulfate fractionation, gel filtration and preparative discontinuous acrylamide gel electrophoresis. Purity was established by analytical gel electrophoresis, using different pH buffers, degrees of cross-linking, and % acrylamide. Bovine and other mammalian CaBPs have been isolated by a similar technique as with chick CaBP but recent modifications have included DEAE-Sephadex chromatography, using ionic strength gradient elution and/or elution with 1 mM CaCl_2 , after Hitchman and Harrison (Can. J. Biochem. 50: 758, 1972). Purity was established by analytical gel electrophoresis and gel electro-focusing techniques.

Wasserman, R. H., R. A. Corradino and A. N. Taylor. (1968) Vitamin D-Dependent calcium-binding protein. Purification and some Properties. J. Biol. Chem. 243, 3978.

Fullmer, C. S. and R. H. Wasserman. (1973) Bovine Intestinal Calcium-Binding Proteins (CaBP): Purification and Some Properties. Biochim. Biophys. Acta, In press.

b. Physical Properties of Purified CaBPs. Most of the effort was devoted to the chick CaBP, the first isolated protein in this series, although information on the mammalian CaBPs is becoming available.

1. Molecular weight. Chick CaBP. Gel filtration on a calibrated Sephadex G-100 column yielded a value of 28,000; a slightly smaller molecular weight (25,000) was indicated by sedimentation equilibrium techniques. Molecular weight determination on SDS gels have recently verified the 28,000 value. Estimation from the amino acid composition also yielded a value of about 28,000. Bovine and other mammalian CaBPs. By calibrated gel filtration, a molecular weight of bovine CaBP was found to be about 11,000. Similar values (in the range of 11,000 - 13,000)

pertain to the isolated CaBPs from guinea pig, horse and pig intestine. These are comparable to the values for the mammalian protein reported by Hitchman and Harrison (loc cit), Drescher and DeLuca (Biochem. 10, 2303, 1971), Schachter (Fat-Soluble Vitamins, H. F. DeLuca and J. W. Suttie, Eds., Univ. of Wisc. Press, p. 55, 1970). Others, for example, Ooizumi et al (J. Vitaminol. 16, 228, 1970) Alpers et al (Gastroent. 62, 559, 1972) and Piazzolo et al (Z. Physiol. Chem. 352, 1480, 1971) reported higher values ($\sim 20,000 - 24,000$) for mammalian proteins.

2. Amino Acid Composition. The amino acid composition of chick CaBP is, as follows, in integer residues per 27,975 m.w.: ala 17, arg 5, asp. a. 34, 1/2 cyst 3, glut. a. 44, gly 13, hist 3, isoleu. 11, leu 31, lys. 24, meth. 8, phenylal 13, pro 3, ser 9, thre 9, trpt 2, try 8, val 5, amide-N 18. The composition of bovine CaBP, in residues per 10,690 m.w., is: ala 3, arg 9, asp. a. 6, 1/2 cyst. 1, glut. a. 18, gly 5, hist 0, isoleu 2, leu 12, lys 12, meth 0, phenylal 5, pro 5, ser 6, thre 2, trypt 0, try 1, val 3, amide-N 5.

3. Sequencing. Chick CaBP and bovine CaBP are being subjected to sequencing by the group of J. T. Potts (Mass. Gen. Hosp., Boston). Early work indicates that the N-terminal is blocked and the C-terminal is lysine. Effort is being given to unblock the N-terminal to allow automatic sequencing.

4. Ca binding parameters. Reassessment of the affinity constant and number of binding sites by equilibrium dialysis, and Scatchard and other plotting procedures, indicated that, for chick CaBP, there are 4 high affinity sites with a k_a of about $10^6 M^{-1}$. The preliminary value for bovine CaBP indicates a k_a of $10^5 M^{-1}$. Similar values were obtained by Schachter (loc cit) for the rat and Hitchman and Harrison (loc-cit) for the porcine protein.

5. Prosthetic groups. No prosthetic groups have been detected on chick CaBP or human CaBP (Alpers et al, Gastroenterology 62: 559, 1972).

6. Cation binding specificity. In the alkaline earth series, the sequence of binding is $\text{Ca} > \text{Sr} > \text{Ba} > \text{Mg}$. When other divalent cations are considered, the order of binding was: $\text{Ca} > \text{Cd} > \text{Sr} \approx \text{Mn} > \text{Fe} > \text{Zn} > \text{Ba} > \text{Co} > \text{Mg} > \text{Ni}$. A relationship between the non-hydrated ionic radii (after Pauling) and binding capacity was apparent.

7. Conformational integrity and Ca binding. High concentrations of urea or lecithin reversibly inhibit Ca binding by CaBP, and CaBP has about 40% helicity from C.D. measurements. Buffer mixtures of high ionic strength also decrease binding.

Wasserman, R. H., R. A. Corradino and A. N. Taylor. (1968) Vitamin D-Dependent Calcium-Binding Protein. Purification and Some Properties. J. Biol. Chem. 243, 3978.

Wasserman, R. H. (1970). Interaction of Vitamin D-Dependent Calcium Binding Protein with Lysolecithin: Possible Relevance to Calcium Transport. Biochim. Biophys. Acta 203, 176.

Ingersoll, R. J. and R. H. Wasserman. (1971) Vitamin D₃-Induced Calcium-Binding Protein. Binding Characteristics, Conformational Effects, and Other Properties. J. Biol. Chem. 245, 2808.

Fullmer, C. S. and R. H. Wasserman. (1973) Bovine Intestinal Calcium-Binding Proteins (CaBP): Purification and Some Properties. Biochim. Biophys. Acta, In press.

Bredderman, P. J. and R. H. Wasserman. Vitamin D-Dependent Calcium-Binding Protein. Chemical Composition, Related Physical Properties and Affinity for Calcium Ions. (In preparation for Biochemistry).

c. Species shown to contain intestinal CaBP are the following:
turtle, frog, toad, chick (and other avian species), rat, bovine, pig, guinea pig, horse, monkey and human.

Kallfelz, F. A., A. N. Taylor and R. H. Wasserman. (1967). Vitamin D-Induced Calcium Binding Factor in Rat Intestinal Mucosa. Proc. Soc. Exptl. Biol. Med., 125, 54.

Taylor, A. N., R. H. Wasserman and J. Jowsey. (1968) A Vitamin D-Dependent Calcium-Binding Protein in Canine Intestinal Mucosa. Fed. Proc., 27, 675.

Wasserman, R. H. and A. N. Taylor. (1971). Evidence for a Vitamin D₃-Induced Calcium-Binding Protein in New World Primates. Proc. Soc. Expt. Biol. Med., 136, 25.

Chapman, M., W. Pond, A. N. Taylor, L. Krook and R. J. Rivers. (1972). Calcium Binding Factor and Rickets in Guinea Pigs. J. An. Sci. 35, 283.

Fullmer, C. S. and R. H. Wasserman. (In press 1973). Bovine Intestinal Calcium-Binding Proteins: Purification and Some Properties. Biochim. Biophys. Acta.

d. Tissue distribution. CaBP is present in all segments of the small intestine, the colon, kidney, and the shell gland (of the laying hen). Despite several efforts, CaBP has not been identified in bone.

Taylor, A. N. and R. H. Wasserman. (1967). Vitamin D₃-Induced Calcium-Binding Protein: Partial Purification, Electrophoretic Visualization and Tissue Distribution. Arch. Biochem. Biophys., 119, 536.

Corradino, R. A., R. H. Wasserman, M. H. Pubols and S. I. Chang. (1968). Vitamin D₃ Induction of a Calcium-Binding Protein in the Uterus of the Laying Hen. Arch. Biochem. Biophys., 125, 378.

Taylor, A. N. and R. H. Wasserman. (1972). Vitamin D-Induced Calcium-Binding Protein: Comparative Aspects in Kidney and Intestine. Am. J. Physiol., 223, 110.

e. Preparation of specific antiserum to CaBP: Use as a specific reagent and for the immunofluorescent localization of CaBP. Chick intestinal CaBP was purified by the previously described procedure, emulsified with Freund's complete adjuvant and injected subcutaneously into rabbits. The resulting antiserum was purified by adsorption with insolubilized protein present in intestinal mucosa of rachitic chicks. The antiserum was demonstrated specific for CaBP by immunoelectrophoresis in three different systems and it also blocked in vitro ⁴⁵Ca binding to purified CaBP.

The antiserum has proven to be a reliable, rapid and very sensitive assay tool for the detection and quantitation of CaBP in addition to its

originally intended function as an immunochemical marker for cellular localization studies. The micro-Ouchterlony (microscope slide) double diffusion in agar technique allows the qualitative detection of CaBP in crude tissue extracts and requires only 10-20 μ l of sample. The sensitivity of the method allows detection of 1-5 μ g CaBP/ml or 10-50 μ g total in the volumes employed. The radial immuno-assay described by Lopez and Golder (Clin. Chim. Acta 21, 517, 1968) was adapted for use with anti-CaBP. Measurement of precipitin rings which form at the equivalence point of CaBP and anti-CaBP antibodies in agar discs yields a quantitative relationship between CaBP concentration and precipitin ring diameter. Again, only very small volumes of sample are required (10 μ l) and the sensitivity permits detection of 0.5-1 μ g CaBP/ml.

Another quantitative immunoassay has been developed based on the electro-immunoassay method of Laurell (Anal. Biochem. 15, 15, 1966). This method which involves the electrophoretic movement of CaBP into agarose containing antiserum, has about the same sensitivity as the radial method, but offers additional advantages in that it is more rapid and produces less ambiguity with very low concentrations of CaBP.

The CaBP present in vitamin D₃-dosed rachitic chick intestine was visualized by employing the indirect fluorescent antibody method. Specific fluorescence, indicating the presence of CaBP, was observed in the PAS-positive goblet cells and also in the microvillar-surface coat region of all intestinal epithelial cells. The latter site represents the absorptive surface of the intestine and from other considerations was indicated as a membrane site significantly affected by vitamin D. As a working hypothesis, it was assumed that the CaBP at the absorptive surface represented the functional CaBP. How CaBP may function to enhance Ca absorption at this locus is presently a matter of speculation.

Taylor, A. N. and R. H. Wasserman. (1970). Immunofluorescent localization of Vitamin D-Dependent Calcium-Binding Protein. J. Histochem. Cytochem., 18, 107.

f. Location of CaBP synthesis on intestinal villi. The question of where CaBP was synthesized, i.e. goblet cells vs. absorptive cells or both, arose but was not answered by the previously mentioned immunofluorescent localization studies. An additional question concerning another type of location of synthesis is: where on the villus is CaBP synthesized? Is it only in the cells located in or near the crypts or are all cells on the villus capable of responding to vitamin D? A modification of the intestinal planning technique of Imond, et al (Exptl. Cell Res. 58, 323, 1969), was used to answer the question. CaBP was formed at all levels of the villi indicating that cells located along the entire length were responsive to vitamin D administration. The active metabolite of vitamin D₃, 1,25-dihydroxycholecalciferol, also produced the same type of response.

Taylor, A. N. Location of Calcium-Binding Protein Synthesis on the Intestinal Villus: Induction by Vitamin D and 1,25-Dihydroxycholecalciferol. (Manuscript in preparation).

g. Kidney CaBP. Studies with embryonic chick kidneys, both the mesonephros and the metanephros, indicated CaBP was present during embryonic life. This is unlike embryonic intestine which contains no CaBP until the day of hatching. Kidney CaBP was present at the time of hatching and if the more sensitive immunoassay was used the protein could be detected in the kidneys of chicks which were subsequently raised up to 6 weeks on a vitamin D-free diet. By using ³H-thymidine as a marker for DNA and, thus, cell turnover, it was shown that the CaBP present at hatch time was slowly diluted by formation of new renal cells devoid of CaBP. At the same time the CaBP-containing cells remained and it was this CaBP, which had been

synthesized in response to vitamin D during embryonic life, that was detectable after 6 weeks without additional vitamin D. The serum Ca concentration was monitored as a function of length of time on the rachitogenic diet and the kidney CaBP concentration. A correlation ($r = 0.82$) was observed between kidney CaBP content and serum Ca concentration. This observation suggested the possibility that kidney CaBP might function in renal tubular reabsorption of Ca.

Taylor, A. N. and R. H. Wasserman. (1971) Vitamin D-Induced Calcium-Binding Protein (CaBP) in Chick Kidney During Embryonic Development and Growth. Fed. Proc., 30, 407.

Taylor, A. N. and R. H. Wasserman. (1972) Vitamin D-Induced Calcium-Binding Protein: Comparative Aspects in Kidney and Intestine. Amer. J. Physiol. 223, 110.

h. Mammary Gland CaBP. The report of Bauman et al (Proc. Latvian Acad. Sci., 294, 133, 1972) indicating the presence of CaBP in rat and bovine mammary tissue prompted a study of that tissue in dogs. A Ca-binding factor with a molecular size similar to dog intestinal CaBP was observed in mammary tissue from a limited number of dogs. The preliminary results do not provide conclusive evidence of the protein nature of the binding factor but they indicate the heat stability is the same as dog intestinal CaBP and the ^{45}Ca binding activity is higher in mammary tissue from lactating than from non-lactating dogs.

Taylor, A. N. and F. A. Kallfelz. A Calcium-Binding Factor in Canine Mammary Gland. (Manuscript in preparation).

i. Dietary calcium and phosphorus, intestinal calcium (^{47}Ca) absorption and levels of CaBP in the intestine. Using the ligated duodenal segment technique, the effect of feeding various levels of Ca and P in the diet on ^{47}Ca absorption was determined. Intestinal CaBP was also determined. The diets were fed to 17 day-old chicks for a period of 10 days, using a 3 x 3 factorial design. Each group received either 0.08, 1.20 or 2.32% Ca

and either 0.25, 0.65 or 1.20%. Those chicks on a calcium deficient diet absorbed calcium at higher rates than those on a normal or a high calcium diet, and those chicks on a low phosphorus diet also absorbed calcium at a higher rate, as noted previously by Carlson in 1953. The correlation coefficient between ^{47}Ca absorption and CaBP was 0.99, and the correlation coefficient between ^{47}Ca absorption and bone ash was -0.94. The inverse relationship between bone ash and calcium absorption suggested a bone factor, after Nicolaysen. This concept needs to be evaluated in terms of the kidney metabolism of 25-hydroxycholecalciferol (a vitamin D metabolite). This study emphasized the relationship between Ca translocation and CaBP.

Morrissey, R. L. and R. H. Wasserman. (1971). Calcium Absorption and Calcium-Binding Protein in Chicks on Differing Calcium and Phosphorus Intakes. *Amer. J. Physiol.* 220, 1509.

j. Stable strontium and the vitamin D-mediated intestinal calcium absorptive mechanism. Studies were undertaken to determine if stable strontium could substitute for calcium in the diet of chicks undergoing adaptation to a low calcium intake. Not only could stable strontium not substitute for calcium, but even though there was ample dietary vitamin D_3 , strontium was found to be very rapidly inhibitory of CaBP synthesis and intestinal calcium absorption. After only three days on the strontium diet, chick intestine was devoid of CaBP and could absorb calcium no more efficiently than vitamin D_3 deficient rachitic chicks of the same age. Accompanying these intestinal inhibitory actions of strontium, the chicks showed severe depletion of bone mineral and the development of lesions somewhat similar to those seen in rickets. If the chicks initially fed the strontium diet for 7 days were returned to a normal diet, they rapidly recovered their ability to synthesize CaBP and absorb calcium and the bone changes were substantially reversed.

A series of experiments were then undertaken to determine the specificity of the strontium effect and the effects of varying the dietary strontium: calcium ratio. It was found that, like strontium, both beryllium and barium inhibited CaBP synthesis. Varying the strontium: calcium ratio in the diet produced some surprising results. A ratio of Sr: Ca in the diet of only 1:5 was inhibitory of CaBP synthesis within 3 to 7 days, with the degree of inhibition increasing with increasing Sr:Ca ratio until complete inhibition was observed at a Sr:Ca ratio of 1:1. However, after 14 days on the respective diets, the CaBP producing ability of the duodenum spontaneously recovered at Sr:Ca ratios of 1:1 or less. In a followup study, radiocalcium absorption was also monitored in chicks fed a Sr:Ca ratio of 1:2. Again, transient inhibition of CaBP synthesis and spontaneous recovery was observed. However, calcium absorptive capacity was essentially normal throughout the experimental period. This apparent discrepancy between duodenal CaBP concentration and calcium absorptive rate cannot be explained at present but one further observation should be considered: the CaBP present in strontium-fed and normal chicks in this study had differing calcium binding activities. One could speculate, therefore, that there may be changes in the activity of CaBP in vivo based on the animal's calcium requirement.

Corradino, R.A. and R. H. Wasserman: (1970) Strontium Inhibition of Vitamin D₃-Induced Calcium-Binding Protein (CaBP) and Calcium Absorption in Chick Intestine. Proc. Soc. Exp. Biol. and Med. 133, 960.

Corradino, R. A., J. G. Ebel, P. H. Craig, A. N. Taylor and R. H. Wasserman. (1971) Calcium Absorption and the Vitamin D₃-Dependent Calcium-Binding Protein. I. Inhibition by Dietary Strontium. Calc. Tis. Res. 7, 81.

Corradino, R. A., J. G. Ebel, P. H. Craig, A. N. Taylor and R. H. Wasserman (1971) Calcium Absorption and the Vitamin D₃-Dependent Calcium-Binding Protein. II. Recovery from Dietary Strontium Inhibition. Calc. Tis. Res. 7, 93.

Corradino, R. A. (1972) Strontium Inhibition of the Vitamin D-Induced Calcium-Binding Protein and the Intestinal Calcium Absorptive Mechanism. Proc. Second International Conference on Strontium Metabolism. Glasgow and Strontian, August, 1972.

k. Cortisone inhibition of Ca absorption. Hydrocortisone administration to vitamin D₃-dosed rachitic chicks causes a decrease in several parameters of ⁴⁷Ca metabolism: mucosal tissue uptake, absorption, tibia deposition and total mucosal cell weight. With the exception of mucosal cell weight, the hydrocortisone appears to be acting predominantly on the vitamin D enhanced portion of each parameter. The drug, however, does not inhibit the vitamin D induced synthesis of intestinal CaBP.

The possibility exists that cortisone acts to alter membrane structure non-specifically and no effect is seen in the rachitic chick because of the already very low level of Ca-transport capacity present, i.e., cortisone does not inhibit Ca transport by way of a specific anti-vitamin D directed action. This hypothesis is supported by the observation that mucosal tissue weight is equally depressed by the drug in rachitic and vitamin D treated chicks, by the observation that the enzyme activities of both alkaline phosphatase and Ca-ATPase are similarly decreased by the drug in brush border preparations from rachitic and vitamin D treated chicks and finally by the investigations of Kimberg et al (J. Clin. Invest. 50, 1309, 1971). Those investigators also indicated that CaBP synthesis was not inhibited by cortisone and they were able to show an effect of cortisone on the rate of iron and galactose transport in addition to Ca transport.

1. Brush border enzyme activity stimulated by vitamin D. The studies of Martin et al (Biochem. Biophys. Res. Comm. 35, 819, 1969) and Haussler and Nagode (J. Cell Biol. 43, 51a, 1969) indicated vitamin D increased brush border Ca-ATPase activity and it was proposed that the

Ca-ATPase might be involved in some manner in mediating the vitamin D enhanced Ca absorption process. Brush borders prepared from chick duodenal mucosa exhibited an increased activity of Ca-ATPase, alkaline phosphatase and leucine aminopeptidase in response to vitamin D₃ administration while ATPase and maltase did not change. However, the time of increased Ca-ATPase activity did not correspond to the physiological response time of the vitamin. CaBP was formed in the intestinal mucosa from these same chicks before increased Ca-ATPase activity was noted. In chicks adapted to a low Ca diet, the only brush border activity showing an increase was leucine aminopeptidase. The Ca-ATPase and alkaline phosphatase did not change over an 8-day experimental period, while the CaBP content of duodenal mucosa increased after 3 days on the low Ca intake. These observations suggest that the increased enzyme activity may be a secondary, non-specific effect of vitamin D and cast doubt on the proposal that Ca-ATPase and/or alkaline phosphatase may be a primary mediator of Ca transport.

These and additional observations related to the action of L-phenylalanine and Ca concentration on Brush border enzyme activity, are consistent with the concept that alkaline phosphatase and Ca-ATPase represent separate enzyme systems.

Taylor, A. N. and R. H. Wasserman. (1970) Vitamin D Stimulation of Calcium-Binding Protein and Brush Border Enzyme Activities. Fed. Proc., 29, 368.

Taylor, A. N. and R. H. Wasserman. Changes in Brush Border Enzyme Activity and CaBP Content of Chick Intestine as Influenced by Vitamin D, Dietary Calcium and Other Factors. (Manuscript in preparation).

m. Vitamin D and phosphate transport. Vitamin D₃ administration to rachitic chicks caused the stimulation of phosphate absorption as measured with ligated segments of intestine in vivo and also if measured with everted sacs of ileum in vitro. The in vivo studies indicated that the vitamin D

effect did not require the presence of Ca^{++} ions in the intestinal lumen and the in vitro findings confirmed this. Thus, in addition to the direct effect of vitamin D on Ca transport, it appears the vitamin also exerts a direct action on phosphate transport. Some of the observed characteristics of the vitamin D mediated transport are, as follows:

(a) the absorption is a saturable process, (b) both the in vivo and in vitro studies indicated a vitamin effect on the mucosal uptake and the serosal release steps, (c) arsenate inhibited phosphate transport, (d) L-phenylalanine the inhibitor of intestinal alkaline phosphatase inhibited phosphate transport in vivo, while it enhanced it in vitro, (e) the in vitro studies indicated the possible involvement of the $(\text{Na}^+ - \text{K}^+) - \text{ATPase}$ in the serosal extrusion of phosphate and the need for medium - Na^+ in the uptake step, (f) the inhibitor of glycolysis, iodoacetate, significantly reduced the phosphate uptake step as measured in vitro, (g) the inhibitor of bone resorption, EHDP (ethane-1-hydroxy-1, 1-diphosphanate), effectively reduced the in vivo uptake and absorption of phosphate, (h) there was no binding of ^{32}P to purified CaBP and time-course experiments indicated CaBP appeared considerably ahead of increased ^{32}P absorption, and (i) preliminary studies designed to detect a phosphate binder have not yet yielded any positive evidence for such a compound.

Taylor, A. N. and R. H. Wasserman. (1972) Intestinal Phosphate Absorption: Effect of Vitamin D and Other Variables. Fed. Proc., 31, 686.

Wasserman, R. H. and A. N. Taylor. (1973) Intestinal Absorption of Phosphate in the Chick: Effect of Vitamin D_3 and Other Parameters. J. Nutr. 103, 586.

Taylor, A. N. and R. H. Wasserman. (1973) In Vitro Intestinal Phosphate (^{32}P) Transport: Effect of Vitamin D and Other Factors. Fed. Proc. 32, 917.

Taylor, A. N. and R. H. Wasserman. The In Vitro Transport of Phosphate in Everted Ileal Sacs from Rachitic and Vitamin D-Treated Chicks. (Manuscript in preparation).

n. Embryonic Chick Intestine in Organ Culture. In order to study the intestinal calcium absorptive mechanism under more precisely controlled conditions, attempts were made to culture vitamin D₃-responsive tissues in vitro. The first attempt was with the avian shell gland, a site of vitamin D₃-induced CaBP synthesis. This seemed a likely tissue for such studies since it is sterile, unlike the highly labile chick intestine. However, attempts to maintain this tissue in culture responsive to vitamin D in the culture medium were unsuccessful.

Another tissue which seemed promising was the embryonic chick intestine. It too is sterile and experiments showed that it contained no CaBP until after hatching but that CaBP could be induced precociously by injection of cortisone into the egg on day 16 of incubation. It was subsequently found that the embryonic duodenum would respond to vitamin D₃ in a simple, immersion culture technique by synthesizing CaBP and stimulating radiocalcium uptake. In later studies, the synthesis of CaBP under these conditions was shown to be de novo and specific for vitamin D-like sterols.

The response to vitamin D₃, i.e. CaBP synthesis, was shown to be absolutely dependent on medium calcium concentration; there was no CaBP produced below approximately 0.2-0.3 mM calcium in the medium. Above this level of calcium, CaBP synthesis reached a peak (between 1.25 and 2.5 mM) and declined at higher levels.

Using the same immersion culture method, the first direct evidence for a role of CaBP in intestinal calcium transport was obtained. Addition of purified chick CaBP to the culture medium stimulated intestinal uptake and transport of radiocalcium in a fashion quite similar to the same responses achieved by the biosynthesis of CaBP in response to vitamin D₃ in the medium.

Attention was then turned to the development of optimal culture conditions. After considerable effort, a technique was achieved which permitted maintenance of large amounts of embryonic duodena on stainless steel grids in serum-free medium with excellent preservation of mucosal structure. Using this grid technique it was found that the CaBP effect on radiocalcium transport was specific for CaBP; neither heat-denatured CaBP nor a bacterial glutamine binding protein stimulated ^{45}Ca transport when added to the culture medium.

The relative potency of vitamin D_3 and some biologically active metabolites was tested in this system. One compound, 1,25-dihydroxycholecalciferol, currently considered the active form of vitamin D_3 in vivo, was found to be enormously potent in the organ-cultured duodenum. A detectable response, i.e., CaBP production, was achieved at a medium concentration of 6.5 pM. Although much less potent in terms of CaBP induction, vitamin D_3 itself was shown to be effective in vitro.

In contrast to the in vivo results of others, 1,25-dihydroxycholecalciferol was shown to exert its biological effects in vitro via an actinomycin D and α -amanitin-sensitive mechanism, i.e., new protein synthesis is involved. Thus, the initial biological effect of 1,25-dihydroxycholecalciferol is probably on the genetic system as was previously shown for vitamin D_3 in vivo.

Recently, using this organ culture system, it was shown that vitamin D_3 also stimulates cyclic AMP production. Thus, vitamin D joins a large number of hormones and drugs which may bring about their actions via stimulation of the adenyl cyclase system. Of considerable interest in the organ-culture studies were the findings that dibutyryl cyclic AMP, a cyclic AMP analogue, and theophylline, which prevents endogenous cyclic AMP breakdown, both stimulated radiocalcium uptake and that their

effects were additive and synergistic to the effect of vitamin D₃ itself. Surprisingly, the vitamin D₃ action of ⁴⁵Ca uptake proceeded while CaBP synthesis was reduced. It seems apparent that there is a relationship between vitamin D₃, CaBP and the adenylyl cyclase system. This relationship will be the subject of continuing investigation.

Salmon and porcine calcitonin and bovine parathyroid hormone were shown to be without effect on either CaBP synthesis or radiocalcium accumulation by organ-cultured duodenum. These observations are at odds with the results of others obtained in vivo but are, perhaps, more significant since indirect effects may have been responsible for the positive effects in vivo.

Corradino, R. A., A. N. Taylor and R. H. Wasserman. (1969). Appearance of Vitamin D₃-Induced Calcium-Binding Protein (CaBP) in Chick Intestine During Development. Fed. Proc. 28, 760.

Corradino, R. A. and R. H. Wasserman. (1970) Induction of Intestinal Calcium Binding Protein by Vitamin D₃ In Vitro. Fed. Proc. 29, 368.

Corradino, R. A. and R. H. Wasserman. (1971) Stimulation of Calcium Transport in Embryonic Chick Intestine by Incubation in Medium Containing Vitamin D₃-Induced Calcium-Binding Protein. Biophys. Soc. Abstr. 11.

Corradino, R. A. and R. H. Wasserman. (1971) Vitamin D₃: Induction of Calcium-Binding Protein in Embryonic Chick Intestine In Vitro. Science 172, 731.

Corradino, R. A. and R. H. Wasserman. (1972) Embryonic Chick Intestine in Organ Culture: Relative Potency of Vitamin D₃ Analogues in Stimulating CaBP Synthesis and the Calcium Absorptive Mechanism. Fed. Proc. 31.

Corradino, R. A. (1973). Embryonic Chick Intestine in Organ Culture: Response to Vitamin D₃ and its Metabolites. Science 179, 402.

Corradino, R. A. (1973) Embryonic Chick Intestine in Organ Culture: Increased Cyclic Amp in Response to Cholecalciferol (Vitamin D₃) and Stimulation of Radiocalcium Accumulation by Dibutyryl Cyclic Amp and Theophylline. Annual Meeting, Endocrine Society, Chicago, June, 1973.

Corradino, R. A. Embryonic Chick Intestine in Organ Culture: A Unique System for the Study of the Intestinal Calcium Absorptive Mechanism. J. Cell Bio. (in press).

Corradino, R. A. 1,25-Dihydroxycholecalciferol: Inhibition of Action in Organ-Cultured Intestine by Actinomycin D and α -amanitin. Nature (in press).

Corradino, R. A. and R. H. Wasserman. Stimulation of Calcium Transport in Embryonic Chick Intestine by Incubation in Medium Containing Vitamin D₃-Induced Calcium-Binding Protein (Manuscript in preparation).

4. Graduate Student and Post-doctoral Support

- a. Dr. A. Bar of Israel; 1 year post-doctoral fellow on an NIH International Fellowship.
- b. Drs. P. J. Bredderman, R. J. Ingersoll, R. L. Morrissey, A. H. Taylor received Ph.D. degrees.
- c. R. J. Ingersoll and C. S. Fullmer received M.S. degrees.
- d. C. S. Fullmer and L. Lippiello are presently working on Ph.D. programs.

5. Status

The investigations reported from this laboratory plus other studies concerned with Ca transport, combined with the very extensive research on the metabolism of vitamin D itself, have contributed considerably to a greater understanding of the fundamental processes of calcium translocation across membranes and the action of vitamin D on this process. This also applies to other important mineral ions, strontium and phosphate. Specifically, some of the active metabolites of vitamin D are now in clinical use for the treatment of certain disease states which in the past were only poorly or not at all responsive to vitamin D treatment. The studies with CaBP and the mechanism of Ca absorption are not yet at a stage to yield a "practical application." However, by utilizing the information gained thus far as a basis for future research, much can potentially be learned and continued progress towards a full definition

of these processes is anticipated. Such knowledge bears on disease states associated with abnormal mineral metabolism in humans and animals, and the potential for decreasing body burdens of alkaline earth radionuclides associated with nuclear debris and fallout.

The importance of protein carriers in the transport and subsequent behavior of diverse mineral elements in the body cannot be overemphasized. The techniques and approaches that have worked so well for the Calcium Binding Proteins should be generally applicable to similar substances, as for example, the sialoproteins which may be involved with plutonium sequestration in bone.

III. Strontium Metabolism. C. L. Comar

1. Introduction

Over the years a great deal of work has been done on various aspects of strontium metabolism and movement in the biosphere. Areas that received particular attention, earlier, were movement in the food chain; metabolism in man and animals; remedial measures; surveys and monitoring. But more recently emphasis has been given to empirical models of retention as a basis for dose calculations; dosimetry of the cells at risk; and risk estimations at low levels.

2. Objectives

A major objective has been to develop predictive equations for estimation of the ^{90}Sr commitment of an individual based on his previous year's ^{90}Sr body burden and on the dietary contamination during the current year - taking into account the reutilization of resolved bone ^{90}Sr and the differences in rates of resorption of Sr and Ca. As a peripheral study, procedures were developed for achieving steady-state levels of a radio-nuclide in the blood of an animal using computer controlled administration.

3. Main Research Accomplishments

Extensive studies in which stable Sr balances and radiotracer balances were determined in growing pigs, led to quantitative estimates of the fractional rates of removal of Sr and Ca from bone and permitted validation of a new prediction equation. This equation is considered more flexible than the previous semi-empirical equations of Kulp, Rivera and Marshall. The equations are given later but the details cannot be presented here and are to be found in two Ph.D. theses: "A Quantitative

Study of Strontium Metabolism in Bone", S. D. Talisayon (1971) and "Alkaline Earth Regulation in Blood", R. H. Bubar (1973).

C. L. Comar, Radiostrontium Availability and Metabolism. Proceedings of the Conference on Biomedical Implications of Radioactive Strontium, Davis, Calif. (In press).

4. Graduate Training

S. D. Talisayon and R. H. Bubar received Ph.D. degrees in 1971 and 1973 on the basis of research performed under this program.

5. Status

There are essentially four versions of prediction models for ^{90}Sr that can be used.

a) Observed Ratio Model:

This model simply states that the $^{90}\text{Sr}/\text{Ca}$ ratio of bone being mineralized at any time cannot exceed the $^{90}\text{Sr}/\text{Ca}$ ratio of the diet multiplied by the $\text{OR}_{\text{bone/diet}}$. This sets the maximum dose rate for any portion of the bone being formed and will always be higher than the average dose rate to bone except in the case of a constant $^{90}\text{Sr}/\text{Ca}$ in the dietary intake from fetal life onward.

b) Observed Ratio-Balance Model:

This model sets forth relationships between the ^{90}Ca contents of human diets and the resulting ^{90}Sr concentration in bone in terms of measured values from year to year. This procedure was essentially suggested by Kulp and associated and developed extensively by Rivera.

It is based on established human data and is an extension of the OR concept which allows for bone growth and remodeling as well as excretion; it does not take into account the re-utilization of bone mineral. It

makes no mathematical generalization as to the form of the retention curve.

c) Observed Ratio-Reutilization Model:

This is the model referred to under Main Research Accomplishments (see later under paragraph 8 of Discrimination Equations and Explanations)

d) Marshall Retention Model (ICRP/20):

This is a most sophisticated model that fits existing data quite well, but there is no provision for taking into account changes in calcium intake.

Because the concept of Observed Ratios is often misunderstood and because the meaning of Discrimination Factors in this system is generally not appreciated, especially the idea of sequential or competing discrimination, a summary is presented. Also, there is given a table of typical Sr and Ca values for adults and infants computed from the literature.

In the near future we expect to test the various models with survey data primarily available from the New York Health and Safety Laboratory.

Discrimination Equations and Explanations

$$1. \quad OR_{\text{sample/precursor}} = \frac{\text{Sr/Ca of sample}}{\text{Sr/Ca of precursor}}$$

OR = Observed Ratio; measure of overall discrimination; valid under conditions of equilibrium or steady state; Sr/Ca of precursor must be that available to organism as a single precursor.

$$2. \quad OR = (DF_1)(DF_2) \dots (DF_n)$$

DF = Discrimination Factor for the individual process that contributes to the overall discrimination. It is usually given a specific name - DF_A , DF_U represent Discrimination Factors due to gastrointestinal absorption and urinary excretion, respectively. The DF value = $\frac{\text{Sr/Ca after process has occurred}}{\text{Sr/Ca before process has occurred}}$. If there is no discrimination, the DF = 1. In a one step process the OR is identical with the DF value.

$$3. \quad DF_A = \frac{Sr_o - F_{Sr}}{Ca_o - F_{Ca}} \div \frac{Sr_o}{Ca_o}$$

DF_A = Absorptive Discrimination Factor; Sr_o and Ca_o = strontium and calcium intakes; F_{Sr} and F_{Ca} = fecal excretions of strontium and calcium.

4. Example of 2-step sequential discrimination

$$DF_A = \frac{SR_o - F_{Sr}}{Ca_o - F_{Ca}} \div \frac{Sr_o}{Ca_o}$$

$$DF_U = \frac{SR_o - F_{Sr} - U_{Sr}}{Ca_o - F_{Ca} - U_{Ca}} \div \frac{Sr_o - F_{Sr}}{Ca_o - F_{Ca}}$$

DF_u = Urinary Discrimination Factor; U_{Sr} and U_{Ca} = renal excretions of strontium and calcium. In non-pregnant and non-lactating individuals these two processes are the major ones operative. These formulas can be used if the excretions can be collected and analyzed or if whole body retentions plus either fecal or urinary excretion can be measured.

5. Example of 3-step discrimination process with 2-steps competing.

$$DF_A = \frac{Sr_o - F_{Sr}}{Ca_o - F_{Ca}} + \frac{Sr_o}{Ca_o}$$

$$DF_U = \left(\frac{Sr_o - F_{Sr} - U_{Sr} - M_{Sr}}{Sr_o - F_{Sr}} \right) \left(\frac{U_{Sr}}{U_{Sr} + M_{Sr}} \right) + \left(\frac{Ca_o - F_{Ca} - U_{Ca} - M_{Ca}}{Ca_o - F_{Ca}} \right) \left(\frac{U_{Ca}}{U_{Ca} + M_{Ca}} \right)$$

$$DF_M = \left(\frac{Sr_o - F_{Sr} - U_{Sr} - M_{Sr}}{Sr_o - F_{Sr}} \right) \left(\frac{M_{Sr}}{U_{Sr} + M_{Sr}} \right) + \left(\frac{Ca_o - F_{Ca} - U_{Ca} - M_{Ca}}{Ca_o - F_{Ca}} \right) \left(\frac{M_{Ca}}{U_{Ca} + M_{Ca}} \right)$$

DF_M = Lactational Discrimination Factor; M_{Sr} and M_{Ca} = secretions into milk of strontium and calcium. These equations are based on assumption of first-order kinetics. The same principles could be applied to other processes such as endogenous secretion into the gastrointestinal tract.

6. Expression of discrimination in terms of comparative rate constants.

$$DF_A = \frac{1 - (F_{Ca}/Ca_o)^K}{1 - F_{Ca}/Ca_o}$$

$K = k_{Sr} + k_{Ca}$ where k_{Sr} and k_{Ca} = fractional rate constants for movement of Sr and Ca across gastrointestinal tract. This equation assumes first order kinetics and that the rate constants are related to each other in the same way at each site of movement.

The equation demonstrates that any treatment that alters absorptive discrimination can do so in two ways: by changing the absorption of both alkaline earths proportionally and by changing the comparative rate constants. These two effects can now be distinguished.

7. Equations for use of urinary excretions as a measure of dietary intake.

$$OR_{urine/diet} = \left(\frac{A_{Sr} B_{Sr}}{A_{Ca} B_{Ca}} \right)^K \left(\frac{U_{Ca}}{Ca_o} \right)^{K-1}$$

Empirical equations:

$$\text{Adults} \quad OR_{urine/diet} = 0.32 \left(\frac{U_{Ca}}{Ca_o} \right)^{-0.50}$$

$$\text{Children} \quad OR_{urine/diet} = 0.54 \left(\frac{U_{Ca}}{Ca_o} \right)^{-0.46}$$

A_{Sr} and A_{Ca} = fractional gastrointestinal absorption of strontium and calcium; B_{Sr} and B_{Ca} = fractional filtration into the glomerulus of strontium and calcium; $K = k_{Sr} + k_{Ca}$ = ratio of fractional rate constants of tubular resorption. The empirical equations were derived from a series of experimental observations.

8. The Observed Ratio-Reutilization Model.

$$Ca_n X_n = (1-f') Ca_{n-1} X_{n-1}$$

$$+ \left(\frac{f' Sr_{n-1}}{Sr_a + f' Sr_{n-1}} \right) \left[Ca_n X_n - (1-f') Ca_{n-1} X_{n-1} \right]$$

$$+ \left(\frac{Ca_a}{Ca_a + f Ca_{n-1}} \right) \left[Ca_n - (1-f) Ca_{n-1} \right] Z \text{ OR}$$

Ca_n and Ca_{n-1} = calcium contents at a later time and an initial time;

X_n and X_{n-1} = Sr/Ca ratios in skeleton at a later time and an initial

time; f and f' fraction of skeletal Ca and Sr, respectively, removed from

the body; Ca_a = amount of Ca absorbed from gastrointestinal tract;

Z = Sr/Ca ratio in the diet between the later and initial times; OR =

observed ratio between diet and skeleton.

Typical Values for Amounts of Stable Strontium and Calcium
Metabolized by Adults on English Diet (16) and Experimental Values
for Infants (age 148 days, weight 7.5 kg) on U.S. Diet (14)

	<u>Adults</u>			<u>Infants</u>		
	Sr	Ca	Sr/Ca	Sr	Ca	Sr/Ca*
Dietary intake (mg/day)						
Dairy products	0.28	644	0.31			
Plant sources	0.43	146	2.1			
Meat, fish, eggs	0.18	69	1.9			
Creta preparata	0.59	270	1.6			
Water	0.19	60	2.3			
Other sources		18				
Total	1.67	1207	1.0	0.66	450	1.0
Absorbed into body from diet (mg/day)	0.3	450	0.5	0.17	240	0.48
Endogenous fecal excretion (mg/day)	0.05	150	0.24	0.013	30	0.30
Unabsorbed in feces (mg/day)	1.37	757	1.3	0.49	210	1.6
Total in feces (mg/day)	1.42	907	1.2	0.50	240	1.4
Excreted in urine (mg/day)	0.24	250	0.69	0.068	21	2.2
Excreted in sweat (mg/day)	0.025	50	0.36	0.007**	13**	0.37**
Plasma (mg)	0.12	350	0.24			
Extracellular fluid (mg)	0.33	1000	0.24			
Bone Surfaces and soft tissue (mg)	1.2	3650	0.24			
Total exchangeable pool (mg)	-----	-----	-----	1.4	3,300	0.38
Bone (mg)	345	1.04×10^6	0.24	20	50,000	0.28
Newborn (mg)	5.6	2.8×10^4	0.14			
Mother's milk (mg/day)	0.043	260	0.12			
Accretion rate in bone (mg/day)		500		0.31	740	
Resorption rate (mg/day)		500		0.23	580	
Half life in exchangeable pool (days)	2.4	3.7		2.2	2.5	
Half life in bone (days)	2300	2770		270	750	

* Value for total diet set at 1

** Includes saliva loss

IV. Environmental Problems. F. W. Lengemann, J. C. Thompson, Jr.

R. A. Wentworth, C. L. Comar.

1. Introduction

The original apprehensions of the public and scientific community about the possible effects of fallout and medical uses of radiation have been carried forward to include concern about a developing nuclear power industry. Our research program has attempted to anticipate such concerns and provide a data base that would enable logical decisions. We consider it important not to be constrained by compartmentalized interests which means that net effects on the population have to be considered. Thus, there has to be taken into account such matters as: (a) possible effects of accidents as well as of routine releases, (b) impact of fuel reprocessing as well as of reactor operation, (c) health effects of non-nuclear options, (d) need for public understanding of biological and environmental costs. In approach, then, we have attempted to provide whatever facts are possible in such a way that those who are responsible for the overall decisions will find them to be meaningful. In particular, it is important to point to those areas where concern is justified as well as to help allay apprehension in areas where concern is not justified.

2. Objectives

A general objective has been to maintain a capability for appraising levels of radiocontaminants in the environment, whether arising from nuclear testing residues or from releases associated with peacetime uses of nuclear energy. Since reactors are usually sited in areas of low population density, that are often agricultural in nature, it appeared important to estimate the magnitudes of agricultural economic losses in areas affected by various

degrees of radionuclide releases. Specific objectives have included:

- (a) development of simple methods to predict the intake commitment from milk for the population after deposition of radiocontaminants on pasture,
- (b) study of radionuclides other than those of iodine and strontium that could be important, particularly taking into account age groups and metabolic conditions that would enhance radiation dosages, (c) development of effective countermeasures for reduction of radiocontaminants in animal products and food.

3. Main Research Accomplishments

A. Intake Commitments -- A relatively simple prediction procedure has been developed from extensive laboratory and field studies that permits estimation of total human intakes of ^{90}Sr , ^{137}Cs , and ^{131}I that will result from environmental contamination. The only samples needed are milk samples collected at a known date and an estimate of the date of deposition. The necessary tables and textual material is being compiled for publication as a T.I.D. document. Emphasis has recently been given to ^{137}Cs because of the recognition of the increased importance of this radionuclide and the fact that suckling individuals may be at increased risk. Its metabolism has been studied as related to age, temperature, sex, weaning, dietary potassium, thyroxine, and propylthiouracil. It has also been determined that significant exposure to cerium can occur in the suckling as opposed to older individuals.

B. Fallout and Peactime Environmental Radiocontamination -- General fallout summaries covering the period from 1957 - 1973 indicate that average radiation exposures to the United States population were considerably higher than exposures anticipated from non-military uses during the next 30 years. Average fallout exposures ranged from 2-25 mrem per person per year during this period whereas exposures from non-military uses are not

expected to exceed 0.5 mrem by the year 2000. Although unique population groups may receive slightly higher exposures in some cases, it will normally be less than typical background variations experienced throughout the United States.

The comprehensive assessments that have been made for radioactive fallout have permitted a better understanding of the pathways and critical exposure concepts of radioactive releases. Considerable time and effort have been directed toward these summaries with our departmental staff making significant contributions in the past few years (UNSCEAR Reports, NAS Radionuclides in Foods 1962 and 1973, JCAE Hearings, AEC Symposium Series, Understanding the Atom Series, and Scientific Reports). This information is essential as increased non-military radiation usages develop. In particular, the increased growth in the nuclear power industry has pointed out the need for greater understanding of low level releases to the aquatic environment; an area which had been given relatively limited study under the fallout program. In the terrestrial environment the absence of specific radionuclide measurements does not prevent making an appraisal of the nature and extent of radio-contamination. The estimating procedure can rely upon the known interrelationships between various dietary components, excretions, accumulation patterns, and secretion levels that have been defined through extensive fallout programs. These efforts have resulted in the development of alternative estimators through such means as milk/pasture ratios, urine/diet ratios, bone/diet ratios, milk/diet ratios, bovine thyroid collections and their correspondence to human accumulation patterns and plant/soil patterns. These and many other techniques permit a rather complete appraisal of terrestrial contamination even when the information or monitoring programs are incomplete.

No similar capability exists for the aquatic environment. The data are limited and many of the interrelationships unknown or unproven at this time. Aside from the use of concentration factors and some indicator products for certain radionuclides (^{65}Zn , ^{90}Sr in shellfish), there are very few known associations capable of providing effective estimates within this environment. This uncertainty will continue until monitoring programs are adequate to develop such data or until some degree of cross correlation or correspondence is achieved between the terrestrial and aquatic environments. Research is necessary to provide this capability and is vitally needed if the full impact of nuclear releases is to be evaluated.

Other special concerns such as radiation effects on benthos, variations in concentration factors, possibilities of synergistic thermal-radiation effects, enhanced primary production due to elemental discharges from nuclear facilities and the impact of accidental releases are now the focal point as our concern for the environment becomes a primary objective. An example of the uncertainties is illustrated by the lack of correspondence between derived and measured concentration factors for some radionuclides in freshwater fish. Time-related changes due to varying rates of deposition (or release) are another uncertainty as has been shown for several Italian lakes. Other factors having a bearing on concentration factors are the quantity and nature of stable elements, amount of chemically related and competing ions, chemical and physical state of the radionuclide in solution, rates of uptake and metabolism, temperature, pH, salinity, presence of binding or complexing agents, etc. It is quite evident that any detailed assessment of radioactive exposure due to reactor releases must take into account the unique features of the surrounding aquatic environment, and cannot be based upon broad averages or derived data.

C. Remedial or Countermeasures -- Concern for the human population in the event of nuclear war or accidents has resulted in a reasonable understanding of the feasibility of radioactive contamination control in the past ten years. Various remedial measures have been evaluated and identified for maximum effect under each situation. For example, as already mentioned, prediction equations are available to relate milk contamination levels to total intake commitments of various radionuclides. Utilizing this information permits a selection of action processes which are designed to minimize intake. Simple actions such as keeping animals off pasture and feeding stored feed for three weeks can mean a total intake commitment that is only 14 percent of the maximum possible commitment. Similar calculations make it possible to determine appropriate feeding practices for the utilization of contaminated forage in order to avoid crop loss, maintain food productivity and keep radioactive intake within established guidelines.

Other factors such as dietary additives and substitutions indicate the possibilities for further reductions in radionuclide intake. Dietary additives such as stable iodine, calcium, strontium, magnesium, phosphorus, alginates and Prussian blue, might be used under severe emergency conditions. However, dietary substitutions and/or alterations would be the preferred procedure in the event of small releases of radioactivity. Examples of reductions due to dietary manipulation are shown below for high and low fallout periods and can be extrapolated to conditions expected for smaller releases of radioactivity.

<u>Diet</u>	<u>1961-63</u> $^{90}\text{Sr/gCa}$	<u>1967</u>
1. Normal	18.6	15.6
2. Milk 90% decontaminated	10.2	10.4
3. Milk eliminated	22.4	24.1
4. Fruits and vegetables selected for low ^{90}Sr	15.6	15.6
5. Combination of diets 2 and 4	6.0	10.4

The simplest and most logical decontamination techniques are normal food preparation and processing practices in the home and factory which remove a significant fraction of the surface-deposited radionuclides. A 30 to 60% removal is readily attainable for ^{131}I , ^{90}Sr , and ^{137}Cs . Greater reductions, up to 80 and 90% are also possible by using more-extensive preparation techniques (several washings, scraping, peeling, cooking, and discarding cooking water).

Special studies designed to evaluate retention and removal of ^{131}I from contaminated vegetables re-inforce the value of standard home preparation procedures. Most of the ^{131}I is retained on the leafy structures (54 to 87 percent) of beans, tomatoes, cabbage, and celery. The amount of removal by ordinary rinse procedures varied with time, with maximal wash-off occurring immediately after contamination (78-97 percent. As the delay times increased, the removal of ^{131}I became smaller, until by 20 hours it was about half the initial removal rate. Further preparatory measures such as multiple rinsing and boiling were generally less effective. It is shown that even simple home preparation and cooking procedures can remove two-thirds to three-fourths of the ^{131}I deposited on fresh vegetables.

Thompson, J. C. Jr. and Sister Marmion Howe. (1973) Retention and removal of ^{131}I from Contaminated Vegetables. Health Physics, Vol. 24, pp. 345-351.

Comar, C. L. and J. C. Thompson, Jr. (1972) Radioactivity in Foods, Chapter in Modern Nutrition in Health and Disease. 5th Ed., Lea & Febiger.

Casarett, A. P. and J. C. Thompson, Jr. (1972) Biological Effects of Radioactive Fallout, Chapter in Progress in Human Biometerology, Part I. Swets and Zeithinger N.V., The Netherlands.

Thompson, J. C. Jr., R. A. Wentworth and C. L. Comar. (1971) The Control of Fallout Contamination in the Post-attack Diet in Survival of Food Crops and Livestock in the Event of Nuclear War, AEC Symposium Series 24, December 1971.

Lengemann, F. W. (1970) Consumption of Radionuclides from Milk by Man After Radionuclide Contamination of Pastures: A Predictive Model. Zentralblatt fur Veterinarmedizin Beiheft 11: 77-87.

Lengemann, F. W. (1970) Radiocesium Retention by Rats as Affected by L-thyroxine and Propylthiouracil. Health Physics 19: 551-556.

Lengemann, F. W. (1970) Interaction of Temperature, Age and Sex on the Retention of Radiocesium by Rats. Health Physics 19: 785-790.

Lengemann, F. W. (1970) ^{134}Cs Retention in Rats from 1 to 25 Months of Age. Radiation Research 42: 167-174.

Inaba, J. and F. W. Lengemann, (1972) Intestinal Uptake and Whole Body Retention of ^{141}Ce by Suckling Rats. Health Physics 22: 169-175.

Lengemann, F. W. and R. A. Wentworth (1972) A Predictive Model for Radiocesium Intake from Milk: Errors Associated with Among-Cow Variation. Health Physics, 22: 135-141.

Inaba, J. and F. W. Lengemann. Whole Body Retention of ^{42}K in the Rat. Health Physics - In press.

Sagan, C. E. and F. W. Lengemann. The Retention and Movement of Cerium-141 in the Gastrointestinal Tract of Adult Rats Irradiated with 800R and Fed Grain-based or Milk Diets. Radiation Research (In press).

Lengemann, F. W., R. A. Wentworth and C. L. Comar. Physiological and Biochemical Aspects of the Accumulation of Contaminant Radionuclides in Milk. In Lactation, edited by B. Larson and V. R. Smith, Academic Press (In press).

4. Graduate Training

C. E. Sagan, a graduate student under the Academic Year Institute, did a research project on cerium in a non-degree program. Jiro Inaba from the National Institute of Radiological Sciences, Japan served for a year as a postdoctoral fellow.

5. Status

Some of the problems that face the nuclear industry are new, others are relatable to previous experience with radioactive materials. Previous technological advances and planning were governed by such factors as efficiency, economy, profitability. However, the nuclear industry now finds that a major concern is public opinion about possible harm to the environment and all planning must take this into account. Some of the specific problem areas viewed in this way are indicated as follows:

A. Location: -- In contrast to fallout which is generally distributed, the impact of contamination from nuclear plants is greatly dependent upon the characteristics of the particular location. Each plant must be considered on its own terms ultimately, although some grouping can be made for development of generalized models e.g., using relatively small lakes, rivers, or coastal waters. At the present time, most decisions on siting have been based on economic factors, much more attention needs to be given to ecological and climatological factors. There is no question

that nuclear plants utilize to a considerable degree the natural resources where they are located. The effects on the environment should be minimized to the extent possible so that it is not subject to undue stress.

B. Critical exposures: -- Each proposed plant site should be investigated to determine the existence of any critical population or ecological group; for example: persons swimming in areas near effluents,

fishermen handling nets, or consumers of large amounts of local products. This will require knowledge of the nature of potential releases.

C. Releases: -- The Actual release of radioactivity, heat and chemicals is the starting point for assessment of environmental effects. Therefore, information as accurate as possible should be established about the levels to be released, the chemical form, the point of entry into the environment, and the possibility of changes in these factors with time.

D. Monitoring: -- Regulation and control depend upon monitoring procedures. To date, such procedures generally have been inadequate because of the following: lack of standardization, lack of thoroughness, few comparisons made among various sites, lack of supervision by independent agencies, need for indicator samples, need for integrating samples, lack of pre-operational data.

Although it is highly unlikely that population radiation exposures from nuclear reactor facilities will exceed the 1 mrem per person per year level, this does not preclude the necessity for an extensive radiological monitoring network. Continuous measurements of radioactive emissions and the subsequent accumulation patterns in the environment are an integral part of federal regulations to insure compliance with federal standards. The sampling system must be consistent with the local environment in terms of ecology and meteorology. Therefore, it must be designed to observe known phenomena and be capable of detecting unknown or unique changes that may occur over the operating life span of the facility. Such investigations are needed in order to: 1) establish accurate baselines of radioactivity during the pre-operational phase so that post-operational comparisons are statistically valid, 2) measure rates of build-up in the various sub-systems of the aquatic and terrestrial environments, 3) closely correlate radio-

activity release rates with accumulation patterns, 4) facilitate development of special area countermeasures for emergency conditions, should they develop.

E. Biological Effects: -- A most difficult problem is the estimation of the actual biological effects that may occur from the operations. This is primarily because of the relatively low levels involved, and secondarily because of the possibilities of interactions among organisms and among pollutants. As the populations exposed increase in size it is necessary to have more definite information about effects at lower and lower levels of exposure. Such data are not possible to obtain by direct experiment. Every advantage must be taken of the considerable experience available to-date in assessment of low levels of radiation.

F. Guidelines: -- It is obviously necessary to have established limits in order to regulate the contamination of the environment and prevent harm. The guidelines must be realistic; the acceptable levels must be low enough to protect the public and yet high enough so that the operations can be carried on and the public enabled to benefit from them. There is usually the tendency to establish levels on the basis of technical feasibility rather than actual potential of harm, especially when the latter is so difficult to estimate. The problem is further aggravated by the emotional pressure to set acceptable levels lower and lower so as to be on the safe side. Finally, if each state exercises jurisdiction, then a chaotic situation may develop.

G. Risk and Benefit: -- Ultimately the final decisions will have to rest upon considerations of the risk to society as balanced against the benefit. This comparison may be very hard to determine because of the many unknowns including the need to make comparisons with health risks

from fossil-fuel sources of electrical power. Fuzzy as this area seems to researchers used to "hard" science it appears that considerable investigative and analytical efforts must be made along these lines.

H. Public Acceptance: -- As previously mentioned, the public must be educated to accept the concept of a reasonable biological cost for the benefit that is desired. We feel that a planned and budgeted effort must be made in the area of public education. It is just not enough for the scientist, competent though he may be, to take a day or two off to give lectures here and there, and appear at public meetings or before Congressional hearings.

I. Emergency Procedures and Counter-measures: -- Firmer estimates are needed of probabilities of accidents and the effects they may have. Procedures should be established for each site taking into account the agriculture of the surroundings so that the effects can be minimized.

6. Status Assessment

The literature contains a reasonable body of data concerning transfer of some radionuclides into milk. Without it, the development of our prediction equation would have been impossible. A severe gap exists in the literature on the relation of the secretion of radiocontaminants into milk with environmental stress. From the location of the investigators reporting it is safe to say that most animals tested were under reasonably similar environmental conditions common to temperate zones. It is necessary to develop a body of data that will relate secretion of these fission products to conditions as extreme as the tropics and the subarctic regions. These data are important since they are the basis for any prediction procedure.

The data on radiocerium metabolism has the central theme that this radionuclide is essentially unabsorbable and that the chief radiation hazard is to the intestinal tract as the material transits after ingestion. Recent work from Japan and the United States has shown that the suckling rat or mouse exhibits a marked hold up of radiocerium. This does result in a markedly greater true absorption but this is still small. Of greater importance is the larger than predicted radiation dose that results. The literature as a whole contains little about how this occurs or how extensive this is likely to be among other poorly absorbed nuclides. The health significance is obvious because it suggests that the suckling is at much greater risk than is the adult.

The literature for radiocesium is rather abundant and because of the gamma characteristics of cesium-137, the prevalence of ^{137}Cs in the environment and the availabilities of whole body counters there is a significant volume of literature relating to man himself. A general characteristic is that the data are not usable to show why something is observed. Most often the human data is searched and analyzed for a particular phenomenon only after laboratory experiments report that a particular stress has an effect on radiocesium metabolism. The prediction of cesium body burdens by particular groups of people necessarily must allow for large variations. These variations are possibly the result of poorly understood influences. The prediction can be made more precise after the important variables are recognized and quantitated. The health significance of radiocesium is finally being properly assessed.

V. Secretion of Milk Constituents. F. W. Lengemann

1. Introduction

In the study of human ecology it has often been observed that milk is important not only for providing essential nutrients but also as a means of transferring to man environmental contaminants deposited on grain or leafy vegetation not ordinarily consumed by man. In the past much attention has been given to the transfer of fission products. Lately, however, attention has shifted to milk as a vehicle, also, for the transfer of pesticides, drugs, corrosion products and other environmental pollutants.

2. Objectives

The study of the transfer of materials into milk has been a special aim of our laboratory. One prime objective has been to quantitate the amounts of various materials (i.e., fission products, nutrients, pollutants) secreted into the milk of several species, to establish kinetic parameters and to learn how to manipulate the system. The data thus developed can be utilized to estimate the mode of transfer, reduce the amount being transferred in the milk, and to develop equations that can predict the amounts of a material that would be expected to appear in milk under given conditions of intake by the lactating animal. A second objective is to develop and define kinetic models which can describe the movement of substances among the various cellular compartments of the mammary gland. This will lead to an understanding of the metabolic processes of the gland.

3. Main Research Accomplishments

In the past three year period we have progressed towards the fulfillment of these objectives by describing the transfer of iodine to the milk

of goats and studying the factors that alter the transfer of iodine into the milk. It is worth noting that this information represents the largest body of such data available and has been used recently by the Radiological Assessment Branch, Directorate of Licensing U.S.A.E.C. to predict intake of radioiodine by infants consuming goats milk. In addition, we have reported on the effectiveness of various levels of perchlorate in reducing the transfer of iodine to the milk of cows. These data allowed an estimate of the kinds of transfer mechanisms involved in iodine accumulation in milk as well as a comparison of the iodine transfer capability of goats and cows.

The transfer of inorganic mercury to the milk of goats has been carried out and is a useful first project in the area of non-radioactive environmental pollutants.

To meet the second objective we have developed techniques for estimating contained-milk volume of the mammary gland using ^{141}Ce and applied it to estimating the milk secretion rates of guinea pigs and goats. The technique is more effective with goats than guinea pigs as judged by the completeness of dispersal of the marker. Using an open 3-compartment model, estimates of the transfer rates of calcium between blood, milk and secretory tissue have been made for the guinea pig. We are now in the process of attempting to develop an external counting procedure that will permit estimation of the transfer constants using large animals in non-sacrifice type studies.

Metabolism of Radioiodide by Lactating Goats Given Iodine-131 for Extended Periods. F. W. Lengemann. J. Dairy Sci. 53: 165-170 (1970).

Effect of Perchlorate on the Milk Plasma Radioiodine Ratio of Intact and Thyroidectomized Goats. D. J. Djurdjevic and F. W. Lengemann. J. Dairy Sci. 53: 808-812 (1970).

Reduction of Iodine Transfer to Milk of Cows after Perchlorate Ingestion. F. W. Lengemann. J. Dairy Sci. Accepted for publication (1973).

Transfer of Inorganic Mercury to Milk of Goats. S. M. Howe, J. McGee and F. W. Lengemann. Nature 227: 516-518 (1972).

A Practical System for Resolution of Two β -Emitting Isotopes by Liquid Scintillation Counting. M. B. Snipes, and F. W. Lengemann. Int. J. Appld. Rad. Isotopes 22: 513-520 (1971).

Guinea Pig Mammary Gland Milk Content by Isotope Dilution Methods Using ^{141}Ce and ^{14}C Polyethylene Glycol-4000. M. B. Snipes and F. W. Lengemann. J. Dairy Sci. 55: 1775-1782 (1972).

Tissue Composition of Lactating Guinea Pig Mammary Glands. M. B. Snipes and F. W. Lengemann. J. Dairy Sci. 55: 1783-1786 (1972).

Measurement of the Contained Milk Volume of the Mammary Gland Using Radioactive Markers. F. W. Lengemann and M. B. Snipes in Mineral Studies With Isotopes in Domestic Animals. IAEA-PL-312-2/8 p. 111-119 International Atomic Energy Agency, Vienna, 1971.

Determination of the Contained Milk Volume of the Mammary Gland of the Goat by ^{141}Ce and ^{14}C -PEG-4000. M. B. Snipes and F. W. Lengemann. J. Dairy Sci. 53: 1596-1602 (1970).

Magnitude of the Error Associated with Estimation of Contained Milk Volume in the Dairy Goat Using Cerium- ^{141}Ce . C. E. Sagan, M. B. Snipes, and F. W. Lengemann. J. Dairy Sci. Submitted for publication.

4. Graduate Training

M. B. Snipes completed a Ph.D. program in this area and C. E. Sagan under a non-degree Academic Year Institute program performed some research.

5. Status

Although it is recognized that many different kinds of compounds and elements appear in milk there is very little information on their quantitative transfer. Most studies in the literature deal with simple recovery and reveal little of the mechanisms involved. The human studies are usually short term and performed on women who are at the end of a lactation; The large animal experiments are usually 7 days in length and describe only the amount in the milk. Rat and mice studies as currently

performed can not yield data on the amount of milk in the gland and often utilize recovery of milk from the stomachs of the suckling young as the sample.

This is a significant field of research that has been neglected. Milk forms a very important part of the diet of the children and adults of most western societies. More important, milk forms the sole intake of nutrients for all infants in all societies. Since hormones, drugs, pesticides, toxic chemicals and fission products have been found in milk, it is obvious that milk is an important link in the chain of transfer and possibly concentration of these materials. In many ways, therefore, milk has public health significance. It is important to learn what principles are involved in the transfer of materials into milk so that in the future the amount of a material which may be secreted into milk can be predicted accurately by relating its characteristics to that of similar compounds or elements.

The work on developing an external counting technique for estimating the transfer constants across secretory epithelium will continue. For this purpose we will use lactating goats and ^{47}Ca to study calcium transfer into the milk of this species and particularly to attempt a better quantitation of the transfer from secretory tissue to blood. In studies with the guinea pig and goat the study of iodine transfer will continue with an intent to obtain transfer rates, estimation of the transfer mechanisms, the effect of protein binding and interaction with protein on the transfer, and to estimate the proportionate contribution of the various possible modes of transfer to the total transfer of the element. Future work could also include locating an ion pump, if any, measuring the potential across the various tissues at various levels of the mammary gland, and the source of the

potential. Of interest also is the study of the modification that milk might undergo as it moves from the alveoli to the cisterna of the gland.

VI. Veterinary Nuclear Medicine. F. A. Kallfelz and R. A. Wentworth

1. Introduction

There has been little development of the use of nuclear techniques in the diagnosis and therapy of diseases of domestic animals. This is mainly because usually the simultaneous opportunity (both in terms of facilities and scientific training) of having available animal and physical science capabilities does not exist. The fact that this Department of Physical Biology and its Laboratory of Radiation Biology are physically and administratively located in the Veterinary College provides a unique situation, especially because there is enthusiastic collaboration with the clinical departments. It is especially important that applications be made on a herd or flock basis to improve production efficiency.

2. Objectives

The stated objectives of the veterinary nuclear medicine effort has been to stimulate the increased use of radiotracer techniques primarily in the diagnosis, but also in the therapy of diseases of domestic animals. That is, to develop techniques of value to the practicing veterinarian, to make him aware of the value and availability of nuclear medical techniques, and to stimulate him to avail himself of these techniques when they are indicated.

3. Main Research Accomplishments

Although much remains to be done, it is felt that a good start has been made in this direction. For example, the T_4 test, or determination of total serum thyroxine by competitive protein binding of labeled thyroxine is now routinely used as a clinical test for the evaluation of thyroid gland function in the dog, cat, horse, and cow. In addition

to the use of this test by the attending veterinarians at the N.Y.S. Veterinary College, we have been also servicing practitioners in New York State and elsewhere, and assisting other laboratories throughout the country in establishing normal values for this test in their own laboratories.

Recently, we have been investigating the value of determining free thyroxine levels as a tool in diagnosing thyroid gland function. In addition to this, various radioimmunoassay systems for insulin, digitoxin, and other hormones and drugs are being developed for use in the veterinary field.

In vivo applications have also been employed over the past three years in clinical veterinary medicine. These include blood volume and ferrokinetic studies in dogs, cats, horses, and cattle; labeled fat absorption studies in dogs and horses; evaluation of gastrointestinal protein loss in dogs, horses, and cattle; evaluation of bone calcium metabolism in dogs, cattle, and horses; and evaluation of renal function in cattle and horses. The above stated techniques have been evaluated both with research animals and with clinical cases as presented to the clinics of the New York State Veterinary College.

A system for making in vivo measurements of bone mineral content of farm animals based upon gamma photon transmission measurements as first described by Sorenson and Cameron in 1963, has been constructed and used for several years in this laboratory. During the past three years a modest effort has been devoted to the continued development of the technique. Objectives have included obtaining a more informative output, improving reliability and repeatability, and in making the system easier to use.

Accomplishments of this period have included: the design and construction of restraint devices for goats and dogs which allow for repeatable positioning; interfacing with a new computer for on-line data acquisition, calculations, and live display; refinements in calculations. The latter include computations of bone mineral mass with confidence limits, bone width, cortical bone thickness and an index of approximate bone mineral density. In addition, investigations into multi-directional scanning as a means of obtaining direct bone mineral density values have indicated this method to be feasible but will require considerable more development effort.

Some Normal Values of Thyroid Function in Horses. F. A. Kallfelz and J. E. Lowe. JAVMA 156: 1888-1891, 1970.

Reversibility of Nutritional Osteoporosis: Physicochemical Data on Bones from an Experimental Study in Dogs. Lennart Krook, Leo Lutwak, Per-Ake Henrikson, Francis Kallfelz, Carl Hirsch, Bertil Romanus, Leonard F. Belanger, John R. Marier and Ben E. Sheffy. The Journal of Nutrition. Vol. 101, 233-246, 1971.

Thyroidectomy and the T-4 Test to Assess Thyroid Dysfunction in the Horse and Pony. J. E. Lowe and F. A. Kallfelz. Equine Practitioners, Nov. Dec. 1970 (1971).

In Vivo Estimation of Bone Mineral Content: A Research and Diagnostic Technique for Veterinary Medicine. F. A. Wentworth, F. A. Kallfelz, F. L. Hiltz, H. Schryver, B. Sheffy, and L. P. Krook. Am. J. Vet. Res. 32: June, 1971.

Correlation Between ^{47}Ca Absorption and Intestinal Calcium-Binding Activity in the Golden Hamster. F. A. Kallfelz and R. H. Wasserman. Proc. Soc. Exptl. Biol. & Med. 139: 77-79, 1972.

Some Recent Technical Advances for the Study of Bone Disease. F. A. Kallfelz. Meth. Achievm. exp. Path., Vol. 6, pp. 139-171. E. Bajusz and G. Jasmin ed. 1972. Montreal (Kroger Basel, 1972).

Bone Calcium Accretion and Mineral Content as a Function of Dietary Ca and P in the Dog. F. A. Kallfelz and L. P. Krook. Fed. Proc. 29: No. 2, March-April, 1970 (Abstract).

Observations on Thyroid Gland Function in Dogs: Response to Thyrotropin and Thyroidectomy and Determination of Thyroxine Secretion Rate. F. A. Kallfelz. Am. J. Vet. Res. 34: April, 1973.

Thyroid Function Studies in Domestic Animals Free Thyroxine Index (FTI₁, T-7). F. A. Kallfelz and R. P. Erali. Am. J. Vet. Res. In Press.

Survival of ⁵⁹Fe Labeled Erythrocytes in Cross Transfused Bovine Blood. F. A. Kallfelz and R. A. Whitlock. Am. J. Vet. Res. In Press.

Veterinary Nuclear Medicine. F. A. Kallfelz and C. L. Comar. Advances in Veterinary Science and Comparative Medicine, Vol. 18, In press.

Reproductive Function in Thyroidectomized Mares and Stallions. J. E. Lowe, R. H. Foote, B. H. Baldwin, R. B. Hillman, and F. A. Kallfelz 109th Annual Meeting AVMA, New Orleans, 1972.

Ferrokinetic Studies in Horses. R. H. Whitlock and F. A. Kallfelz 109th Annual Meeting A.V.M.A., New Orleans, 1972.

4. Graduate Training

During the past three years, Dr. Jesus D. Alcantara from the Department of Animal Industry of the Republic of the Phillipines and Dr. Vladimar Mitin of the Department of Physiology, University of Zagreb, Zagreb, Yugoslavia have completed sabbatic leaves in the Nuclear Medicine unit.

5. Status

It is felt that a continuation and expansion of the use of nuclear techniques in veterinary medicine is essential. The field of veterinary nuclear medicine is now being recognized by some as a valid clinical specialty area, but the number of techniques routinely available and used is still small, and much work must be done to increase the size of the armamentarium of the veterinary nuclear medicine practitioner, particularly in the area of competitive protein binding techniques, including radioimmunoassay, and in the development and use of imaging procedures. In this regard, while assay systems for corticosteroids,

estrogens, progesterones, pituitary sex hormones, have been described, they have certainly not been developed to the level of being routinely available for use to the veterinary clinician.

Clinical nutrition is also an area that is much in need of development in the veterinary field. It is felt that many of the above mentioned techniques could be used in biochemical profiling procedures to assess the nutritional and health status of animals on a herd or flock wide basis.

This gamma photon technique is the most accurate in vivo method of measuring bone mineral "content" to date. However, to attain the full potential of such a technique, especially for making comparisons between animals or in growing animals, methods must be developed to measure bone mineral "density". It would appear that the next step would be to use the theoretical considerations worked out to design and test design concepts for a multi-directional or perhaps a revolving scanner. It is envisioned that, while this is not a large effort item under this contract, progress will continue to be made.

PHASE III

NEURAL AND BEHAVIORAL RESPONSE TO RADIATION

E. L. Gasteiger and D. N. Tapper

During the period covered by this report the work was confined to three aspects with the intention that during the last year only those activities necessary for orderly termination and maximum exploitation of previous findings would be undertaken.

I. BEHAVIORAL ASSAY OF RADIOPROTECTIVE AGENTS

1. Introduction

Previous work in this laboratory had developed measurements of the behavioral response of rats to relatively low levels of ionizing radiation that were reasonably reproducible. It was thought that these techniques, especially that of radiation conditioned gustatory aversion, could be useful as a measure of the ability of agents to protect against radiation.

The effectiveness of radioprotective agents had been measured only in terms of their ability to reduce the number of deaths resulting from radiation exposure. Since irradiation of living systems produces a broad spectrum of effects from lethality, through graded anatomical, physiological and biochemical changes, to subtle modifications of behavior, use of the lethality measure ignores more graded and possibly more sensitive measures. Behavior of mammals can be evaluated both

quantitatively and qualitatively by operant conditioning techniques. Furthermore, behavior can be modified by radiation exposures as little as 10 to 50 R.

2. Objectives

It was proposed that currently used behavioral responses to ionizing irradiation be used to assay the effectiveness of radioprotective agents. Also, as an accessory benefit, it was hoped such experiments would give insight to the mechanisms by which ionizing radiations affect behavior.

3. Main Research Accomplishments

The radioprotective agent WR 2721 (S-2- (3-aminopropylamino) ethylphosphorothioate) has been tested for its ability to reduce x-ray conditioned aversion behavior. Conditioned aversion was developed by exposing water-deprived rats to 48 R of X-ray immediately after they were allowed to drink 5.0 mM Na saccharin solution from tubes. The resulting aversion of saccharin was measured 24 hours later by recording their licking for water and saccharin, presented alternately. Under these conditions the saccharin consumption was reduced more than 60%. After a series of tests to determine the effect of intraperitoneal administration of WR-2721 on licking behavior and on survival to 1000 R whole body x-irradiation, the following regimen was established. Rats were injected with WR-2721 (100 mg/kg body weight). One hour later, they were permitted to drink saccharin solution and then exposed to 48 R x-irradiation. Control animals were injected with saline. Three separate experiments (132 rats total) gave reductions of saccharin consumption of 70, 60, and 50% for controls and only 35, 20, and 10% for WR 2721 treated animals.

For each experiment parallel lethality studies were conducted.

In these experiments the assay time was reduced to 6 days, a period less than half that used with the lethality measure. Furthermore, the behavioral measure was five to ten times more sensitive than the lethality method. Therefore, there is no question but that the behavioral technique can be useful for the assay of radioprotective compounds.

Gasteiger, E. L., Behavioral Assay of a Radioprotective Agent. Rad. Res. 47, July (1971).

4. Status

Due to withdrawal of governmental support for development of radioprotective compounds the above results have appeared too late for possible use in mass screening programs. The promise that protective agents will be used in conjunction with x-ray therapy of cancer patients may make further exploration of behavioral assays a worthwhile endeavor. Only one behavioral measure was used here. Others, such as immediate avoidance of an irradiated area, may be even more sensitive and less time consuming. Some x-ray induced behavioral measures may never respond to protective agents thus providing a clue to the site or mechanism of action of the radiation for a specific behavioral effect.

One disadvantage of the behavioral measure is that the sensitivity of the measure in assaying the effectiveness of a radioprotectant may be reduced by modification of the behavior by direct toxic action of the agent. In view of the very limited number of observations in

this area a fair assessment of its promise cannot be made. Certainly, trial of other behavioral measures for assaying protective agents is greatly warranted.

II. FACTORS AFFECTING ASSAY OF PERFORMANCE DECREMENT (ETI)

FOLLOWING MASSIVE RADIATION DOSES

1. Introduction

Early transient incapacitation (ETI) is characteristic of monkeys, miniature pigs, and rats following supralethal doses of ionizing radiation. After recovery these animals go through a phase of partial or complete incapacitation prior to death. The effect of split doses of pulsed γ -n reactor radiations on rats had been studied in our laboratory by Dr. A. Casarett. The small size and low cost of the rat makes it a useful animal for evaluating operant conditioning and physiological factors related to the development of early transient incapacitation (ETI).

2. Objectives

Since the basis of the above-described striking phenomenon is unknown and the physical means were available at Cornell, studies were undertaken to observe the modification of behavior in pulsed animals in the normal non-conditioned state, the effects of operant conditioning parameters on ETI, and the physiological changes which occur following pulsed irradiation of rats. This necessitated establishment of a favorable operant conditioning measure and determination of dose-response curves for that measure.

3. Main Research Accomplishments

Control and pulsed rats were compared by recording the presence or absence of components of motor activity every 20 sec for a total 30 min

period following pulse. The components of behavior noted were moving head, sniffing, moving body, reaching, rearing, grooming and respiration. Sham irradiated animals showed two to three times more activity than the pulsed animals. Both groups continued to be active throughout the 30 minute period although some reduction in motor activity occurred in the second half period and the controls had an earlier quieting after the fifth minute. Exception to the preceding was the near absence of rearing and grooming in the pulsed animals with an indication that this activity became more frequent in the second half period.

From such subjective observations no evidence was obtained to suggest the presence of early transient incapacitation except perhaps the late appearance of the more complicated behavior of rearing and grooming. ETI may very well be confined to more complex behavioral patterns such as these two and those developed in operant conditioning paradigms.

For a relatively precise measure of ETI the jump box paradigm of Casarett was modified so that rats would be shocked once per 3 sec if they remained on the floor of the operant conditioning box; they could rest 6 sec on a shelf which they could reach above the floor before being dropped on to the floor. This permitted measures from 0 to 20 shocks per minute for quantifying the ability of the animals to perform the jump box task. In this way 41 rats were used to obtain dose-response curves for 6 levels of irradiation.

Threshold for ETI in rats under the above conditions was less than 2,570 rads, with only one third decrement in behavior for 2,570 rads and complete recovery in 8 min. Doses of 5,833 rads and above caused more than 80% decrement within 3 min of pulse and gave the following

mean times for recovery to 50% performance levels: 5,833-7; 6,820-9; 8,433-11; 11,040-15; and 13,600 rads -- no recovery in 30 min. The curves from which these values were obtained tended to be sigmoidal with a hint that ETI develops swiftly and reaches a maximum by the third minute after the pulse.

The influence of motor activity and the strength of electrical shock on recovery from incapacitation was tested. Motor activity was not necessary for recovery to develop. Irradiated animals could be left unmolested on the shocking grid for 15 min during which time they never climbed onto the shelf. However, upon receiving shocks at 15 min they immediately executed the escape behavior as though they were fully recovered. Another interesting factor was that many animals, after γ -n pulse, would remain on the grid taking the 1 ma shock with which they were trained despite passage of normal recovery time. Upon increase of the shock to 2.5 ma these animals immediately showed full recovery.

From the observations on non-conditioned animals and from the example of dependence of the behavior of pulsed animals on shock strength, one can conclude that characterization of ETI at this stage of our knowledge is more dependent on the operant conditioning paradigm and the parameters used than on the irradiation modified state of the animal.

Several pilot studies were carried out on changes in physiological parameters such as heart rate, blood pressure and breathing which might be altered by massive doses of radiation. Should blood pressure be markedly reduced, these parameters would change and a transient depression of behavior might be expected due to O_2 lack. For the first 6 to 8 minutes after pulse all irradiated animals displayed rapid respiration and then suddenly shifted to their normal rates. Measurement of heart rate and blood pressure was difficult but in a limited number of

successful experiments no marked changes occurred. A direct test of the hypoxia theory was subsequently made by comparing recovery time of animals working in 100% O₂ with those in air. No significant differences were found. An easy explanation of ETI is not at hand.

Casarett, A. and C. L. Comar. Incapacitation and Performance Decrement in Rats Following Split Doses of Fission Spectrum Radiation. Rad. Res. 53: (1973).

Casarett, A., Swim Tank Measurement of Radiation-Induced Behavioral Incapacitation (submitted for publication) (1973).

4. Status

For the rat, availability of dose-response curves for a sensitive and easily mastered operant conditioning paradigm provides a base for study of behavioral and physiological factors important to an understanding of ETI. It is abundantly clear that characterization of ETI depends on the behavioral paradigm used. Performance of discrimination behavior was disrupted more readily than escape behavior. Escape behavior in a jump box was more readily disrupted than swim tank escape.

Observation of animals in these various paradigms reveals that motor behavior is not disrupted and that there is no apparent muscular weakness during ETI. On the sensory side rats, although incapacitated, still squeak and attempt to escape from the electric shock; there is no gross shift in sensory threshold. It is as though they suffer from disorganization of cognition, disorientation or disequilibrium.

No definitive studies are available on the physiology of ETI. Although hypotension has been favored as an explanation of the reduced performance, our studies do not support this view. Furthermore, the O_2 therapy experiment suggests that simple hypoxia due either to hypotension or brain swelling would not explain ETI. It is indicated that numerous physiological parameters should be examined in a search for clues to the cause of performance decrement; the rat is a favorable animal for such studies.

III. TASTE STIMULI: QUALITY CODING TIME

1. Introduction

Measurement of the x-ray induced saccharin aversion phenomenon was refined in our laboratory and applied in studies of taste categorization. Taste stimuli classified together by this means were DL-alanine, glycine and sodium saccharin as contrasted to D-glucose or potassium chloride. In the course of these studies the surprising speed at which rats responded to the difference between the various taste stimuli was noted and estimated as less than 600 msec. Rejection of taste stimuli was based on quality identification, neurally encoded within this brief period.

2. Objectives

The characteristics and possible mechanisms of the above recognition phenomenon have been sought through use of the x-ray conditioned aversion response and through comparison to knowledge of other sensory systems.

3. Main Research Accomplishments

Thirsty rats learned to lick at watering tubes and their licking rate and pattern were recorded for analysis. When the rate and pattern were well established the rats were allowed to drink 300 mM NaCl solution and then were immediately exposed to 200 R whole body x-irradiation. The next day water and 300 mM NaCl were presented alternately through the drinking tubes. First, water was presented and readily lapped. Then 300 mM NaCl was presented in place of water and within four licks some animals rejected the NaCl solution. This represented a total time of 260 msec for the animals to receive the sensory information centrally, perceive its character and arrest the motion of the tongue.

Electrophysiological studies of mammalian gustatory function have attempted to equate "what" and "how much", with patterns of discharge from the tongue to the chorda tympani. The response to a liquid placed on the tongue characteristically contains three time periods: a latency of 25-60 msec, a phasic discharge lasting 200 to 400 msec, and a prolonged tonic discharge lasting several seconds. Nearly all studies have either ignored the phasic period or considered it as part of the tonic period which has been thought to be most important for recognition of chemical stimuli. Such a view is no longer tenable since our studies reveal that both recognition and response can occur well within the phasic period. Similarly, speedy responses occurred in animals conditioned by x-irradiation against DL-alanine. Even earlier recognition was observed when the concentration of the averting stimulus was increased.

Tappper, D. N. and R. Van Scoy. Development and Extinction of Radiation-Induced Saccharin-Avoidance Behavior. Rad. Res. 31: July (1967).

Tappper, D. N. and B. P. Halpern. Taste Stimuli: A Behavioral Categorization. Sci. 161: 708-709, (1968).

4. Status

The use of licking behavior in rats and controlling it by x-ray conditioned aversion has by no means been exhausted. The speed of response should be further studied both in terms of behavior and short latency electrophysiological discharges which can code the characteristics of the stimulus. Neurophysiologically, the licking response is a motor oscillator subjected to delicate control. Its locus and organization should be sought.

PHASE IV
BIOLOGICAL EFFECTS OF IMPLANTED NUCLEAR ENERGY
SOURCES FOR ARTIFICIAL HEART DEVICES

F. A. Kallfelz, P. H. Craig, A. P. Casarett, C. L. Comar

1. Introduction

The three year review of this phase was undertaken in a previous year; however, a brief statement is included here for the sake of completeness.

2. Objectives

The summary objectives of this program have been the development of a suitable animal model and the study, under proper conditions, of the biological effects of radiation from a simulated 30-50 watt ^{238}Pu power source on the mammalian organism.

3. Main Research Accomplishments

The observations are made on Labrador Retriever dogs implanted in the left flank with a 333 ml stainless steel sphere containing a radiation source consisting of ^{252}Cf to provide the neutron flux and ^{90}Sr - ^{90}Y Bremsstrahlung sources to provide the photon flux from a ^{238}Pu power source.

A total of 60 animals have been implanted with radiation sources ranging from 1 X (nominal sources delivering the same radiation flux is a 30 watt ^{238}Pu source) to 70 X. These have been implanted in 5 groups of 12 dogs each. In group I, 4 dogs received blank capsules, four dogs received 15 X sources and four dogs received 70 X sources. In each of the other

four groups, 4 dogs received blank capsules, four dogs received 1 X sources, and four dogs received 5 X sources.

Continuous clinical evaluation of these animals has been carried out beginning with the acquisition of base line data before implantation and continuing throughout the period of implantation, which has ranged from 3 months to 3½ years to date.

Of the dogs in group I, nine have been killed at various times from 3 months to 3½ years after implantation due to deterioration of the preparation, and complete gross and microscopic post-mortem examinations have been obtained. Group II dogs have also been killed at the end of 2 years of implantation and complete post-mortem examinations are available on these animals as well. Group III animals constitute a 10 year study group, Group IV, 7½ years, and Group V, a five year group.

The results from this study to date indicate that the mammalian organism may be able to tolerate the radiation dose expected from a ^{238}Pu power source large enough to supply operating energy for a completely artificial heart.

Kallfelz, F. A., C. L. Comar, A. P. Casarett, P. H. Craig, Radiological Effects of Simulated Nuclear Power Sources for Artificial Hearts: A Preliminary Report., ANS Transactions 13, No. 2, 499-500, 1970.

Kallfelz, F. A., P. H. Craig, A. P. Casarett, and C. L. Comar. Biological Effects of Radiation from Simulated ^{238}Pu Power Sources in Dogs. Research Animals in Medicine (In Press).

4. Status

Although it appears that mammalian organisms may well be able to tolerate the radiation dose to be expected from a 30 - 50 watt ^{238}Pu power source for the time periods used, it is necessary that these studies be

carried out for much longer periods of time before a definitive answer can be given. It is evident that this research has great significance in the field of medicine since the development of major cardiac assist devices requires the availability of a usable power source. In the future, combined studies of heat and radiation sources located in the animal body in a site similar to the intended implantation site in the human should definitely be carried out.

Present plans are to continue this project for a total period of ten years since present data indicates that the most probable effects to be seen will be a shortening of the life span of these animals or, possibly, an increase rate of tumor induction in later life. In addition, it is felt that a study should be made of the combined effects of heat and radiation using an actual ^{238}Pu source implanted, using a suitable heat exchanging device. A preliminary proposal for this study was submitted in January 1972, but has not been funded as yet.

Graduate Training

- 1970 M.S. Drake, Rosemarie Sheila. Effects of oxytocin and an antagonistic analogue on the electrophysiology of the myometrium.
- Ph.D. Morrissey, Robert L. Regulation of intestinal calcium absorption.
- 1971 Ph.D. Bredderman, Paul John. Chemical composition and some physical properties of the calcium binding protein.
- Ph.D. Faull, John Richard. An electrophysiological comparison of two theories of gustation.
- M.S. Galera-Garcia, Ceasar. Responses of tactile pad receptor to sinusoidal mechanical stimulation.
- Ph.D. Kirkpatrick, Jay Franklin. Radiation response of the pre-implantation mouse embryo in vitro.
- Ph.D. Mann, Michael David. A study of the cutaneous subdivision of the dorsal spinocerebellar tract.
- Ph.D. Snipes, Morris Burton. A kinetic model for calcium transport in the guinea pig mammary gland.
- Ph.D. Talisayon, Serafin Demandante. A quantitative study of strontium metabolism in bone.
- 1972 Ph.D. Bubar, Richard Harris. Part I - Computer regulation of blood levels of a radionuclide. Part II - Regulating response of the goat to an intravenous impulse of stable calcium.
- Ph.D. Parker, Edward Joseph. The distribution dose and effects of 90-Sr-90-Y in the male mouse following intraperitoneal injection of equilibrium mixtures.
- M.N.S. Fullmer, Curtis Sheridan. Bovine calcium binding protein: isolation, purification and partial characterization.
- 1973 Ph.D. Morgan, Robert Andrew. The effect of tritium oxide on preimplantation mouse embryos.