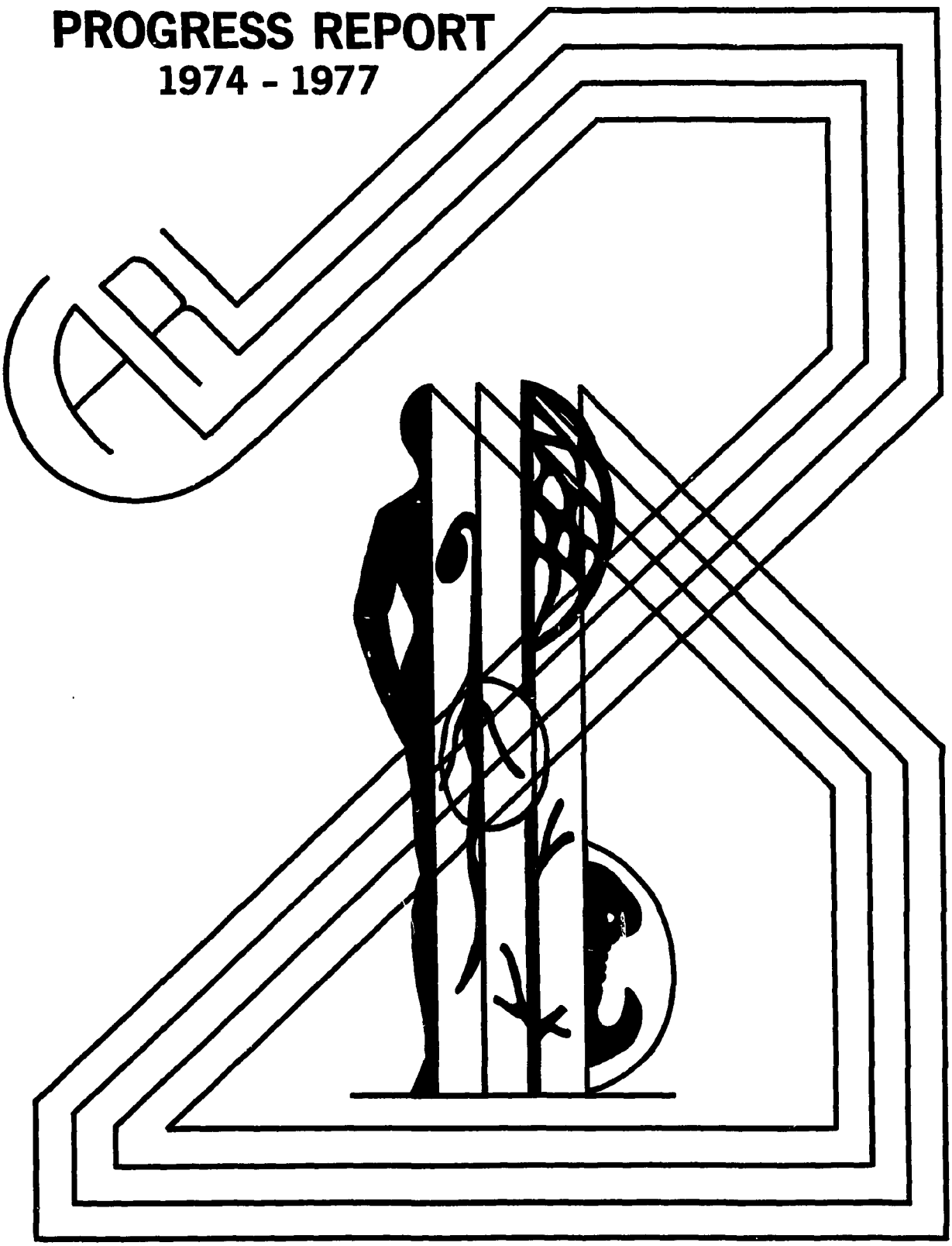
ORO-754

COMPARATIVE ANIMAL RESEARCH LABORATORY

# PROGRESS REPORT
## 1974 - 1977



COMPARATIVE ANIMAL RESEARCH LABORATORY/OPERATED BY THE UNIVERSITY OF TENNESSEE
FOR THE ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION
1299 Bethel Valley Rd., Oak Ridge, TN 37830

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June 1977

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# Introduction

In 1948 the U.S. Atomic Energy Commission and the University of Tennessee established a cooperative research program to study the effects of fallout radiation on a herd of Hereford cattle accidentally contaminated during the testing of the first atomic bomb at Alamogordo, New Mexico. This cooperative program led to the establishment of the UT-AEC Agricultural Research Laboratory; the research program was quickly expanded to include radioisotope studies and radiation effects on agricultural products. From its inception the Laboratory has contributed significantly to an understanding of the transport of radionuclides into man's food chain and the biological effects of external radiation on animals and plants.

At the request of the AEC, the Laboratory has assumed a new direction since 1973 which is reflected in a new name—the Comparative Animal Research Laboratory. The principal concerns of the current research program are the estimation of risk to man from effluents of energy production and the means of extrapolating from experimental data to man. We believe that only through interspecies comparisons can a more rational and accurate prediction be achieved. Our experiments still serve many of the Laboratory's original goals: to determine the effects of toxic agents on the production of plant and animal foodstuffs and to clarify the metabolism of radionuclides and other chemicals that might appear in man's food.

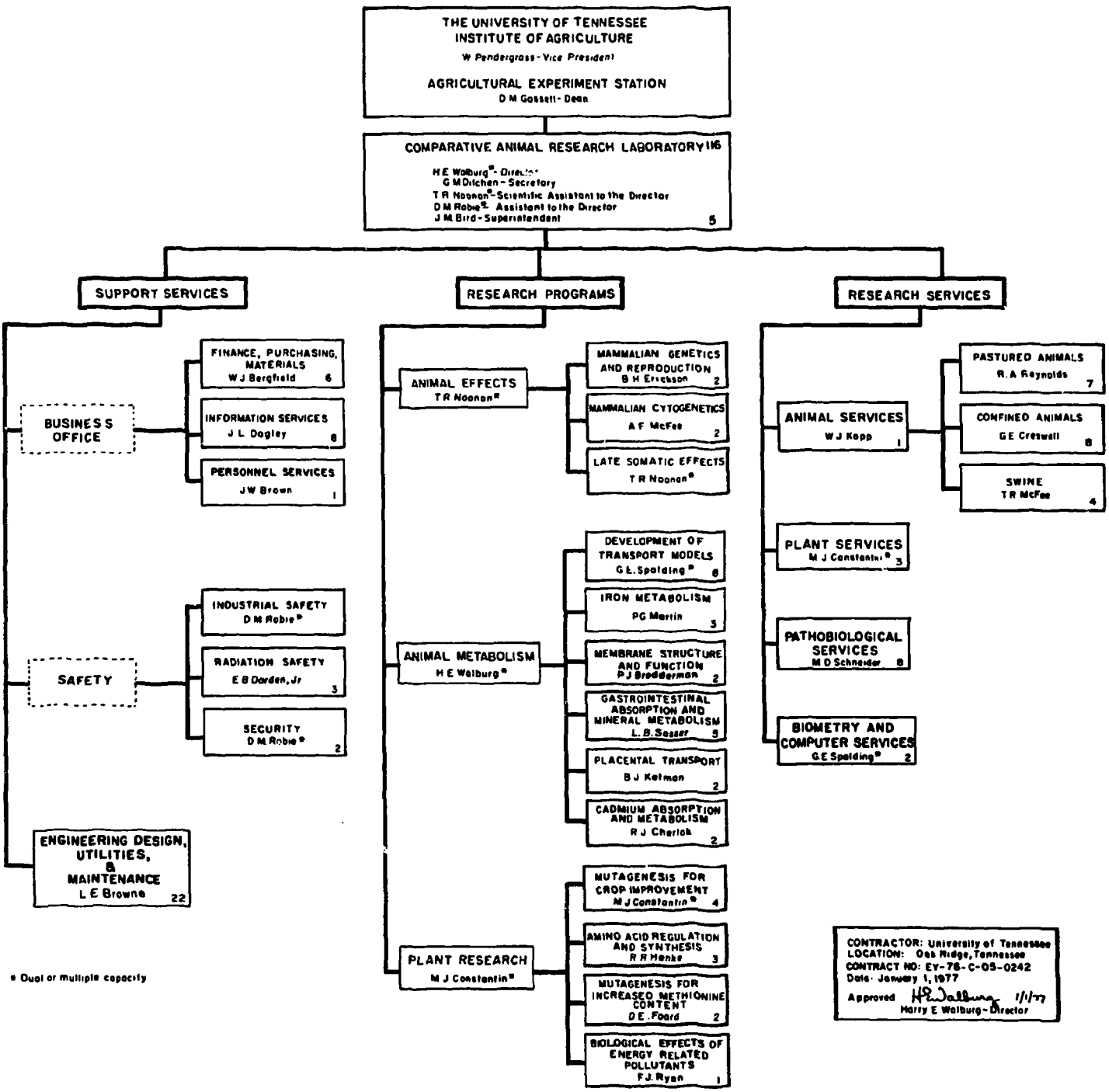
In the transition to a new program direction the uniqueness of the Laboratory has been maintained—the capacity to maintain and study reproducing herds of large domestic animals. A physical plant and equipment representing an investment of over $4 million provide unusual facilities for large animal surgery, necropsy, and metabolism and a unique variable-dose-rate irradiation facility as well as other more common laboratory, plant, and animal research facilities.

It has been exciting to lead a laboratory staff filled with new purpose and enthusiasm through the torturous steps of program redirection: the planning, administrative changes, and selection of appropriately trained staff; facility and equipment additions, land use planning, and initiation of research; the scientific and administrative reviews; and the first excitement of critical questions answered often with unexpected results. Progress has often been frustratingly slow, because the plans were exceedingly large and the staff exceedingly small (85-110).

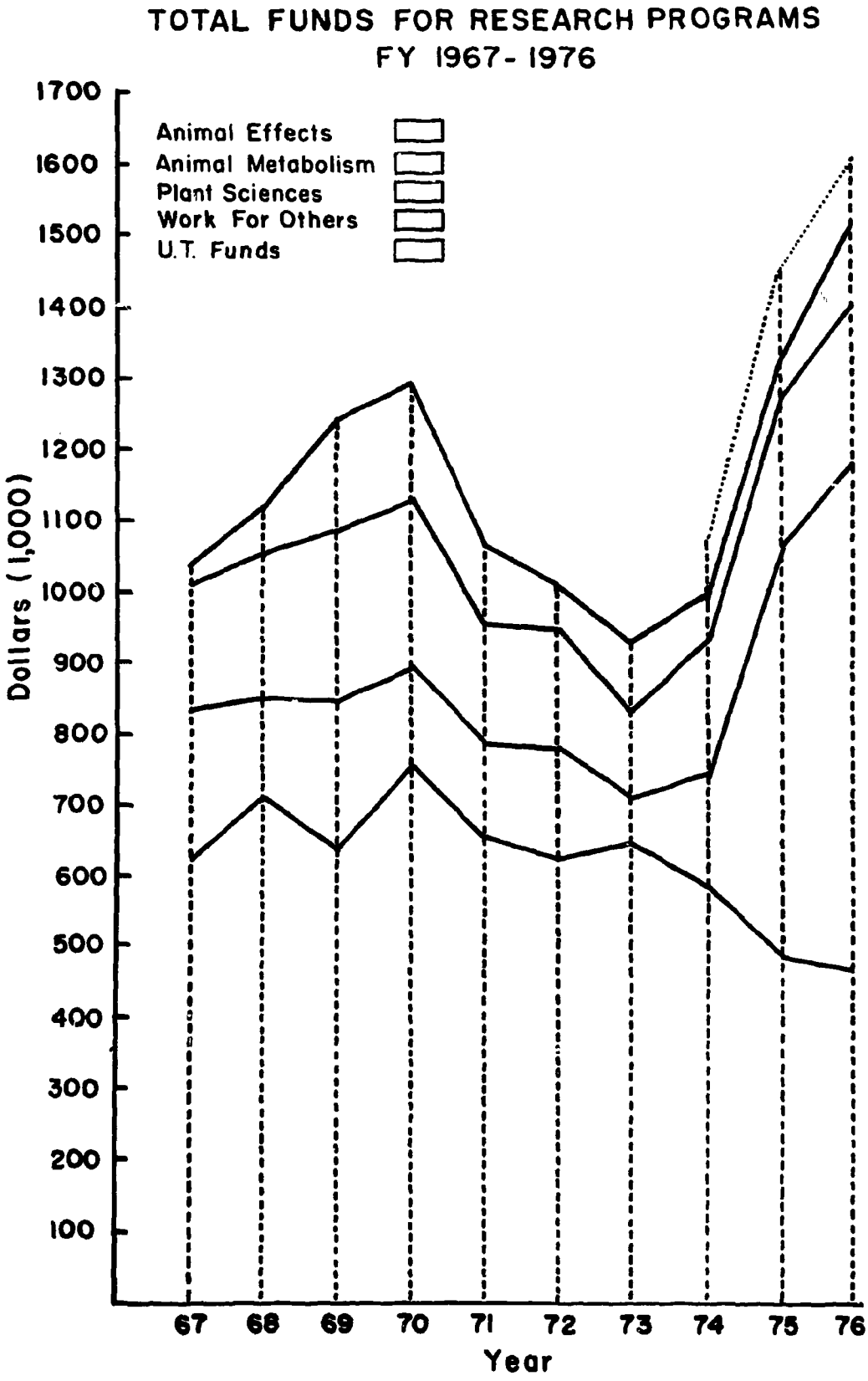
As we approach the end of the fourth year in our new course, research goals are still being refined and in some cases redirected to accommodate the shifts in mission orientation of our federal providers. However, we feel that sufficient progress has been made to justify publication of a progress report for the period. While some of the reports represent research initiated prior to fiscal year 1974, most reflect our new program direction and the greater number have been completed during the last two fiscal years (FY 1976 and 1977).

We still have much to do, but if the enthusiasm of the staff and the funding continue we expect to accomplish the goals we established in 1973.

# Organizational Chart



# Financial-Data



# Professional Staff Additions

W. J. Bergfield, Budget Officer. B. S., Business Administration, Eastern Illinois University (currently pursuing M.B.A., The University of Tennessee).

P. J. Bredderman, Associate Professor. B. S., Agriculture; M. S., Animal Breeding; Ph.D., Physical Biology, Cornell University.

J. W. Brown, Personnel Officer. B. S., Psychology, The University of Tennessee (currently pursuing M.B.A., The University of Tennessee).

L. E. Browne, Chief Engineer. Certified Professional Engineer, State of Tennessee.

M. F. Callaham, Senior Research Assistant. B. S., Microbiology; M. S., Biochemistry, Mississippi State University.

R. J. Chertok, Associate Professor. B. S., Biology, University of South Carolina; M. S., Zoology; Ph.D., Physiology, University of Miami (FL).

J. L. Dagley, Information Officer. B. S., Business Administration, The University of Tennessee.

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G. H. Eisele, Assistant Professor. B. S., Animal Science, Delaware Valley College; M. S., Animal Breeding and Reproductive Physiology; Ph.D., Physiology, The University of Tennessee.

D. E. Foard, Associate Professor. B. A., Biology; M. A., Biology, University of Virginia; Ph.D., Botany and Genetics, North Carolina State University.

R. R. Henke, Assistant Professor. A.A.S., B. S., Biology, Rochester Institute of Technology; Ph.D., Botany, Miami University (OH).

B. B. Hitchcock, Senior Research Assistant. B. S., Animal Science, University of Connecticut; M. S., Food Science, Michigan State University.

B. J. Kelman, Assistant Professor. B. S., Physiology; M. S., Veterinary Medical Science; Ph.D., Veterinary Medical Science, University of Illinois.

W. J. Kopp, Professor. B. S., Dairy; D.V.M., Iowa State University.

F. M. Osborne, Purchasing Agent. Certified Purchasing Manager.

D. M. Robie, Administrative Assistant to the Director. B. A., Biology, Gannon College (PA).

F. J. Ryan, Assistant Professor. B. S., Chemistry, State University of New York (Buffalo); Ph.D., Chemistry, Northwestern University.

M. D. Schneider, Professor. M. S., Parasitology, University of Wisconsin; Ph.D., Microbiology, University of Chicago; D.V.M., Auburn University.

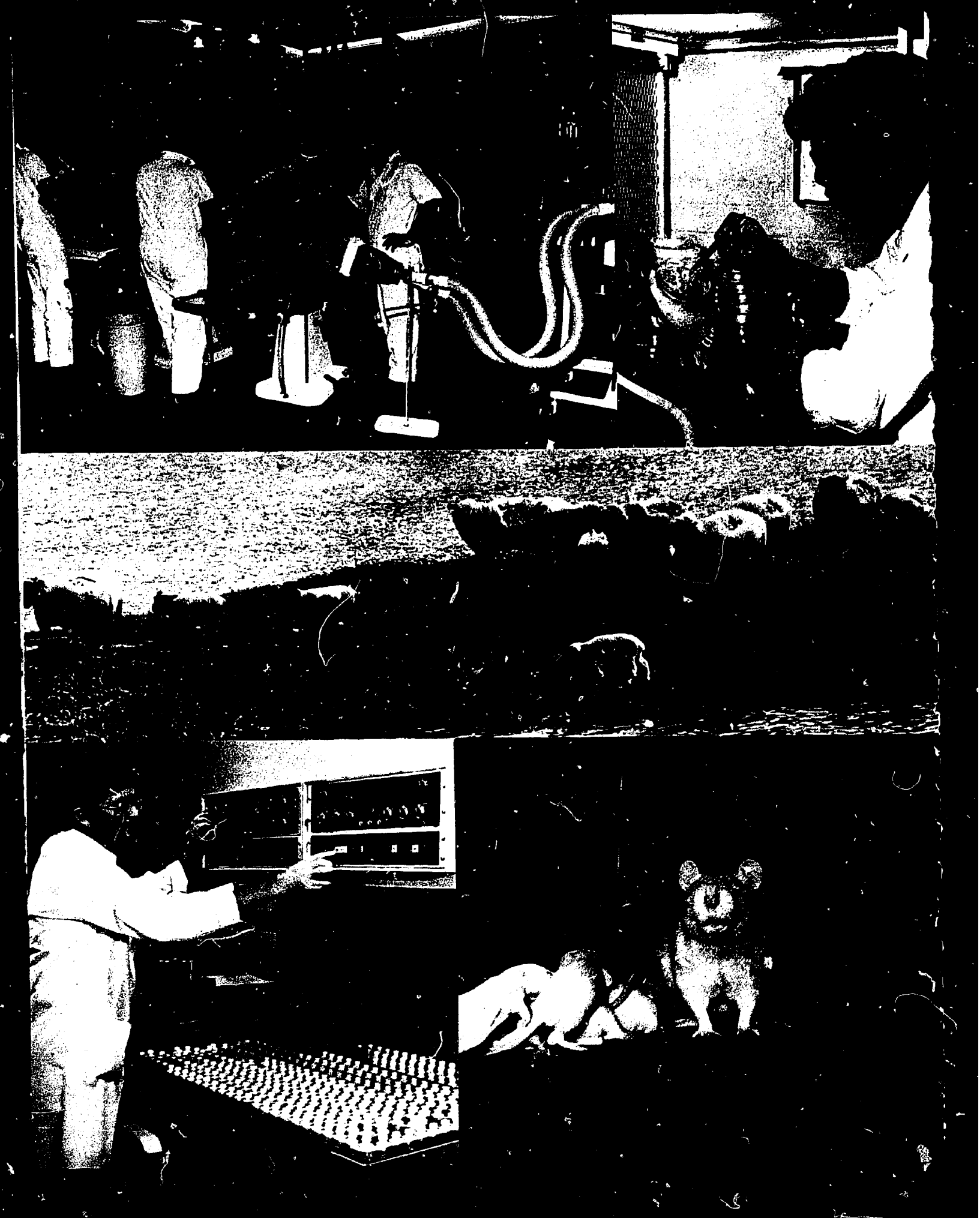
H. E. Walburg, Director. A. B., Zoology, Dartmouth College; M. S., Biology, Virginia Polytechnic Institute; D.V.M., University of Georgia; Ph.D., Veterinary Medicine, University of Illinois.

K. M. Wallace, Health Physicist. B. S., Educational Administration, Lincoln Memorial University; M. A., Educational Administration, George Peabody College.

B. K. Walter, Senior Research Assistant. B. S., Mathematics (equiv.), Goteborg Gymnasium; M. S., Medical Research (equiv.), University of Goteborg.

J. F. Weiss, Assistant Professor. B. A., Chemistry, New York University; M. A., Chemistry, Columbia University; Ph.D., Inorganic Chemistry, University of Arizona.

W. V. Wong, Biostatistician. B. A., Statistics, National Chengchi University, Taipei, Taiwan, China; M. S., Statistics, University of Minnesota.



# Research Programs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# Animal Metabolism

**H. E. Walburg, Head**

**Development of Transport Models** G. E. Spalding, Group Leader
**Iron Metabolism** P. G. Martin, Group Leader
**Membrane Structure and Function** P. J. Bredderman, Group Leader
**Gastrointestinal Absorption and Mineral Metabolism** L. B. Sasser, Group Leader
**Placental Transport** B. J. Kelman, Group Leader
**Cadmium Absorption and Metabolism** R. J. Chertok, Group Leader

## DERIVING AND ESTABLISHING AN SPF HERD OF YORKSHIRE SWINE

W. J. Kopp

The original herd of Yorkshire swine at CARL is plagued with chronic diseases as are 80% of the swine throughout the nation. These include mycoplasma pneumonia and atrophic rhinitis which are transmitted by animal-to-animal contact. The presence of these diseases results in poor feed conversion, low daily gains, increased death loss, higher susceptibility to other respiratory ailments, and poor experimental subjects. These diseases can be eliminated by surgically deriving colostrum-free pigs into a sterile environment and rearing them in isolation. Intensive preventive medicine is then practiced to prevent contact or contamination from diseased swine. Only swine derived in a similar manner are allowed to enter this Specific Pathogen Free (SPF) herd. In addition to ridding the swine of the aforementioned chronic diseases, the animals are freed of sarcoptic mange, lice, hemorrhagic enteritis, and Brucellosis.

Surgical derivation of colostrum-free SPF pigs is accomplished by performing a caesarean section on the pregnant sow at the 111th day of gestation with delivery directly from the uterus into a 4-ft × 100-ft plastic bag sterilized with potassium permanganate and formaldehyde and containing the necessary surgical equipment. The bag is inflated with a forced circulating air system filtered at both intake and exhaust. Four pairs of "dry box" gloves are placed two pairs to the side for use by the surgeon and assistants. After preparation of the surgical site on the right lateral abdominal area and application of surgical adhesive to the skin and bag, both are brought together for secure attachment. Surgery is performed within the bag, and the delivered pigs are revived to a state of active respiration. After all pigs are derived and located in the distal end, the bag is ligated at the middle and severed on the proximal side. The sterilized bag is then removed to an area of surgery remote from the dam while her incision is closed to permit rebreeding at a later date.

The pigs are removed to a disinfected room in another building where each animal is placed in a separate cage in an incubator for the first 3 wk of rearing. Diet consists of a commercial synthetic milk formula. Sanitary control is maintained by personnel change of outer clothes, boots, and wearing of rubber gloves and by maintaining an intense facility sanitation program.

At 3 wk of age the pigs are weaned from the liquid diet to solid feed and colonized by movement to brooders that hold three or four pigs apiece. At approximately 6 wk of age, they are moved to another area of the building with less climate control to acclimate them to the outside environment. When moved to the outdoor SPF facility, they are suddenly introduced to a foreign bacterial flora often resulting in a transient enteritis. Adaptation usually occurs readily with only minor adverse reactions.

Confinement to concrete flooring has magnified a skeletal defect of genetic origin common in the Yorkshire breed. Bilateral asymmetrical toes with the medial toe considerably smaller than the lateral requires that the bulk of the body weight be supported on this one digit. This results in excess callus formation on the foot pad, fissures between sole and pad, and sand cracks of the hoof wall, creating embarrassment to locomotion that progresses with age. Elimination of this defect can only occur through careful selection of animals with good foot conformation, a time-consuming task.

The old line of Yorkshires will eventually be entirely depopulated and the premises cleaned and vacated for at least 6 wk before repopulation from the SPF colony.

(a) Preparation of everted gut sacs to study the transport of environmental pollutants across intestinal tissue. (b) Perfusion technique used to determine the means by which environmental pollutants gain access to the developing guinea pig fetus. (c) Skeleton of a 3-day-old pig used in a detailed analysis of the localization of various radionuclides. (d) Measuring effects of hyperkalemia produced by intravenous infusion of KCl on electrocardiograms of normal and magnesium-deficient calves.

## COMPUTER FACILITY

G. E. Spalding

In September 1976, CARL acquired a Data General Corporation Eclipse S/200 minicomputer to replace purchased outside timesharing services. The system included 128 KB of core memory, a 10-MB disk, a 75-ips–800-BPI magtape drive, a 165-cps line printer, a 400-cps paper tape reader, a 2400 baud system console (CRT), and three 110-baud teletype terminals.

The RDOS operating system for the Eclipse was supplied by the manufacturer and includes support for foreground/background programming and various system utilities and editors. Also supplied were Multi-User Extended BASIC and FORTRAN 5 programming languages, and a batch processor.

The Basic Timesharing System (BTS) is typically operated in the foreground throughout the normal work day. This system is accessible to three concurrent users to be utilized for data input and BASIC program development and execution. Various scientific applications as well as the laboratory cost-accounting and inventories systems use BTS. The background is typically used for system maintenance, batch processing, and FORTRAN program development and execution. Modeling of research data on the metabolism and transport of energy-related environmental pollutants accounts for nearly 100% of background utilization. During off hours, both grounds of the Eclipse are utilized for the development and execution of modeling programs.

Since acquiring the Eclipse we have been attaining far better service and more computer power than was available to us under our previous outside timesharing service, and at significantly lower cost.

## BIOLOGICAL DATA RETRIEVAL SYSTEMS

G. R. Eisele, F. R. Mraz, and H. E. Walburg

Although both the number and use of computer data bases are increasing rapidly, the data often do not serve the needs of research biologists. We have developed a data base to fill this need by using specific biologically important fields which can be searched independently or simultaneously.

Since the accessibility of most existing data retrieval systems is based on key word indexing, a thesaurus must be used to compile a list of proper terms. This controlled vocabulary of terms or descriptors directs the format of the search strategy to be used. The 1974 INIS [IAEA-INIS-13 (Rev. 6)], for example, has 12,519 accepted terms with additions and deletions from year to year which may or may not appear in other data base centers. An "overkill" or saturation of related terms must be introduced for one to retrieve all information available on a specific subject. The various data retrieval systems used give an array of information from reference citations to abstracts containing no numerical data.

Our numerical data retrieval system contains over 1200 articles on the biological aspects of plutonium which are classified under specific fields, e.g., species, sex, age, route given, etc. Each field receives an answer to its specific question, and if that information is not given in the original reference it is so noted.

In cooperation with other Oak Ridge information groups, evaluation is continuing of those existing computer programs which can best be modified to permit specialized storage, retrieval, and output of numerical data while providing detailed coverage needed by various groups. This approach in data retrieval is unique and necessary for research biologists interested in dosimetry and toxicology of various environmental pollutants.

## COMPANAL, A COMPUTER PROGRAM FOR COMPARTMENTAL ANALYSIS OF BIOLOGICAL TRANSPORT DATA

G. E. Spalding

COMPANAL is a FORTRAN program designed to facilitate the development of compartmental-model analogs of biological transport systems. The program accepts data from various biological "compartments" (e.g., blood, liver) and from multiple routes of administration of a test dose (e.g., oral, intravenous), and then determines a set of transfer coefficients yielding the best least-squares fit of the model to the data.

The model is defined to COMPANAL by coding the set of differential equations describing it and by specifying the routes of administration used in the experiment, with their appropriate sets of initial conditions.

COMPANAL contains several features which greatly aid in model definition and parameter estimation:

1. Initial estimates of parameters (transfer coefficients) need be only very rough guesses. Initial estimates which differ from final estimates by several orders of magnitude are usually acceptable.

2. One has the option of using log-transformed parameters in the first several parameter-estimation loops, i.e., $\ln(\lambda_{ij})$ is the parameter of interest rather than $\lambda_{ij}$. When parameter estimates are much different from their true (i.e., least-squares) values, this greatly increases the reliability of successive estimation of parameters.

3. One can define the scope of a model as much greater than one wishes to work with initially, and can include only the desired aspects on any particular run or analysis. For example, the investigator may ultimately wish to develop a model which will be regressed to data from both oral and intravenous routes of administration, but he may wish initially to examine only the oral data, and to examine only transport in the GI tract. As the model-definition process

progresses, he can readily include more data, up to the limit of the experiment(s) being analyzed.

4. One has the option of basing the regression of log-transformed data. When data are expressed as percent-of-dose, one generally finds that the standard deviations of data points are proportional to their means—this violates the assumptions upon which the least-square criterion for best fit is based. In such cases, regression based on log-transformed data is appropriate, as this transformation tends to produce standard deviations which are homogenous and independent of the mean.

5. The presence of "wild" parameters, particularly noticeable during early stages of parameter estimation, can slow or prevent the model from converging to a least-squares solution. To overcome this, COMPANAL employs an algorithm identifying parameters which appear to be wild during each parameter estimation loop. When such parameters are found, their identity is placed in a "hold" list. The program then proceeds to reestimate the "good" parameters. The reestimation loop may or may not detect more wild parameters; if it does, the newly declared wild parameters are added to the hold list, and a further reestimation loop is executed. The program proceeds in this manner until either no new wild parameters are found or only two good parameters remain. The program then proceeds to reexamine the hold list, including the best two or more parameters in each further reestimation loop; this continues until all, or all but one, of the held parameters have been adjusted. If a parameter was not reestimated at any time within the estimation loop, it is tagged as a "must include" parameter for the next loop.

6. To prevent divergence of the model to a least-squares fit, new parameter estimates are tested to determine whether they would, if accepted, lead to an increase in the residual sum of squares. If such an increase is found, then the magnitude of the correction vector is reduced, and new estimates are computed and tested. If, after several attempts, the problem cannot be corrected, then the sign of the original correction vector is reversed, and testing proceeds as above.

7. When a least-squares solution has been found, COMPANAL estimates standard errors for each parameter and outputs a table of observed vs. computed values of the input data.

8. COMPANAL will automatically tag suspected data outliers. The investigator has the option of excluding these data points during the late stages of parameter estimation.

Although COMPANAL smooths the way toward a fruitful effort to define compartmental analogs of biological transport systems, it is unfortunately not a "black box" that will automatically proceed to the desired result. Considerable judgment must be exercised in accepting, rejecting, or modifying a model or aspects of that model. This judgment must be based upon an adequate understanding of the biological system under study as well as mathematical and statistical considerations.

## USE OF DERMESTID BEETLES FOR QUANTITATIVE SEPARATION OF BONE AND MUSCLE RADIOACTIVITY IN SMALL RODENTS

H. E. Walburg, G. R. Eisele, and F. R. Mraz

One of the principal problems in determining tissue localization of radionuclides in small rodents is the separation of muscle from bone in the carcass remaining after evisceration. While in many cases soft tissue activity is small compared to bone activity, the muscle mass is large (for the mouse, 88% of eviscerated carcass weight) and considerable error can occur from estimations based on activity of small samples extrapolated to the entire carcass. Direct measurement of bone and muscle activity requires separation of these components, a task simply accomplished in large animals where cooking and physical separation are feasible. In small rodents such as the mouse and rat, size precludes such an approach. The larvae of dermestid beetles can be used to clean animal carcasses leaving an intact skeleton (Fig. 1). Accurate determinations of radioactivity require that no muscle activity remain on the skeleton and more importantly that the frequently higher bone activity not contaminate the residue (beetles, larvae, and their excrement).

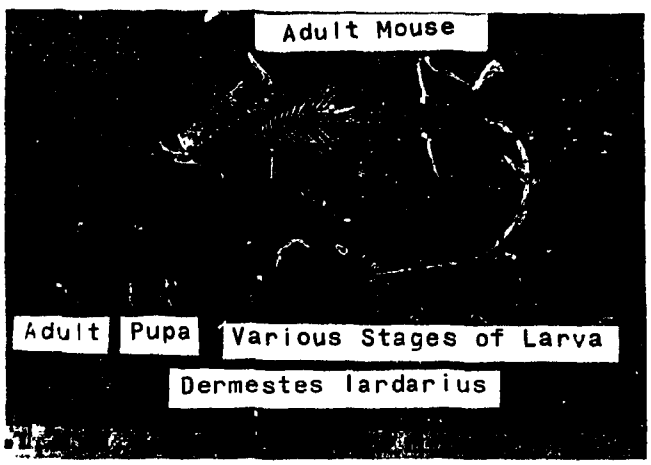



Fig. 1 Dermestid beetles with intact cleaned skeleton of adult mouse.

To determine whether quantitative separation of muscle and bone can be achieved by dermestid digestion, 20 adult $C_3H$ female mice were injected with 1 $\mu$Ci $^{95}$Nb oxalate via tail vein. Forty-eight hr later the mice were killed, eviscerated, and the carcasses placed in pint Mason jars with larvae of *Dermestidae lardarius*.

Dissection of the mice resulted in a loss of only 4.9 ± 0.4% (S.E.) of the radioactivity in the whole animal, presumably due to tissue, fluid, and hair loss associated with the procedure. Dermestid digestion likewise resulted in only a small loss of radioactivity, 5.7 ± 0.4% (S.E.) of the

activity in the eviscerated carcass. These losses are acceptably small and do not appear to introduce any bias into the determination of tissue localization of radionuclides. The actual muscle activity measured by counting the activity in beetles, larvae, and excrement from dermestid digestion was 9.66 ± 0.41% (S.E.) of the dose injected. Muscle compartment radioactivity estimated by counting the activity in approximately 0.35 g of gluteal muscle was multiplied by the ratio of total muscle weight to sample weight (approximately 9.0). The estimated value for muscle compartment radioactivity was 9.02 ± 0.29 (S.E.). A comparison of these determinations for muscle activity using a T-test for paired samples shows that the difference between the two methods is not significant (T = 1.65) at the 5% level. It was further determined that the power of the T-test was such that if the average percentage dose determined by actual counting of the beetles, larvae, and excrement from dermestid digestion was 2% higher than the theoretical determination the test would have a 90% probability to detect such a difference when the significance level is 5%.

Thus dermestid digestion appears to be an effective method for quantitatively separating muscle and bone radioactivity in small rodents and is currently being used in our actinide and heavy metal metabolism studies.

## LIQUID SCINTILLATION α COUNTING AND SPECTROSCOPY AND ITS APPLICATION TO BONE AND TISSUE SAMPLES

J. F. Weiss and W. J. McDowell*

Because of the increasing concern with plutonium release to the environment, the need for fast analysis of α emitters, especially in low level samples, has intensified. To meet this need, we have developed a methodology for fast analysis of α emitters without using chromatography or electroplating. This method has a 100% counting efficiency; 100% plutonium recoveries extend to low level samples, and α energy discrimination is possible. Liquid scintillation counting of α emitters allows 100% counting efficiency even in the presence of some quenching, elimination of backscatter and self absorption, and geometry correction. Recent advances in electronic techniques have now enabled backgrounds in liquid scintillation counting to be reduced sufficiently for accurate analysis of α emitters at environmental levels, with energy discrimination.

Our method combines tertiary amine-based solvent extraction and high resolution liquid scintillation counting. Beginning with a sample in solution, only two extractions, one stripping step, and one volume reduction are required to prepare a low level sample in a form ready to count. The time required is about 1 hr compared to 10–16 hr for ion exchange and electroplating. Counting requires simple equipment, has low background, allows for chemical separations, and can give a peak to peak energy resolution of 200–300 keV. Our work has utilized only $^{239}Pu$. The method and variations thereof will allow selective nuclide counting in a mixture of isotopes and elements in samples of diverse origin.

## THE DETERMINATION OF TOTAL PROTEIN—A BETTER METHOD

P. J. Bredderman

We have initiated a research effort to develop expertise and analytical methodology for the investigation of molecular mechanisms of trace metal intestinal transport. Thus far we have succeeded in developing a new method for determining total protein which overcomes many of the deficiencies of previous methods (Fig. 2). These deficiencies

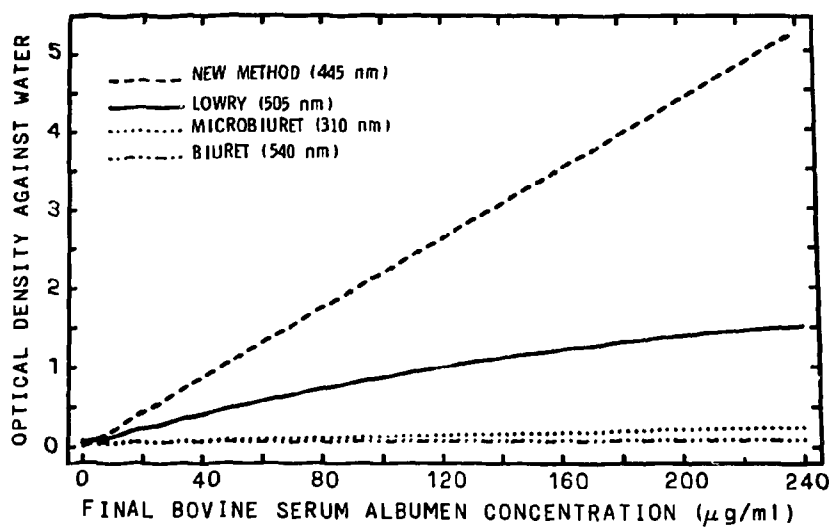



Fig. 2 Comparison of new method for determination of total protein with existing methods.

*Oak Ridge National Laboratory.

include lack of required sensitivity; lack of accuracy or absoluteness (i.e., the specific absorbance varies from protein to protein); nonlinear response (i.e., the specific absorbance is a function of protein concentration so that a standard curve is required); lack of specificity (i.e., substances other than proteins interfere directly or indirectly); and incompatibility with conditions required to solubilize membrane proteins.

Our method is similar in principle to that of Westley and Lambeth (1960) but differs in significant ways which allow it to be used for both soluble proteins and SDS-solubilized membrane proteins. In addition, both its sensitivity and linear range (0.010 to 1.5 mg/ml sample) are somewhat greater. The process uses stable stock solutions; color develops quickly and is stable. It is also reasonably short and simple to perform, requiring no special equipment. The reaction, based on copper binding, actually measures the concentration of polypeptide linkages, and is therefore essentially independent of protein amino acid composition. In addition to its superior linearity, it is also of greater sensitivity than the commonly used method of Lowry et al. (1951). Like all methods that involve copper binding, it lacks specificity; interfering substances are removed by a prior treatment with phosphotungstic acid.

### References


Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* 193: 265-275.

Westley, J., and J. Lambeth. 1960. Protein determination on the basis of copper-binding capacity. *Biochim. Biophys. Acta* 40: 365-366.


## THE ISOLATION OF RENAL BRUSH BORDER VESICLES


R. J. Chertok and M. F. Callaham


The nephrotoxic action of heavy metals is well documented. Chronic cadmium exposure in man and experimental animals, for example, produces a renal tubular syndrome that often includes proteinuria, renal glycosuria, aminoaciduria, hypercalciuria, impaired concentrating ability, and impaired acid excretion. It has been reported that cadmium selectivity inhibits amino acid transport but does not affect maximum tubular transport of glucose when corrected for glomerular filtration rate. Under the conditions of the experiment, the relative increases in amino acid excretion reflected intrarenal rather than extrarenal causes. This report further suggests that the generalized aminoaciduria results from inhibition of amino acid carriers at the luminal membrane. This inhibition is similar to that suggested for mercury and uranium.

The luminal surface of the renal proximal tubule is characterized morphologically by the presence of a brush border. Since amino acids are reabsorbed in the proximal tubules, this suggests that the brush border is intimately involved with the reabsorptive mechanisms. With these concepts in mind, a comprehensive study of the effects of heavy metals on the amino acid transport in isolated renal vesicles of the brush border membrane has been initiated.

To date several methods have been published for isolating renal vesicles. Some of the methods are very tedious and time consuming while others use fairly drastic procedures such as osmatic shock for rupturing the cells. These drastic procedures could result in the loss of some functional proteins which may be vital for the transport studies. One problem which appears to be common in the published procedures is the contamination of the brush border vesicles with vesicles of the baso-lateral membrane. In a study of amino acid transport this is a serious drawback since the two kinds of membranes have different amino acid transport characteristics.

In the first phase of this program our objective is a gentle isolation of renal brush border vesicles which are relatively free of subcellular contaminants including baso-lateral membranes. Presently the procedure consists of isolating the proximal tubules, rupturing the cells by gentle homogenization, and eliminating the subcellular particles by differential centrifugation. Attempts are being made to separate the baso-lateral and brush border membranes by taking advantage of the difference in surface charges. The procedure currently under investigation involves partitioning of the membranes in two-polymer aqueous phases of dextran and polyethylene glycol. Throughout the procedure the presence of contaminants is assayed by quantitation of marker enzymes.

## NEW APPROACHES TO THE ISOLATION OF INTESTINAL BRUSH BORDERS


P. J. Bredderman


This project is the first phase of research designed to study the metal-protein and protein-protein associations that occur during trace-metal transport between lumen and absorptive epithelium of the intestine. It is envisioned that in addition to the soluble metal-binding proteins already recognized, both peripheral and integral membrane proteins are transport components. Conventional approaches to brush border isolation, which use low ionic strength media containing chelating agents, are unsatisfactory for this type of study because they result in the extensive loss of peripheral membrane proteins. For this reason we have been exploring the use of pressure homogenization to free the brush borders (or their membranes). We have thus far determined the following factors to be critical to the reproducible release of intact brush borders in good yield, as assessed by phase contrast microscopy:

1. The tissue must be expelled from the pressure vessel during decompression. No tissue disruption occurred during rapid decompression from 900 psi of nitrogen to 1 atmosphere in less than 1 sec when the tissue was not

expelled, irrespective of whether or not the tissue was immersed in liquid.

2. Pre-equilibration under pressure does not have a noticeable effect and can be omitted.

3. Relatively low pressures must be used (250 psi) to avoid disintegration of brush borders.

4. To assure both uniformity of treatment of the entire sample and treatment reproducibility, the full discharge pressure gradient must be achieved instantaneously and maintained with a pressure regulator.

5. Pressure homogenization must be sharply focused at an orifice to achieve uniformity of treatment and to avoid plugging.

6. Time factors and animal-to-animal variation should be minimized. Still to be investigated are the effects of tissue pretreatments and homogenizing medium composition.

Subsequent phases of this work will include brush border purification using a liquid polymer, two-phase system. This will be followed by procedures for solubilization of membrane proteins in a way compatible with the production of polyspecific antisera which in turn will be used in cross-line immunoelectrophoretic studies of protein-protein associations.

## MEASURING TRANSPORT ACROSS PERFUSED PLACENTAS OF SWINE AND SHEEP

B. J. Kelman and B. K. Walter

The use of interspecies comparisons of placental function is particularly important in delineating placental function in humans. A great diversity of placental types among species is apparent on a morphological basis. In fact, there are almost as many types of placentas as there are viviparous animals. Because of this, it is difficult to extrapolate from one species to the next on a predictable basis. In order to predict characteristics of a particular species based on others, we feel that success is most likely when several species are tested for the characteristic in question, the unique features of the species are recognized, and this information is used to extrapolate to other species.

Three species (guinea pig, sheep, and swine) which possess histologically different placentas are currently under investigation. The guinea pig with a hemochorial (or hemoendothelial) placenta has a placenta which appears histologically close to the human. Sheep and swine placentas are quite different, allowing physical separation of fetal and maternal sides. Although perfusion techniques have been reported in the literature for sheep, none have previously been reported for swine and none have been reported which allow easy adaptation to more than one species.

To investigate placental transport of metals in different species, a perfusion technique was developed which is easily adaptable to larger animals such as sheep and swine. The fetal circulation of placentas from each species is perfused in situ with modified plasma from the corresponding species. At appropriate times, the pollutant under study is injected into the maternal circulation and the resulting transfer is examined under the conditions desired. Perfusion pressure, tritiated water clearance (to serve as a baseline measurement in order to account for changes in maternal blood flow to the placenta and differences in placental exchange surfaces), maternal blood pressure, cardiac rate, electrocardiogram, and respiratory rate are monitored continuously throughout all the experiments.

## CLEARANCE OF TRITIATED WATER AS A BASELINE MEASUREMENT FOR TRANSPORT OF MATERIALS ACROSS PERFUSED PLACENTAS

B. J. Kelman

Although there are limited data available on placental transport of such materials as water, minerals, amino acids, and fatty acids, almost none of these data reflect the advantages of any baseline measurements. To date, only one investigator has proposed a nondestructive method of continuously measuring maternal blood flow to the placenta, and this has not been used in small animals. In perfused placentas, some measurement of maternal blood flow is important because of the potential effects of changes in maternal blood flow on the transfer of materials across the placenta. Without measurements which account for variability in maternal blood flows and placental exchange surface areas, existing data are difficult to interpret.

Since water crosses the placenta in a flow-limited manner, the clearance of tritiated water across the placenta was investigated as a baseline measurement for placental transport of environmental pollutants. When the rate at which tritiated water crosses the placenta was expressed as clearance (milliliters of maternal plasma containing an amount of radiolabel equal to that entering the perfusate per minute), perfusate rate was linearly related to tritium clearance in all experiments in which maternal vital signs (electrocardiogram, cardiac rate, blood pressure, respiratory rate) remained stable and within normal limits. This relationship held over a wide range of perfusion rates and supported the concept that tritiated water equilibrates across the placenta, making tritium clearance highly sensitive to changes in perfusion rates on both fetal and maternal sides.

To clarify the relationship between tritiated water clearance and maternal blood flow to the placenta, a series of experiments was conducted to quantify the relationship

between uterine blood flow and the clearance of labeled water using radiolabeled microspheres. The data indicate that clearance of labeled water is closely correlated with uterine blood flow late in gestation and confirm that the clearance of labeled water serves as a reasonable baseline measurement for the transfer of substances across the placenta.

## CHANGES IN THE SMALL INTESTINE DURING PREGNANCY

Polly G. Martin, Nancy Kuemmerle, and Joyce King

Evidence from rodent studies indicates that during pregnancy the small intestine increases in weight with concurrent increases in length and surface area of the villi. These changes have been attributed to hypertrophy rather than to a combination of hyperplasia and hypertrophy.

Results of a study on subcellular iron distribution in mucosal cells from the small intestine of pregnant and nonpregnant, iron-deficient and normal rats indicate that the amount of DNA (cell numbers), RNA, and protein was greater in pregnant animals per unit of length than in comparable nonpregnant rats (Table 1). Protein/DNA ratios, indicators of concentration per cell, were greater in pregnant iron-deficient (21.7) and normal rats (22.9) than in nonpregnant iron-deficient (20.1) and normal rats (19.7). RNA/DNA ratios ($P < 0.05$) were greater only in the mucosal cells from pregnant normal animals (1.86) when compared to the other three groups (1.69 to 1.74). Whether or not the proteins are transport proteins is an interesting question.

Since the pregnant animal is in a distinct physiological state with an increased demand for all nutrients during the last third of gestation, an increase in number of absorptive cells would create a mechanism for the additional uptake and transfer of more nutrients as the rates of absorption are reported to be the same in pregnant and nonpregnant animals. What appears more puzzling is the greater quantity of DNA (cells) and protein per unit length from the iron-deficient animals, particularly the iron-deficient pregnant rat ($P < 0.05$). These data suggest an additional mechanism is functioning for enhanced absorption of nutrients due not only to pregnancy but also to iron stress.

Table 1 RNA, DNA, and Protein in Mucosal Cells from 40 cm of Small Intestine

| Treatment | No. | DNA (mg) | RNA (mg) | RNA/DNA | Protein (mg) | Protein/DNA |
|---|---|---|---|---|---|---|
| Normal nonpregnant | 37 | $10.0 \pm 0.5_1$* | $18.8 \pm 1.1_1$ | $1.74 \pm 0.03_1$ | $199 \pm 9_1$ | $19.7 \pm 0.6_1$ |
| Normal pregnant | 37 | $11.8 \pm 0.5_2$ | $21.7 \pm 1.0_1$ | $1.86 \pm 0.05$ | $259 \pm 14_2$ | $22.9 \pm 0.8_2$ |
| Iron-deficient nonpregnant | 27 | $11.2 \pm 0.6_{1,2}$ | $19.2 \pm 1.2_1$ | $1.69 \pm 0.04_1$ | $213 \pm 11_1$ | $20.1 \pm 1.3_{1,2}$ |
| Iron-deficient pregnant | 26 | 13.6 ± 0.4 | 23.8 ± 0.7 | $1.74 \pm 0.02_1$ | $289 \pm 10_2$ | $21.7 \pm 0.9_{1,2}$ |

*Numbers with same subscript are not significantly different ($P < 0.05$).

## ABSORPTION OF MERCURY FROM LIGATED SEGMENTS OF THE RAT GASTROINTESTINAL TRACT

L. B. Sasser, G. E. Jarboe, B. K Walter, and B. J. Kelman

Methylmercury is readily absorbed from the gastrointestinal tract, but sites of absorption have not been identified. Results of our attempt to kinetically analyze mercury transport in the rat suggested that methylmercury was absorbed from two or more large gastrointestinal compartments, and the stomach appeared to be a major site of absorption. Consequently, the ligated segment technique was used to determine the major sites of inorganic mercury and methylmercury absorption, and to compare the gastrointestinal absorption of the two chemical forms in the rat. Three 8–10-cm ligated intestinal segments and the stomach were dosed with either $CH_3{}^{203}HgCl$ or $^{203}HgCl$, and absorption and distribution were determined 4 hr later.

Methylmercury was more readily absorbed (15–35 times greater, depending on the absorption site) than inorganic mercury from all ligated segments. The relative order of methylmercury absorption from ligated segments was as follows: duodenum > stomach = ileum > jejunum. The duodenal segment absorbed over 80% of the methylmercury dose, greater ($P < 0.01$) than any other segment. Nearly 60% of a similar methylmercury dose was absorbed from the stomach, not statistically different from the ileal segment. Absorption from the jejunum was 35%, less ($P < 0.01$) than any other segment. Gastrointestinal retention was inversely proportional to absorption. Significant differences in absorption of inorganic mercury between gastrointestinal segments were not detectable. Endogenous excretion of both forms of mercury into intestinal tissue was equal. Our data indicate that the absorption of methylmercury from the stomach is significant relative to other parts of the gastrointestinal tract and that the stomach must be considered as a major site of absorption.

## EVALUATION OF HAZARDS DUE TO RADIOMERCURY RELEASE FROM STORED TISSUES AND CAGED ANIMALS TREATED WITH METHYLMERCURY

B. J. Kelman

While methylmercury is more volatile than inorganic or elemental mercury, it is the form least likely to be lost from biological systems since it binds specifically to sulfhydryl groups. Methylmercury tends to be associated with those sulfhydryl groups having the greatest affinity for it when its concentration relative to the number of available sulfhydryl groups in tissue is small. The stability of these S-Hg bonds determines the rate at which mercury is released from whole animals, excised tissues, and excreta. In this study, we determined the amount of mercury respired by rats administered low doses of methylmercury and the amount of mercury released to the atmosphere from tissues labeled with methylmercury.

We found that 310-g rats injected with 20 $\mu$Ci of $^{203}$Hg (2.18 $\mu$g total Hg) in the form of methylmercury chloride respired 10.0 ± <0.1 mg Hg (mean ± SE) per animal. This amount was clearly insignificant as a source of stable or radioactive mercury contamination to the laboratory environment.

No significant amount of mercury was lost from excised tissues, excreta, or standards containing mercaptoethanol stored at refrigerator or room temperatures. Conversely, a substantial amount of mercury was released from unsealed standards containing no sulfhydryl groups. Regardless of the specific proteins which bind mercury, the strength of the mercury-sulfhydryl bonds was therefore sufficient to prevent loss of mercury under normal laboratory conditions. It is apparent that once methylmercury is bound by proteins, there is little hazard from inhalation intoxication from gaseous methylmercury.

## EFFECT OF RED-CELL-BOUND MERCURY ON MEASUREMENTS OF TISSUE MERCURY DISTRIBUTION

B. J. Kelman, G. E. Jarboe, and L. B. Sasser

Blood is the transport vehicle of methylmercury in the body with almost complete binding to hemoglobin. Although some variation in the percentage of mercury contained in red cells has been reported among species, most data indicate that red cells contain over 90% of the total blood methylmercury. Because of the high affinity of red cells for methylmercury, gross errors in tissue content of methylmercury could result unless corrections are made for methylmercury in red cells retained in tissues. Results from most studies using radioisotope techniques to show methylmercury distribution and tissue content do not reflect residual red cell methylmercury.

In this experiment, we sought to determine whether the relative contribution of red cell methylmercury to total tissue methylmercury was sufficiently great to affect the measurement of total tissue methylmercury. Red cells were mechanically removed from tissues by flushing the circulatory systems of rats previously injected with methylmercury.

The data clearly show that significant quantities of red-cell-bound methylmercury were removed from the body tissues when whole animals were perfused with saline. It was confirmed that the methylmercury removed from tissues was bound to red cells rather than washed from the tissue itself. Large decreases in tissue methylmercury were observed in all cases except those in which the perfusion technique did not efficiently remove red cells.

It is therefore necessary to adjust tissue methylmercury content for red-cell-bound methylmercury when studying methylmercury distribution. We recommend in vivo labeling of red cells with $^{59}$Fe or in vitro labeling of red cells with $^{51}$Cr as the preferred labeling techniques in most situations.

## TRANSPORT OF ORGANIC AND INORGANIC MERCURY ACROSS THE PERFUSED PLACENTA OF THE GUINEA PIG IN LATE GESTATION

B. J. Kelman and L. B. Sasser

Both organic and inorganic forms of mercury can be found in mercury-polluted environments. These forms of mercury are capable of crossing the placenta and adversely affecting the developing fetus. It is apparent from the literature that less inorganic than organic mercury is absorbed by the fetus at all gestational ages. It is also apparent that more inorganic mercury is absorbed by placental tissue than by the fetus in contrast to organic mercury where fetal and placental tissues absorb similar amounts. Such studies are generally interpreted as supporting the hypothesis that placental tissues restrict the passage of inorganic mercury to the fetus even though almost all studies of mercury transfer to the fetus are confounded by an inability to separate fetal uptake and placental transport. Unless this distinction is made it is difficult to differentiate among restricted transfer of inorganic mercury across the placenta due to a "placental barrier", restricted transfer due to low maternal plasma levels, and low fetal uptake of inorganic mercury.

In our study, we perfused the fetal side of the guinea pig placenta with modified guinea pig plasma. In situ perfusions were done at approximately 60 days gestation. The dams were injected I.V. with high specific activity $^{203}HgCl_2$ or $CH_3{}^{203}HgCl$, and tritium clearance was used as a baseline measurement in all experiments. Perfusion pressure, maternal cardiac rate, ECG, and blood pressure were monitored. A schematic diagram of the perfusing system is presented in Fig. 3.

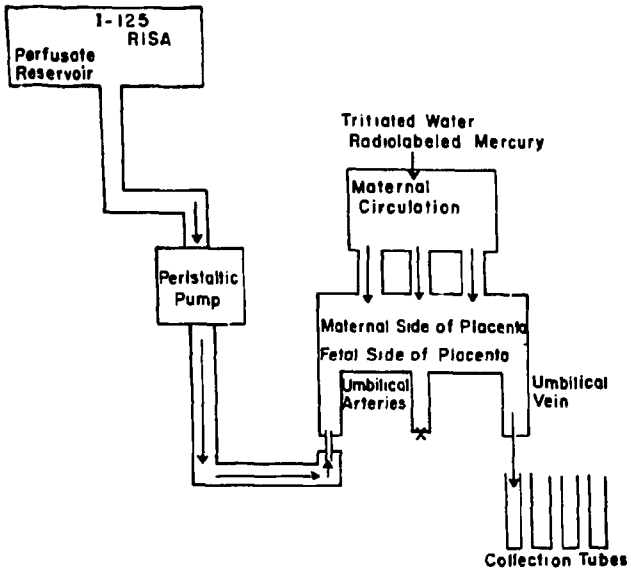



Fig. 3 Schematic diagram of the perfusing system.

Our data show that neither organic nor inorganic mercury clearance was correlated with the clearance of labeled water. Further, neither form of mercury clearance was sensitive to changes in a wide range of perfusion studies. Thus it appears that transplacental exposure of the fetus to mercury occurs in a different manner than occurs with labeled water.

Large methylmercury clearances were measured in these experiments indicating that methylmercury crosses the placenta of the guinea pig with relative ease, independent of the presence of the fetus. A growing body of data from the literature indicates that there are some gross similarities between methylmercury movements across the placenta and other cations such as cadmium. Further investigation of this relationship is warranted since the literature contains data supporting the hypothesis that calcium is transported from dam to fetus across the placenta of the guinea pig by an active mechanism, which implies the existence of a specific carrier.

The clearance of methylmercury was more than 12 times the clearance of inorganic mercury. Since the literature contains data indicating that the fetal absorption of organic mercury is 5–17 times greater than that of inorganic mercury, the differences in their clearances were sufficiently great to account for reported differences in fetal absorption of organic and inorganic mercury.

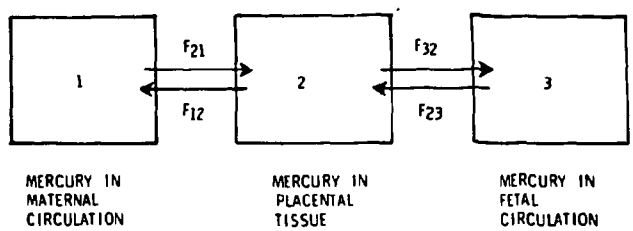
In addition to the differences in magnitude between organic and inorganic mercury clearances, there was considerably less variation in organic than inorganic mercury clearances. However, the variation seen in both clearances was surprisingly large. The source of these large variations was not readily apparent. They did not appear to be due to analytical procedures since measurements of calcium clearances under similar conditions showed much less variation. The large variations in mercury clearances, insensitive to changes in flow rates, suggest that the placenta functions as a third compartment located between the fetal and maternal circulations which releases methylmercury in large discrete units. Since placental tissue absorbs much more inorganic than organic mercury, it seems likely that the placenta acts as a larger third compartment located between fetal and maternal circulations for inorganic than for organic mercury. Steps in the placental transport process involving uptake and release of both forms of mercury may occur through the same or similar mechanisms, the principal differences being in the rate of release of mercury to the fetal circulation.

## A MODEL FOR THE TRANSPORT OF MERCURY ACROSS THE PLACENTA

B. J. Kelman

Based upon a combination of data from this laboratory and that previously reported in the literature, it is now possible to qualitatively summarize information available for the transfer of mercury between fetal and maternal circulations (Fig. 4). Compartment 1 represents mercury in the maternal circulation; compartment 2, mercury in placental tissue; and compartment 3, mercury in the fetal circulation.

SUMMARIZATION OF DATA CURRENTLY AVAILABLE FOR THE TRANSFER OF MERCURY BETWEEN FETAL AND MATERNAL CIRCULATION




Compartment 1 represents mercury in the maternal circulation compartment 2, mercury in placental tissue; and compartment 3, mercury in the fetal circulation. The flow of mercury from dam to fetus ($F_{21} + F_{32}$) is greater for organic than for inorganic mercury. No data are available on the relative flow of organic and inorganic mercury from fetus to dam ($F_{12} + F_{23}$), but the flow of organic mercury from dam to fetus is greater than the flow from fetus to dam ($F_{21} + F_{32} > F_{12} + F_{23}$). The magnitude of compartment 2 is greater for inorganic than for organic mercury. In experiments at our laboratory, perfusing solution is passed through the placenta only once so that compartment 3 never becomes large enough for $F_{23}$ to be significant. If $F_{12}$ is also small, then $F_{32}$ must be the component of $F_{21} + F_{32}$ which is decreased in the case of inorganic mercury, resulting in the increased size of compartment 2.

Fig. 4 Model for transport of mercury across the placenta.

The flow of mercury from dam to fetus ($F_{21} + F_{32}$) is greater for organic than for inorganic mercury. Since the clearance of both mercury forms is independent of perfusion rate, they may move across the placenta by mechanisms with some properties in common. The similarities between methylmercury and calcium transport suggest that some compartment of $F_{21} + F_{32}$ may occur by mediated transport and may not be continuous with respect to time.

No data are available on the relative flow of organic and inorganic mercury from fetus to dam ($F_{12} + F_{23}$), but the flow of organic mercury from dam to fetus is greater than

the flow from fetus to dam ($F_{21} + F_{32} > F_{12} + F_{23}$). The magnitude of compartment 2 is greater for inorganic than for organic mercury. In our experiments, perfusing solution is passed through the placenta only once so that compartment 3 never becomes large enough for $F_{23}$ to be significant. If $F_{12}$ is also small, then $F_{32}$ must be the component of $F_{21} + F_{32}$ which is decreased in the case of inorganic mercury, resulting in the increased size of compartment 2. We hope to reach conclusions about the magnitude of $F_{12}$ by measuring the clearance of mercury from fetus to dam.

Further investigation of the relationship between organic and inorganic mercury clearances seems warranted since both forms of mercury are present in the environment and constitute a potential hazard to the developing fetus. As previously indicated, there seems to be limited evidence supporting the existence of a specific carrier and possibly an active transport mechanism for organic mercury. Elucidation of the relationship between such a mechanism and the movements of inorganic mercury across the placenta should be valuable in understanding the risk of exposure incurred by the fetus from each form of mercury when both are present in mercury-polluted environments.

## FETAL UPTAKE OF METHYLMERCURY IN MID THROUGH LATE GESTATION IN GUINEA PIGS, SHEEP, AND SWINE

B. J. Kelman, B. K. Walter, S. E. Steinmetz, G. E. Jarboe, and L. B. Sasser

There is a considerable body of literature in which fetal uptake and/or consequent effects of methylmercury are described. However, most studies have been done at a single period in gestation in mice and rats. The number of studies involving the uptake of methylmercury as a function of gestational age is very limited, and almost none examine the problem in terms of more than one species. Further, only one or two correct reported values of methylmercury in tissues for methylmercury bound in red cells. Since a high percentage of methylmercury is bound to hemoglobin in red cells, especially after I.V. injection, considerable error can be introduced by not correcting tissue mercury for red-cell-bound mercury.

Existing gaps in the literature have warranted a more complete study of methylmercury uptake by fetuses. To accomplish this, we have nearly completed a series of experiments in which fetal uptake and tissue distribution of mercury were measured following low maternal dosing with methylmercury in guinea pigs, sheep, and swine. In all measurements, appropriate corrections were made for red-cell-bound methylmercury. Pregnant animals were injected with methylmercury 24 hr before experiments, and fetal uptake was measured at 3–5 gestational ages covering the last two-thirds of gestation.

After 24 hr, the fetuses of all three species absorbed similar amounts of mercury when adjustments were made for weight differences between species. Further, tissue mercury concentrations, adjusted for weight changes, were similar at corresponding gestational ages. It was apparent that differences in the number of tissue layers or distance separating maternal and fetal circulations in the placentas of different species had only a limited effect on the amount of methylmercury to which the developing fetus was exposed.

The ability of the fetus to absorb mercury increased from the beginning of the second third of gestation until approximately 80% of gestation. In the last 20% of gestation fetal absorption of methylmercury decreased until it reached slightly more than 50% of the mercury absorbed at 80% of gestation.

Although fetuses absorbed less methylmercury at the end of gestation, there appeared to be a shift of mercury from liver, kidney, and carcass to the blood and brain. The shift of methylmercury to blood and brain may indicate that the developing fetus becomes more sensitive to methylmercury late in gestation than is apparent by examining simple uptake of methylmercury. Since this phenomenon was evident in three species with very different types of placentas, one of which is similar to the human placenta, there is reason to conclude that a shift of mercury to blood and brain may also occur in humans late in gestation.

## THE INFLUENCE OF SELENIUM ON THE DISTRIBUTION OF METHYLMERCURY AND MERCURY CHLORIDE IN THE PREGNANT RAT

L. B. Sasser and G. E. Jarboe

The protective effect of selenium against mercury toxicity has been well documented although its mechanism remains unclear. Since mercury has embryotoxic and teratogenic actions on the fetus, our studies were conducted to determine if Se could reduce the transfer of mercury to the fetus and thus possibly reduce the toxic effects of mercury.

Rats were orally dosed each day of gestation with either 0, 20, or 100 $\mu$g of selenium as rodium selenite. On the 18th day of gestation, the rats were dosed orally or intravenously with 5 $\mu$Ci of either $CH_3{}^{203}HgCl$ or $^{203}HgCl_2$; on day 21 they were killed. Maternal and fetal tissues were removed and assayed for $^{203}Hg$.

Methylmercury was distributed more evenly throughout maternal and fetal tissues than was mercuric chloride. A 500-fold difference existed between maternal liver and fetal brain mercuric chloride concentrations and less than a 10-fold variation was seen in methylmercury tissue concentrations. Mercury concentrations in fetal tissue were approximately five times greater following intravenous doses of methylmercury than of mercuric chloride. The mercuric

chloride concentration of the placenta, however, was 15 times greater than that of methylmercury. Over 15% of either an oral or intravenous dose of methylmercury was transferred to the fetus as $^{203}$Hg. Total $^{203}$Hg uptake from mercuric chloride was 4 and 0.05% of the intravenous and oral dose, respectively.

Although there were no marked changes in the mercury contents of tissues of rats treated with 20 μg of selenium, a 3-fold increase occurred in maternal brain tissue when the dietary selenium level was increased to 100 μg per day. We also observed a slight increase in mercury uptake in brains of rats on the high selenium treatment following intravenous doses of mercuric chloride, but selenium did not alter the uptake of either form of mercury by fetal tissues.

It is difficult to explain the protective action of selenium against mercury toxicity because of the apparent increase of mercury in the brain. The duration of this increased retention is sufficient to rule out any subsequent rapid decline in retained mercury as a result of selenium. These data suggest that the role of selenium in protecting against mercury toxicity is not a direct effect on distribution, retention, or gastrointestinal absorption and that selenium does not alter the fetal distribution of mercury.

## TRANSPORT OF CADMIUM ACROSS THE PERFUSED PLACENTA OF THE GUINEA PIG IN LATE GESTATION

B. J. Kelman and B. K. Walter

Previously existing data indicate that little cadmium is transported to the developing fetus in most species. Unfortunately, severely limited data are available about cadmium transport across the placenta since all previous studies of cadmium transport to the fetus are confounded by an inability to separate fetal uptake and placental transport.

In our study, we perfused the fetal side of guinea pig placentas of about 60 days gestation in situ. The dams were injected with tritiated water (to indicate changes in maternal blood flow) and high specific activity $^{115m}CdCl_2$. Perfusion pressure, maternal cardiac rate, ECG, blood pressure, and respiratory rate were monitored. A schematic diagram of the perfusing system is the same as that presented in Fig. 3 except that radiocadmium is injected into the maternal circulation instead of radiomercury.

Our results show that the widely accepted premise that there is a strong "placental barrier" to the movements of cadmium across the placenta is not correct. The clearance of cadmium (milliliters of maternal plasma containing an amount of radiocadmium equal to that entering the perfusate per min) at an umbilical flow of approximately 2.5 ml/min was more than twice as large as the clearances of methylmercury or calcium measured under similar conditions. Since both methylmercury and calcium seem to cross the placenta relatively easily, it is clear that cadmium in maternal plasma crosses the placenta with little difficulty. Further, cadmium clearance is linearly related to umbilical flow rate whereas methylmercury and calcium clearances are not. Thus it appears that cadmium crosses the placenta of the guinea pig rapidly by simple diffusion, unlike the situation with methylmercury or calcium. Table 2 shows the relationships between cadmium clearances and perfusion rates measured in a typical experiment.

Even though the cadmium present in maternal plasma readily crosses the guinea pig placenta, the fetus is still exposed to little cadmium from an I.V. dose. Fig. 5 shows the radiocadmium in maternal blood and plasma as a function of time in a typical experiment. It is apparent that cadmium, introduced by I.V. injection into the maternal bloodstream, is quickly removed from both maternal whole blood and plasma. Other studies have shown that this cadmium is bound mainly in the liver and kidney by a protein, metallothionien. The implication of this data is that although the fetus is normally exposed to very little cadmium, any pathological condition such as disease or malnutrition which might affect maternal production of metallothionien could expose the developing fetus to greatly increased amounts of cadmium.

**Table 2 Relationships Between Cadmium Clearance and Perfusion Rate**

| Dam | Placenta | a* | b* | P< | r | Range of perfusion rate | Cd clearance at 2.5 ml/min ± SE |
|---|---|---|---|---|---|---|---|
| 1 | 1 | 0.187 | 0.044 | >0.05 | 0.28 | 1.76–2.95 | 0.300 ± 0.068 |
| 1 | 2 | −0.056 | 0.130 | 0.001 | 0.67 | 1.78–2.91 | 0.271 ± 0.017 |
| 2 | 1 | 0.043 | 0.025 | 0.005 | 0.49 | 1.15–3.47 | 0.106 ± 0.008 |
| 2 | 2 | 0.099 | 0.018 | 0.05 | 0.31 | 1.68–3.44 | 0.144 ± 0.011 |
| 3 | 1 | −0.122 | 0.289 | 0.001 | 0.62 | 1.89–3.50 | 0.601 ± 0.041 |
| 3 | 2 | 0.065 | 0.410 | 0.001 | 0.85 | 1.27–2.11 | 1.089 ± 0.044 |
| 4 | 1 | 0.128 | 0.030 | 0.01 | 0.57 | 1.76–2.83 | 0.202 ± 0.008 |
| 5 | 1 | −0.109 | 0.164 | 0.001 | 0.91 | 1.38–3.19 | 0.302 ± 0.011 |
| 6 | 1 | 0.069 | 0.159 | 0.001 | 0.72 | 1.70–2.46 | 0.466 ± 0.034 |
| 6 | 2 | 0.049 | 0.036 | 0.05 | 0.50 | 1.40–2.00 | 0.137 ± 0.028 |

*Y = a + bx, where Y = cadmium clearance and x = perfusion rate.

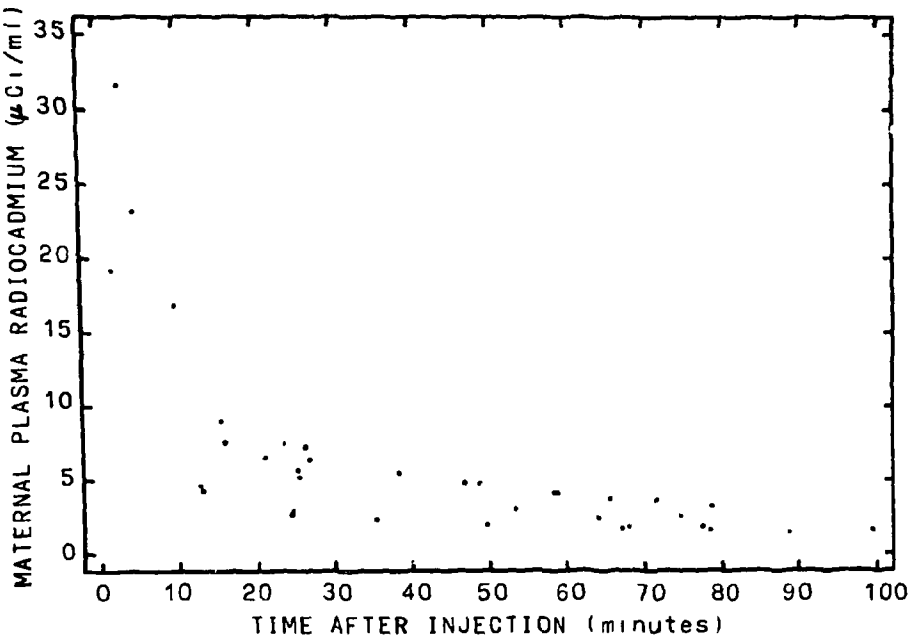



Fig. 5 Maternal plasma radiocadmium as a function of time after injection.

## INTESTINAL ABSORPTION AND RETENTION OF CADMIUM IN NEONATAL RATS

L. B. Sasser and G. E. Jarboe

Although Cd is poorly absorbed, it accumulates in the body throughout life and is not extensively excreted. Heavy metals such as Ce, Pu, Zr, Nb, and Pb are more readily absorbed in newborn rats than in adults, but little is known about Cd absorption in the newborn. If Cd absorption were significantly elevated during this period, then exposure to Cd during infancy could pose an increased risk of Cd toxicity or result in a greater lifetime Cd body burden.

Newborn rats were dosed with $^{115m}CdCl_2$ (5.6 μg/kg) either at 2 or 24 hr of age and killed at intervals between 1 and 21 days of age. Juvenile rats were similarly dosed at 6 wk of age and killed 1 to 4 days after dosing.

Juvenile rats absorbed only 0.5% of the Cd dose, whereas rats dosed at 2 and 24 hr of age absorbed approximately 12 and 5% of the dose, respectively. During the first 24 hr after dosing, 2-hr-old pups absorbed about 8% of the Cd dose or approximately six times more than did 24-hr-old pups.

Cadmium was tenaciously retained in the gastrointestinal tract of newborn rats, with over 60% of the Cd dose remaining in the gastrointestinal tract 15 days after dosing. Nearly 70% of this retained Cd was located in the upper and mid sections of the small intestine 15 days after dosing. By day 21 practically all of the Cd retained in the gastrointestinal tract had been excreted. Weaning 16-day-old pups enhanced Cd excretion, whereas continued nursing without solid food prolonged Cd retention. In contrast, nearly 95% of the Cd dose was excreted in 1 day by rats dosed when 6 wk old.

The prolonged retention of Cd by the intestinal tract of the newborn is probably the result of adsorption of Cd to intestinal mucosa and uptake by the intestinal epithelial, crypt, and lymphatic cells. The age of excretion of this Cd load corresponds to the age of extrusion of intestinal villi from the rat intestine which may be caused by a change from milk to solid food. This may have a significant impact on the interpretation of short term balance studies of Cd and other heavy metals.

If Cd absorption is greater and intestinal retention is prolonged in the human infant, Cd exposure in infancy could result in greater lifetime Cd accumulation because of its long biological half-life.

## LITTER SIZE, BODY WEIGHTS, AND BLOOD VALUES OF RAT FETUSES AFTER INGESTION OF CADMIUM BY THE DAM DURING GESTATION

Polly Martin, Brenda Hitchcock, and Joyce King

Preliminary studies of increased cadmium absorption by animals suffering from iron deficiency suggest that pregnancy and its resultant anemia merit special investigation to determine whether women bearing children are more susceptible to cadmium pollution. In a preliminary study, 6 bred normal rats ingesting water containing 200 ppm Cd beginning at day 0 of gestation produced no viable young. Five females died between days 12 and 16 of gestation and one littered with four dead puplets; therefore, in the following studies normal and iron-deficient rats received water containing either 0, 50, or 100 ppm Cd for 20 days of a 22-day gestation period.

The effect of chronic Cd ingestion during gestation was more severe in iron-deficient rats than in normal females. No viable fetuses were present at day 21 gestation in iron-deficient rats drinking the Cd water. Implantation sites indicated reabsorption began around day 12, a period of increased need for iron. Nondosed iron-deficient rats produced litters of average size (11) for their deficiency state with fetal Hb of 3.4 (Table 3). At 100 ppm, fetal Hb's were 3.7 and fetal weights 3.9 g.

Table 3 Statistics on Fetuses and Dams after Chronic Ingestion of Cd

| Groups | Treatment | Litter size | Fetal weight (g) | Fetal Hb* | Fetal Ht† | Dam Hb | Dam Ht |
|---|---|---|---|---|---|---|---|
| Iron-deficient | 0 | 11 | 4.3 | 3.4 | 14.8 | 7.7 | 23.2 |
| | 100 | 12 reabsorbed | – | – | – | 6.1 | 17.3 |
| | 50 | 13.5 reabsorbed | – | – | – | 5.6 | 16.7 |
| Normal | 0 | 14 | 5.0 | 7.2 | 25.0 | 11.3 | 31.9 |
| | 100 | 14 | 3.9 | 3.7 | 16.5 | 8.5 | 24.1 |
| | 50 | 14 | 4.7 | 4.8 | 18.5 | 9.9 | 28.0 |

*Hb = hemoglobin, expressed as g/100 ml of blood.
†Ht = hematocrit, packed cell volume.

In previous work, Cd appeared to divert iron from the fetus, and this blockage in addition to limiting the iron supply may cause the reabsorptions. The data on fetuses from normal dams indicate that as concentration of Cd increased in the water, hemoglobin levels decreased in the fetuses with a frank iron-deficient state occurring at the highest level of ingestion. Whether the results are a direct effect of Cd being deposited in the fetus or an indirect effect of iron, zinc, and copper being diverted from the fetus is being clarified in ongoing studies.

## UPTAKE AND DISTRIBUTION OF AN ORAL DOSE OF CADMIUM IN POSTWEANLING MALE AND FEMALE, IRON-DEFICIENT AND NORMAL RATS

Polly G. Martin, Brenda Hitchcock, and Joyce King

Children between 6 mo and 2 yr old experience rapid growth and commonly suffer from iron deficiency. They thus constitute an area of concern when considering the greater absorption of many pollutants which occurs in anemic animals. To study the parameters of anemia, age, and sex on Cd absorption, postweanling (42-day-old) male and female, iron-deficient and normal rats were given an oral dose of either carrier-free $^{109}Cd$ (3 $\mu$Ci) or a dose containing 0.025 mM Cd plus $^{109}Cd$. Animals were sacrificed 3 days after dosing. Whole bodies, livers, kidneys, washed intestinal tract, and saline rinses were counted for activity to determine distribution, uptake, and transfer of Cd and transit time through the gastrointestinal tract. Uptake and transfer of an oral dose of Cd were enhanced by iron deficiency and were greater in the female than in her male counterpart. Although the percentage of radioisotope uptake from the tracer dose was greater than from the dose containing cold Cd, the percentage transferred was similar in the two groups (Table 4). The greatest amount of Cd was deposited in the liver. The comparable group receiving a tracer dose showed about one-half of these depositions. When Cd absorption was based on body weights, differences between males and females and iron-deficient and normal animals became even greater.

The relationship between concentration and transfer is noted in many nonessential minerals that combine diffusion and active transport. In the case of essential minerals such as iron, however, the percentage of absorbed dose decreases. The preliminary results of the study pose several further questions: (1) Is the intestine of iron-deficient rats more permeable than that of normal rats, allowing greater diffusion of Cd across the membranes; or is there a common transport mechanism shared with iron (unsaturated during anemia) at both the uptake and transfer steps that results in more absorption of Cd? (2) Is the same mechanism which allows greater uptake and transfer of a mineral such as iron in the female rat compared to the male also operable for Cd absorption? Experiments to clarify these possibilities are currently being considered.

Table 4 Uptake and Transfer in Postweanling Rats of an Oral Dose of Cd

| | % of uptake* | % transfer† | % transfer/ 100 g body weight |
|---|---|---|---|
| Trace | | | |
| Normal–M | 1.447 | 0.664 | 0.390 |
| Normal–F | 1.799 | 1.372 | 1.070 |
| Iron-deficient–M | 8.930 | 3.990 | 2.890 |
| Iron-deficient–F | 11.100 | 5.210 | 5.163 |
| 0.025 mM | | | |
| Normal–M | 1.943 | 1.441 | 0.822 |
| Normal–F | 2.521 | 2.210 | 1.581 |
| Iron-deficient–M | 5.614 | 3.279 | 2.555 |
| Iron-deficient–F | 8.423 | 5.749 | 5.572 |

*Uptake = amount in body–gastrointestinal contents (small intestine, cecum, and large intestine were rinsed in saline).
†% transfer = amount in body–gastrointestinal tract and contents.

## POSTNATAL VIABILITY AND GROWTH IN RAT PUPS AFTER ORAL CADMIUM ADMINISTRATION DURING GESTATION

Polly G. Martin

We have previously shown that (1) Cd is absorbed to a greater degree in iron-deficient pregnant rats; (2) Cd passes through the placenta; (3) viable fetuses are present at day 21; and (4) iron and zinc are reduced in Cd-dosed fetuses. No data are available, however, on postnatal viability, growth, Cd retention, or blood values in pups that received a dose of Cd in utero after oral administration to the dam.

In our study, pregnant, iron-deficient, and normal rats were dosed on day 18 of gestation with 8 $\mu$Ci $^{115}$Cd plus 0.1 mM Cd or served as nondosed normal or iron-deficient controls. All animals were allowed to litter. Viability of pups from iron-deficient dosed dams was severely affected with only two of six pregnant females producing live young, nine and seven pups, respectively (Table 5). Two of the iron-deficient dosed produced stillbirths, and two sacrificed the day after the scheduled delivery date contained dead fetuses. In normal dosed animals, one of the six produced a litter that was dead. When the weights of normal dosed pups were compared to normal nondosed pups the reductions were greater (26 vs. 30.8 gm, respectively) than those noted in the iron-deficient dosed (18.6 vs. 20.6 for iron-deficient pups). Liver weights from normal dosed pups were 90% of liver values of normal nondosed animals, whereas the reduction was greater to 70% when comparing iron-deficient groups. This is undoubtedly a reflection of the greater reduction in iron and zinc concentrations in the livers from iron-deficient dosed fetuses than was observed in normal dosed pups. Iron-deficient pups contained 50% more Cd activity than normal pups although at day 21 of gestation both groups contained similar amounts of Cd.

The data suggest the action of Cd on postnatal viability and growth is possibly indirect with the diversion of iron from the fetuses in those animals which are already iron-deficient and produce pups so low in hemoglobin that they are born dead, die shortly after birth, or are not delivered. In the normal animal, Cd absorption and transfer to the fetus appear to contribute to a less thrifty pup.

**Table 5 Statistics on Litter Size, Viability, and Growth of Rat Pups Dosed In Utero with Cadmium**

| | No. dosed | No. living litters | No. per litter | Alive at day 14 (standardized to 7) | Average weight at day 14 (g) | Pups' Hb | Liver weights (g) |
|---|---|---|---|---|---|---|---|
| Normal pregnant nondosed | 6 | 6 | 12.1 | 7 | 30.8 | 8.4 | 0.902 |
| Normal pregnant dosed | 6 | 5 | 11.0 | 7 | 26.0 | 7.2 | 0.813 |
| Iron-deficient pregnant nondosed | 5 | 5 | 12.2 | 7 | 20.6 | 4.2 | 0.810 |
| Iron-deficient pregnant dosed | 6 | 2 | 8 (9, 7) | 5 (3, 7) | 18.6 | 4.2 | 0.572 |

## ABSORPTION AND DISTRIBUTION OF AN ORAL DOSE OF CADMIUM IN PREGNANT (DAY 18) AND NONPREGNANT, IRON-DEFICIENT AND NORMAL RATS

Polly G. Martin

Although contradictory data exist on Cd absorption, its ultimate effects, and its possible passage through the placenta, enough evidence has been gathered to indicate that a small amount of Cd can be deposited in the fetus of normal animals. This factor coupled with data indicating increased absorption of Cd during the iron-deficient state suggested that studies on Cd distribution in iron-deficient animals should be undertaken. Pregnant and nonpregnant, iron-deficient and normal animals were assigned to groups receiving a dose of 8 $\mu$Ci $^{115}$Cd plus 0.025 mM Cd, 0.050 mM Cd, or 0.100 mM Cd, or to a group receiving no Cd. Pregnant animals dosed on day 18 of gestation and nonpregnant animals dosed at a comparable age were killed after 3 days.

The stress of Cd given orally was greater on the iron-deficient females (Table 6). Fetuses were all viable at day 21 of gestation. Although the percent of the dose deposited in fetuses was not different in any of the groups (Table 7), variations were great. The amount in the placenta was as much as or more than that in the fetus in both normal and iron-deficient rats with concentrations of Cd in the groups receiving 0.100 mM Cd greater than in the group receiving 0.025 mM Cd. Examination of the placentae indicated only 6 out of the hundreds evaluated were

**Table 6 Viability Data**

| Treatment | 0.025* | 0.050 | 0.100 |
|---|---|---|---|
| Normal nonpregnant | 8/8† | 8/9 | 8/8 |
| Normal pregnant | 10/10 | 7/7 | 9/9 |
| Iron-deficient nonpregnant | 6/8 | 7/7 | 5/6 |
| Iron-deficient pregnant | 8/9 | 7/7 | 7/10 |

*Cd concentration: 8 $\mu$Ci $^{115}$Cd + (a) 0.025 mM Cd, (b) 0.050 mM Cd, (c) 0.1 mM Cd.
†Number survived/number dosed.

Table 7 Distribution of an Oral Dose of Cd Administered on Day 18 of Gestation in the Rat

| Treatment | 0.025 | 0.050 | 0.100 |
|---|---|---|---|
| | % in Liver (A) | | |
| Normal nonpregnant | 0.09 ± 0.10 | 1.35 ± 0.16 | 1.94 ± 0.20 |
| Normal pregnant | 1.37 ± 0.09 | 1.72 ± 0.34 | 2.86 ± 0.30 |
| Iron-deficient nonpregnant | 1.38 ± 0.17 | 2.23 ± 0.34 | 2.78 ± 0.64 |
| Iron-deficient pregnant | 2.24 ± 0.16 | 2.36 ± 0.24 | 3.29 ± 0.23 |
| | % in Kidney (B) | | |
| Normal nonpregnant | 0.068 ± 0.003 | 0.075 ± 0.004 | 0.093 ± 0.007 |
| Normal pregnant | 0.086 ± 0.006 | 0.124 ± 0.046 | 0.092 ± 0.011 |
| Iron-deficient nonpregnant | 0.113 ± 0.009 | 0.109 ± 0.010 | 0.157 ± 0.011 |
| Iron-deficient pregnant | 0.123 ± 0.009 | 0.115 ± 0.005 | 0.106 ± 0.008 |

% in Fetuses and Placentae (C)

| | 0.025 | | 0.050 | | 0.100 | |
|---|---|---|---|---|---|---|
| | Fetus | Placenta | Fetus | Placenta | Fetus | Placenta |
| Normal pregnant | 0.095 ± 0.037 | 0.154 ± 0.010 | 0.230 ± 0.066 | 0.203 ± 0.038 | 0.226 ± 0.047 | 0.260 ± 0.028 |
| Iron-deficient pregnant | 0.196 ± 0.031 | 0.195 ± 0.013 | 0.120 ± 0.018 | 0.235 ± 0.022 | 0.189 ± 0.031 | 0.254 ± 0.028 |

hemorrhagic, although this effect has been reported after I.V. injections of Cd where the placenta is insulted with massive amounts of Cd instantaneously which results in disruption of the vascular system and fetal death. The amount of Cd deposited in the liver increased with increasing dose (Table 7). The iron-deficient animals accumulated greater amounts than did comparable normal animals. When adjusted for liver weight, differences were even greater. In contrast the kidney sequestered increasing amounts of Cd but the percent of the dose varied only slightly between groups and doses with no obvious trends (Table 7).

The amount in the body increased from 0.34% in normal nonpregnant females to 1.40% in iron-deficient pregnant animals. The mechanism for handling increasing levels of Cd in the blood must be different between the two organs. It is evident from these data that Cd given orally is absorbed in greater amounts by anemic animals, especially during pregnancy. When Cd is taken up by the blood from the intestine at a low steady rate and is circulating at low concentrations, the placenta appears permeable and Cd is deposited in the fetus.

## INTESTINAL ABSORPTION OF CADMIUM IN VITRO

R. J. Chertok, L. B. Sasser, M. F. Callaham, and G. E. Jarboe

Cadmium, a toxic heavy metal, is an increasing environmental problem, but the mechanism of its intestinal absorption is poorly understood. Pathological changes have been demonstrated in the human from both acute and chronic exposures to cadmium. From limited data on experimental animals, it appears that Cd absorption from the gastrointestinal tract ranges from 0.5 to 10% of the ingested dose. Our experiments were designed to determine or characterize the mode of cadmium movement from the intestinal lumen into the intestinal tissue and the subsequent movement of cadmium from the tissue to the contraluminal surface.

The everted gut sac technique was used in these experiments. The proximal half of the small intestine was removed from mature male rats, everted, and cut and tied into four sacs of equal length. The sacs were filled with 0.5 ml of isotonic saline containing a trace quantity of tritiated dextran which was used as a marker for water movement. The sacs were placed in a bathing medium of isotonic saline, $^{115m}Cd$, and cadmium in appropriate concentrations. The sacs were incubated at 37°C for 30 min and removed; samples were then taken from the bathing medium, sac fluid, and sac tissue and analyzed for $^{115m}Cd$.

The uptake of cadmium into the intestinal tissue from the solution bathing the luminal surface exhibited first order kinetics with respect to cadmium concentration (1 μM–4.6 mM) of the bathing solution at 30 min incubation. This is indicative of a diffusion process although an active process has not been ruled out experimentally.

The accumulation of cadmium in the fluid bathing the contraluminal surface of the sac after 30 min was directly proportional to the intestinal tissue content of cadmium up to a concentration of $1 \times 10^{-6}$ m Cd/g tissue. At higher tissue concentrations of cadmium, however, there was a marked increase in the accumulation of cadmium in the sac fluid. To determine if this increase in tissue efflux of Cd to

the contraluminal fluid could be a reflection of an increase in tissue free cadmium after saturation of cadmium binding sites, time-based experiments were performed where samples were taken at 5-min intervals to 30 min. Results show that the tissue reaches a cadmium concentration of approximately 1 μm/g tissue wet weight and remains at this concentration. This steady state was attained in 10 min at a cadmium concentration of $1 \times 10^{-2}$ M in the bathing solution and 20 min at a Cd concentration of $1 \times 10^{-3}$ M. Lower concentrations of cadmium did not attain this steady state level within 30 min. These results may explain the variability reported in the literature of the transfer of cadmium to the circulatory system after an oral dose. Cadmium moves into the intestinal tissue at a rate proportional to the dose; most of it is retained in the tissue until the cadmium binding sites are saturated; after which the cadmium then moves up to the contraluminal surface of the tissue. At very low doses cadmium is taken up by the tissue with very small amounts passing into the circulatory system while at much larger doses the cadmium moves into the tissue and saturates the binding sites resulting in a subsequent increase in the amount passing into the circulatory system.

## THE EFFECT OF CALCIUM AND PHOSPHORUS ON THE ABSORPTION AND TOXICITY OF CADMIUM

L. B. Sasser, R. J. Chertok, M. F. Callaham, and G. E. Jarboe

There is mounting evidence that elevated Cd intake can adversely affect Ca metabolism, but the role of Ca and P in Cd absorption and toxicity is not clearly understood. In an attempt to clarify this role, mature male rats in our experiments were pretreated for four wk with diets containing (1) adequate Ca and P, (2) low Ca and P, (3) high Ca and P, (4) high Ca and low P, or (5) low Ca and high P. Rats from each diet were divided into three groups and each given a single oral dose of 10 μCi $^{115m}$Cd in solutions containing either 0, 5, or 25 mg of stable Cd. Six animals of each group were killed at either 6, 24, 48, 72, or 120 hr after dosing, and tissues were counted for $^{115m}$Cd.

Analysis of variance showed significant ($P < 0.05$) interrelationships between Cd dose and Ca-P levels of the diet for liver and kidney Cd concentration but not for Cd dose and total absorbed Cd. Low dietary Ca, regardless of the level of P, significantly ($P < 0.05$) increased both Cd absorption and liver and kidney Cd concentrations. Diets high in both Ca and P increased total Cd absorption only at the highest level of Cd intake. This high Ca diet also caused a significant increase in mortality in the highest Cd groups which may be related to higher liver Cd concentrations three days after dosing. Kidney Cd content (% dose) remained constant for all three doses of dietary Cd, whereas liver Cd content increased with increasing Cd in the diet. This suggests that excess Cd was initially retained in liver tissue, thus regulating the transfer to kidney tissue. It was concluded that the level of Ca in the diet can account for changes in Cd absorption and possibly Cd toxicity, while P levels have little influence.

## INTERACTIONS OF CADMIUM, ZINC, AND IRON IN THE IRON-DEFICIENT RAT

Polly Martin, Brenda Hitchcock, and Joyce King

The movements of essential trace minerals between maternal organs (adult females), between maternal and fetal systems, and within fetal systems after an oral dose of cadmium were investigated in pregnant, nonpregnant, iron-deficient, and normal rats. Hb values increased significantly from 8.2 to 10.2 in the iron-deficient dams and from 9.1 to 11.3 in normal animals. Fetal Hb values remained unchanged at 3.5 and 6.4, respectively. Levels of iron and zinc were reduced to a greater degree in whole bodies and livers from fetuses of iron-deficient dams with concentrations decreasing as levels of Cd increased. In nonpregnant rats dosing with Cd did not result in changes of Hb values [9.8 (iron-deficient) and 12.0 (normal)], but zinc concentrations in the liver increased 144% and 190%, respectively, over nondosed levels. In adult females iron concentrations in the liver remained stable. Although differences were apparent in zinc and iron levels in the kidney among the four treatment groups, no movement of these two minerals in or out of the kidney was noted at any level of Cd ingestion. The effect of Cd on the fetus may be indirect with the reduction in zinc and iron levels (dose dependent) causing the poor littering performance by the dam, decreased viability in the pups (especially the iron-deficient), and reduced growth rates.

Results suggest that in the pregnant animals, zinc and iron are diverted from the fetus, with zinc accumulating in the liver of the dam (200% greater than in controls), and with iron being incorporated into the maternal hemoglobin (Hb).

## SUBCELLULAR DISTRIBUTION OF IRON IN THE INTESTINAL MUCOSAL CELLS OF PREGNANT AND NONPREGNANT, IRON-DEFICIENT AND NORMAL RATS

Polly Martin, Nancy Kuemmerle, and Joyce King

To obtain a more complete understanding of the movement of physiological doses of iron across intestinal membranes, orally administered $^{59}$Fe was measured in five fractions of a homogenate from the small intestine of normal pregnant, iron-deficient pregnant, normal nonpregnant, and iron-deficient nonpregnant rats on adequate and low iron diets. Hemoglobin values were 10.8, 8.5, 12.4, and 7.6, respectively. Rats were dosed on day 18 of gestation or

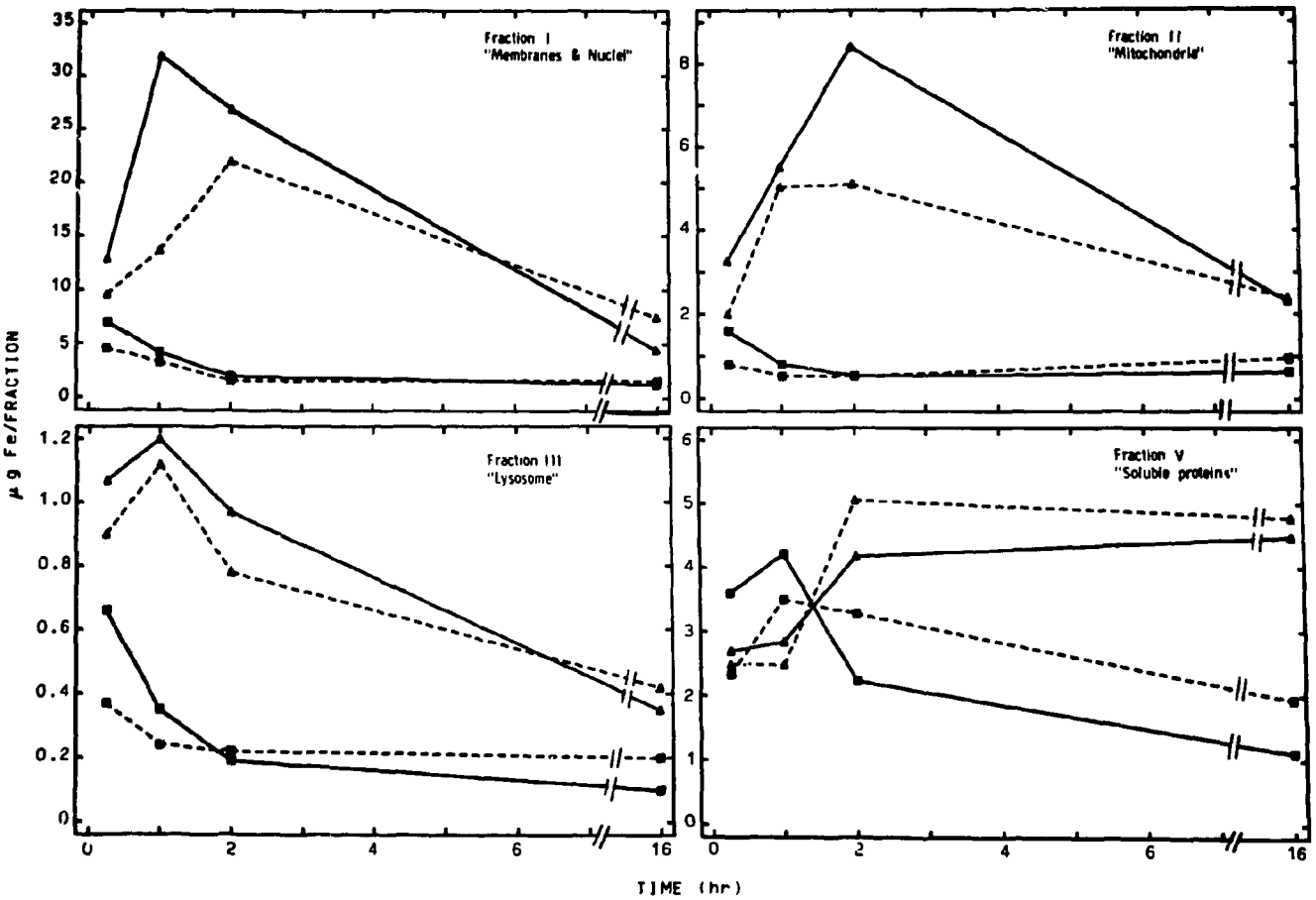



Fig. 6 Subcellular distribution of iron in four fractions of mucosal cells from the small intestine. The groups are: iron-deficient nonpregnant △- - -△; iron-deficient pregnant △—△; normal nonpregnant □- - -□; normal pregnant □—□.

at comparable ages for nonpregnant rats with 500 μg iron and 5 μC $^{59}$Fe and sacrificed after ¼, 1, 2, and 16 hr.

Based on the percent of $^{59}$Fe found in mucosal cell homogenates, differences in distribution between the groups were most pronounced at ¼, 1, and 2 hr with greater differences between the iron-deficient pregnant and nonpregnant rats than between the two normal groups (Fig. 6). By 16 hr, the milieu of the intestine had returned to an unstressed condition with distribution of iron between the fractions of the mucosal cells similar among the four groups (Fig. 6) with the exception of Fraction V "soluble proteins" ($P < 0.05$). The distribution of Fe was reversed from that observed at the earlier sampling times with the fraction from iron-deficient animals containing greater amounts than that from normal cells.

The greater association of iron with the membranes and nuclei in iron-deficient animals up to 2 hr after dosing suggests a mechanism which enables the anemic rat to immediately stimulate uptake (70%) and transfer (60%) (Table 8) during these initial hours in an effort to replenish depleted iron stores. This phenomenon is not observed in the fraction from iron-replete rats at any sampling period. The amount of iron associated with the fraction in iron-replete rats does not vary significantly with time as uptake and transfer are not greater than 15% of the dose at the end of 2 hr and transfer is maximal in the pregnant rat at 16 hr with 30% of the dose in the body. The minimal quantity of iron associated with the fraction in replete animals may constitute a negative feedback which inhibits excess uptake and transfer of iron.

## EFFECTS OF THYROID STATUS ON DIGESTIVE TRACT FILL AND FLOW RATE OF UNDIGESTED RESIDUES IN CATTLE

J. K. Miller and E. W. Swanson*

We have consistently found greater rumen fill in cattle with severe thyroid irradiation damage than in normal

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Table 8 Uptake* and Transfer† of $^{59}Fe$ after an Oral Dosing of Pregnant and Nonpregnant, Iron-Deficient and Normal Rats

| | 1/4 | | 1 | | 2 | | 16 | |
|---|---|---|---|---|---|---|---|---|
| | | | | % uptake | | | | |
| Normal nonpregnant | (7)‡ | 7.5 ± 1.0 | (11) | 7.0 ± 0.7 | (4) | 11.2 ± 3.1 | (4) | 15.5 ± 1.0 |
| Normal pregnant | (4) | 9.0 ± 1.3 | (9) | 14.2 ± 1.6 | (3) | 15.0 ± 3.6 | (5) | 30.8 ± 2.8 |
| Iron-deficient nonpregnant | (3) | 12.4 ± 0.1 | (6) | 25.2 ± 3.3 | (3) | 70.5 ± 5.1 | (4) | 23.6 ± 6.0 |
| Iron-deficient pregnant | (10) | 17.9 ± 1.9 | (6) | 46.2 ± 2.7 | (3) | 70.8 ± 4.4 | (3) | 90.6 ± 1.6 |
| | | | | % transfer | | | | |
| Normal nonpregnant | | 2.3 ± 0.2 | | 2.5 ± 0.3 | | 5.2 ± 1.0 | | 11.2 ± 1.0 |
| Normal pregnant | | 4.4 ± 1.2 | | 8.6 ± 1.1 | | 15.0 ± 1.0 | | 29.2 ± 1.0 |
| Iron-deficient nonpregnant | | 9.4 ± 0.3 | | 19.9 ± 2.0 | | 58.7 ± 4.0 | | 78.6 ± 6.0 |
| Iron-deficient pregnant | | 13.0 ± 1.3 | | 36.2 ± 3 0 | | 62.0 ± 3.0 | | 87.5 ± 2.0 |

*Uptake = % in whole body−gastrointestinal tract contents.
†Transfer = % in whole body−% in gastrointestinal tract and contents.
‡Number of animals in the group.

cattle at slaughter. Since hypothyroid cattle ate less, the greater fill indicated a prolonged retention of feed residues. This was confirmed by administration of a nonabsorbed passage rate marker ($^{144}Ce^{144}Pr$) as single or daily doses (Miller et al., 1974). Single oral doses were used to follow movement of feed residues through digestive tracts of normal and hypothyroid cows. The rate constant for marker flow in hypothyroid cows was less than half that for normal cows (Table 9). During daily administration, hypothyroid cows excreted only 71% as much of the marker in feces as normal cows, but their digestive tracts contained twice as much marker at slaughter.

Thyroxine replacement therapy (8 g thyroprotein daily) for 70 days raised plasma thyroxine to the normal range, increased feed intake, and reduced retention of feed residues to near normal in hypothyroid cows. These results indicate retention time of feed residues in the bovine digestive tract is affected by thyroid status. Retention time can in turn influence digestibility of organic constituents as well as absorption of mineral elements (Madsen et al., 1975).

Table 9 Effect of Thyroid Status on Feed Intake, Digestibility, and Retention in the Bovine Digestive Tract

| Item | Normal | Hypothyroid | Hypothyroid + thyroxine |
|---|---|---|---|
| Plasma thyroxine (μg/100 ml) | 4.9 | 0.5 | 4.2 |
| Hay dry matter intake (kg/day) | 9.0 | 5.0 | 7.5 |
| Dry matter digestibility (%) | 54.2 | 57.2 | 60.7 |
| Rumen contents (% of body weight) | 7.1 | 13.6 | 9.2 |
| Recovery of passage marker | | | |
| Feces (% of total dose) | 76.6 | 54.4 | 78.4 |
| Digestive tract (% of total dose) | 21.5 | 43.5 | 18.9 |
| Rate constant, k ($Hr^{-1}$)* | −0.0485 | −0.0224 | −0.0410 |

*Equation fitted to cumulative recovery of marker in feces was $\hat{Y} = A - Be^{-kt}$

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## UPTAKE AND DISTRIBUTION OF ORALLY ADMINISTERED PLUTONIUM COMPLEX COMPOUNDS IN MALE MICE


J. F. Weiss


In an era of widespread energy shortages and consequent increased potential use of plutonium for fueling reactors, the problem of increased releases to the environment of plutonium-contaminated materials must be considered. Under these circumstances the oral route to man from contaminated foodstuffs and water takes on added significance since recent work indicates an appreciable uptake of plutonium by plants grown on contaminated soil (Dahlman et al., 1976). The movement of plutonium in wet humid environments may be affected by the formation of soluble organic complexes in the soil or water. The gastrointestinal uptake of plutonium in soluble complexed form has not been adequately studied. To this end we have dosed mature male mice with four soluble plutonium complex compounds with graduated dissociative stabilities to determine what effect the chemical nature of soluble plutonium has upon uptake, retention, and excretion of the element from a single oral dose.

The mice were dosed by gavage with 1.25 μCi each of $^{239}Pu$ citrate, acetylacetonate, tartrate, and EDTA chelate. They were then killed 1, 2, 3, or 4 days after dosing; lungs, femur, intestine, liver, and the remainder of the carcass were taken for plutonium analysis. Feces and urine samples

were also taken from each chemical group for 4 consecutive days and analyzed. Plutonium analyses were performed by methodologies developed for fast $\alpha$ emitter sample analyses. Our results indicate that there is little difference in plutonium absorption among these four soluble complexes. The EDTA chelate shows somewhat greater absorption than the others but is also more rapidly and more completely excreted over the 4-day period. Retention of the other three complexes was greater than for EDTA by the 4th day and about equal in carcass, liver, and bone, and little statistical evidence for decorporation of plutonium could be found for any compound except for EDTA over the 4-day period studied.

Thus it is most likely that plutonium does not become unbound from EDTA to any extent while in corporeal residence and that the other complexes with lower order formation constants dissociate and the plutonium becomes bound to tissue components. The order of dissociative stability of these compounds is EDTA > citrate > acetylacetone > tartrate; the largest difference is between the first two. This seems to suggest that the binding of plutonium (IV) to various tissue components is very strong in terms of thermodynamic binding constants.

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## CROSS PLACENTAL TRANSFER OF PLUTONIUM IN MICE


J. F. Weiss and H. E. Walburg


An attempt has been made to define the dose-uptake relation for plutonium in fetal tissue of pregnant mice. Data suggesting the possibility of an inverse dose-uptake relation and hence greater risk to unborns at environmental dose levels (Finkel, 1947) have presented an increased "need to know" about plutonium transplacental movement. BALB/c mice were dosed by tail vein injection during late pregnancy with three concentrations of $^{239}$Pu (IV) citrate. Forty-eight hr following injection, the mice were killed and tissues readied for analysis. Analyses were performed according to methodologies developed for counting fast $\alpha$ emitter samples.

Results of this work indicate that the percentage of plutonium incorporated into fetal tissue rises with decreasing dose to levels of 0.1 $\mu$Ci per animal. At lower dose levels, fetal as well as placental and maternal femur content decrease proportionally along with administered dose. The highest dose level used showed significantly reduced plutonium activities in fetus, placenta, and maternal femur, and significantly increased activity in the remainder of the carcass. Figure 7 shows our data and indicates that in order to make reasonable estimates regarding placental transport in man or other species at environmental levels, one must extrapolate only from low dose data. It can be concluded

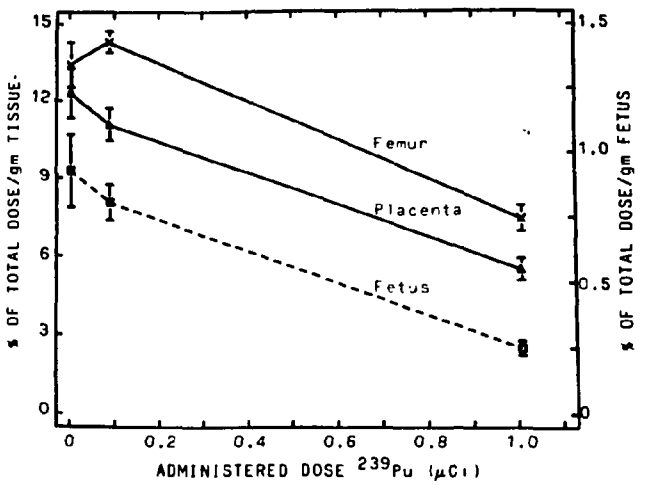


Fig. 7 Relation between administered dose of $^{239}$Pu and content in femur, placenta, and fetus.

that the deposition of plutonium in the feto-placental unit of mice is an inverse function of the dose at levels of about 0.1 $\mu$Ci/mouse and above. One explanation could be competitive inhibition of active transport mechanisms by an excess number of atoms present from large doses, the so-called mass effect. Another explanation could be that a body compartment, most likely the liver, is retaining a greater percentage of the dose at higher levels and that at the higher dose levels, the femur and placenta are thus never exposed to as proportionately high a level of plutonium as they are at lower dosage levels.

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## STUDIES ON THE BINDING OF PLUTONIUM TO VARIOUS PROTEINS IN VITRO


J. F. Weiss


The increasing possibility of plutonium release to the environment has sparked new concern about oral uptake of the element from contaminated food and water supplies. Heavy metal uptake from the gastrointestinal tract is often initiated by binding of the metal to proteins of the mucosal lining. An attempt to define the extent of the interaction of plutonium (IV) with proteins has been initiated to deter-

mine which parameters most strongly affect intestinal absorption of this toxic heavy metal pollutant.

Competitive binding studies employing ultrafiltration membranes to separate protein-bound plutonium from nonprotein-bound soluble complexed plutonium are being performed. The oxalate system was chosen as the best operating system for maintaining both protein and complexed plutonium in solution. Oxalate does not complex plutonium so strongly as to preclude interaction between some proteins and the metal; thus the system allows for ligand transfer under moderately complexing conditions in a well defined buffer system at constant pH. Two classes of proteins are being investigated: the nonspecific metal binding proteins such as the globulins and albumins and the specific metal binding types such as transferrin and trypsin. Proteins of the first type exhibiting some affinity for plutonium in the oxalate system are chicken conalbumin and bovine serum albumin. No interaction was noted for casein, $\beta$ lactoglobulin, beef liver catalase, and bovine $\gamma$ globulin in the oxalate system at pH 6.5 where these proteins exhibited distribution coefficients of one or less. Very preliminary results indicate quite a large differential binding capacity for plutonium among proteins of similar type.

## IODINE METABOLISM IN THE PREGNANT OVINE

S. L. Hansard, J. K. Miller, and F. C. Madsen*

To study the absorption, tissue distribution, and transplacental movement of radioiodine, 18 gravid ewes were intravenously administered tracer doses of $^{125}I$. Fetal lamb nonthyroid tissues concentrated two to ten times more radioiodine per unit weight than corresponding maternal tissues during 72 hr after $^{125}I$ administration. Concentrations of $^{125}I$ in maternal nonthyroid tissues and fluids ranked: abomasal contents > abomasal tissue > bile > blood plasma > kidney > liver > pituitary = adrenal = pancreas > heart > rib end > spleen > rib shaft > gastrocnemius muscle. Fetal tissue and fluid concentrations through the second third of development were more uniform than maternal concentrations, ranking: blood > gastrocnemius muscle = kidney = adrenal = abomasum plus contents > ribs > heart = spleen = liver. Maternal $^{125}I$ excretion and thyroid uptake decreased with advancing gestation as more $^{125}I$ was transferred to products of conception (Table 10). A marked increase in total fetal $^{125}I$ at 12 wk of development was due primarily to uptake by the fetal thyroid. Fetal abomasal content $^{125}I$ concentrations averaged only 5 to 17% of maternal concentrations through the 12th wk but were two to three times maternal concentrations by the 20th wk of development.

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Thus cycling of iodine through the gastric stomach, an important mechanism for conserving iodine in the adult (Miller et al., 1974), did not develop until the final third of gestation. In addition to its thyroidal function, iodine is also concentrated, although to a lesser extent, by other tissues of the developing fetus and may play a microbicidal role (Pincus and Klebanoff, 1971).

**Table 10 Radioiodine Contents of Maternal and Fetal Tissues, Fluids, and Excreta 48–72 Hr after Dosing During Different Stages of Gestation**

| Item | Week of gestation | | | | |
|---|---|---|---|---|---|
| | 6 | 10 | 12 | 15 | 20 |
| | (% of maternal dose) | | | | |
| Maternal thyroid | 33.6 | 38.4 | 29.8 | 21.3 | 12.5 |
| Maternal feces | 1.0 | 2.8 | 2.4 | 2.0 | 4.4 |
| Maternal urine | 19.2 | 17.7 | 13.8 | 21.5 | 12.8 |
| Products of conception | 0.3 | 3.3 | 11.7 | 14.2 | 34.0 |
| Placenta | 0.2 | 1.0 | 1.6 | 1.5 | 1.5 |
| Fluids | 0.1 | 0.9 | 1.4 | 2.1 | 3.4 |
| Total fetus | 0.01 | 1.4 | 8.7 | 10.6 | 29.1 |
| Fetal thyroid | <0.01 | 0.9 | 7.4 | 8.9 | 12.0 |

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Pincus, S. H., and S. J. Klebanoff. 1971. Quantitative leukocyte iodination. *New Eng. J. Med.* 283:744.

## IODINE METABOLISM IN THE BOVINE AS INFLUENCED BY THYROID STATUS AND THIOCYANATE

J. K. Miller and E. W. Swanson*

Hypothyroidism in the bovine, whether induced by $^{131}I$ thyroid irradiation damage, surgical thyroidectomy, or the goitrogen thiocyanate (SCN), alters radioiodine metabolism in several ways (Miller et al., 1975). Effects shared by both hypothyroidism and SCN (although not always to the same degree) are (1) elevated concentrations in blood plasma (Table 11), (2) reduced plasma protein-bound radioiodine, (3) higher excretion in urine, and (4) lower excretion in feces. Differences include (1) a sharp reduction of the gastric secretion of radioiodine by SCN, while hypothyroidism has little apparent effect, and (2) a marked reduction of total radioiodine in digestive tract contents by SCN but an increase by hypothyroidism.

Higher urinary and lower fecal radioiodine excretions by hypothyroid animals probably resulted from lower

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Table 11 Iodine Metabolism in Normal, Thyroid-Damaged, and Thiocyanate Fed Cattle

| Item | Thyroid intact | | Thyroid damaged |
|---|---|---|---|
| | Control | SCN | |
| Plasma radioiodine (% of dose/liter) | 0.22 | 0.30 | 0.44 |
| Plasma protein-bound radioiodine (% of total radioiodine) | 80.0 | 55.0 | 12.0 |
| Urinary radioiodine (% of dose) | 35.8 | 47.4 | 45.7 |
| Fecal radioiodine (% of dose) | 38.0 | 31.0 | 23.0 |
| Gastric recycling (% of daily dose) | 450.0 | 90.0 | 500.0 |
| Total GI tract radioiodine (% of daily dose) | 75.9 | 41.1 | 149.2 |

protein-bound radioiodine as a percentage of total plasma radioiodine. Labeled thyroxine is excreted primarily in the feces but when given as iodine, urinary excretion predominates. Total gastrointestinal content radioiodine would be expected to increase in thyroid-damaged cows because of prolonged retention of feed residues (Miller et al., 1974), but to be reduced by SCN due to inhibition of gastric recycling of iodine.

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## THE ROLE OF THE ABOMASUM IN CONSERVATION OF IODINE IN THE BOVINE


J. K. Miller and E. W. Swanson*


The cow's gastric stomach (abomasum) is a major site for reentry of circulating iodine into the digestive tract. As much as five times the cow's daily iodine intake may be secreted into its abomasum during 24 hr. This was measured in relation to a nonabsorbed reference material in abomasal contents recovered after daily oral radioiodine administration for 8 days (Miller et al., 1975). In other cows fitted with abomasal cannulae, over 65% of a single intravenous radioiodine dose was recovered in abomasal drainage during 6 hr (Miller et al., 1974). Passage of digesta into the intestines and subsequent reabsorption of iodine were prevented in these animals by ligatures between the abomasum and duodenum.

High dietary iodine, which effectively blocked thyroid uptake of radioiodine, did not reduce abomasal radioiodine


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Table 12 Effect of Dietary Iodine and Thiocyanate on Thyroid Radioiodine Uptake and Recovery of Radioiodine from Cannulated Abomase During 6 Hr after Intravenous Administration

| Treatment | Thyroid | Abomasal content |
|---|---|---|
| | (% of dose) | |
| Control | 1.7 | 66.7 |
| 100 mg I/day | 0.13 | 63.6 |
| 1000 mg I/day | 0.0002 | 69.6 |
| 10 g NaSCN/day | 0.57 | 26.0 |

secretion (Table 12) but thiocyanate, the goitrogen, reduced radioiodine uptake by both the thyroid and abomasum. The abomasum may serve as a reservoir which concentrates circulating iodine and then gradually returns it to the bloodstream for uptake by the thyroid. This iodine concentrating action of the abomasum may promote conservation of iodine by transferring it from vascular to extravascular compartments, thus preventing its excessive loss in urine.

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## EFFECTS OF SIMULATED FALLOUT RADIATION ON IODINE METABOLISM IN DAIRY CATTLE


J. K. Miller, M. C. Bell,* and L. B. Sasser


Effects of simulated fallout radiation on metabolism of radioiodine, the component of most concern in early fallout, were investigated in lactating cows. Combined whole body and gastrointestinal tract irradiation resulted in a 53% drop in milk yield, but only 24% less radioiodine was secreted into milk. These results were obtained when 12 Holstein cows 2 months in lactation were each given 200 $\mu$Ci $^{131}$I intravenously 2 wk before and 16 days after fallout radiation was simulated in six of the cows by exposure to 120 R whole body $^{60}$Co and feeding 1 Ci $^{90}$Y.

Feed intake by irradiated cows dropped sharply within a week after $^{90}$Y labeled sand was fed, and when the


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second $^{131}I$ dose was given they were consuming only 30–50% of the normal amount. Compared to pretreatment measurements, plasma $^{131}I$ concentrations were increased somewhat by radiation treatment, but the percentage of plasma radioiodine in protein-bound form was reduced sharply. This may have resulted from reduced feed intake since a number of hormones, including thyroxine, have been greatly reduced by starvation in rats.

The drop in total milk $^{131}I$ (Table 13) was due entirely to lower milk yield since $^{131}I$ concentration in milk was raised by over 33%. Urinary $^{131}I$ excretion decreased after

**Table 13 Effects of Simulated Fallout Radiation on Milk Yield, Total Radioiodine Excretion, and Contents in Blood Plasma and Thyroid Glands of Holstein Cows 168 Hr after Single $^{131}I$ Doses**

| Item | Pretreatment | | Posttreatment | |
|---|---|---|---|---|
| | Irradiated | Control | Irradiated | Control |
| Milk yield (kg/day) | 17.0 | 16.8 | 8.0 | 14.6 |
| Milk $^{131}I$ (% of dose) | 11.2 | 8.6 | 9.4 | 8.4 |
| Urinary $^{131}I$ (% of dose) | 38.7 | 39.0 | 36.8 | 44.4 |
| Fecal $^{131}I$ (% of dose) | 30.8 | 35.8 | 28.2 | 28.4 |
| Blood plasma $^{131}I$ (% of dose) | 0.4 | 0.5 | 0.4 | 0.4 |
| Plasma protein-bound $^{131}I$ (% of total plasma $^{131}I$) | 82.6 | 83.8 | 31.8 | 82.6 |
| Thyroid $^{131}I$ uptake (% of dose) | 12.8 | 11.8 | 10.3 | 8.0 |
| Total $^{131}I$ accounted for (% of dose) | 93.9 | 95.7 | 85.1 | 89.6 |

irradiation during a period when it increased in controls, but fecal $^{131}I$ was little affected. Almost 15% of the $^{131}I$ dose after irradiation was not accounted for in the tissues, fluids, and excreta examined (Table 13). Digestive tract injury from internal irradiation may have resulted in higher radioiodine retention since diseased or injured tissues in the bovine have been found to contain over twice the iodine concentrations of normal tissues (Miller et al, 1973). Increasing milk radioiodine concentrations would increase the time before milk of radiation-injured cows should be used for human consumption.

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## EFFECTS OF FEEDING IODINE BINDING AGENTS ON IODINE METABOLISM IN THE CALF

J. K. Miller

Although absorption of iodine can be drastically reduced by feeding known iodine binding agents such as anion exchange resins, effects of natural feed ingredients may be much less (Miller et al., 1975). Each additional amount of resin from 0.3 to 3.5 g/kg body weight fed to calves reduced urinary and increased fecal excretions and lowered blood plasma levels of both oral $^{131}I$ and intravenous $^{125}I$ doses. By feeding 3 to 10 g cottonseed meal/kg body weight, excretion of oral $^{131}I$ given daily was raised 7 to 94% in feces and reduced as much as 35% in urine, but plasma $^{131}I$ was not changed. Introducing 1 g resin/kg body weight daily into the diet increased fecal $^{131}I$ excretion three to five times that with cottonseed meal alone and reduced both plasma and urinary $^{131}I$. The same amount of resin fed daily had similar effects on excretion of $^{131}I$ injected subcutaneously each day.

The effects of resin consumption on excretion of orally and parenterally administered radioiodine probably resulted from the large and rapid excretion of circulating iodine into gastric stomach contents (Miller et al., 1974). Results from resin addition indicate the probability of effecting iodine depletion by a highly efficient iodine binder in the gastrointestinal tract. Although effects on fecal radioiodine loss were demonstrable when cottonseed meal was fed in excess of protein requirements, plasma radioiodine was not reduced. Thus unless a natural feed ingredient had sufficient iodine binding capacity to substantially increase iodine loss, iodine conserving-recycling systems in the body (Miller et al., 1974) may keep plasma iodine in physiologically effective amounts.

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## INCREASED CERIUM-144 UPTAKE IN FETAL RATS AFTER THE ADDITION OF CARRIER

F. R. Mraz and G. R. Eisele

Moskalev (1974) reported that in the presence of "weighable" quantities of stable cerium, radioactive cerium disappears more slowly from the blood; and its level rises in the soft tissues and declines in bones. We therefore considered the possibility that this slower disappearance rate of $^{144}Ce$ from the blood would result in an increased fetal uptake if placental transfer of $^{144}Ce$ occurred at a constant rate.

Eight 5½-mo-old Sprague-Dawley rats on their 17th day of gestation were given via tail vein 50 $\mu$Ci of carrier-free $^{144}Ce$-$^{144}Pr$ as $CeCl_3$ containing 0.11 $\mu$g Ce, and 8 rats were administered the radionuclide with an additional 143 $\mu$g of carrier cerium. They were sacrificed 48 hr after dosing and the individual fetuses were removed from their membranes, weighed, dried, ashed at 600°C, and assayed for $^{144}Ce$ content.

Fetuses from dams that were given the carrier cerium contained 0.23% of the $^{144}$Ce maternal dose, which was significantly ($P < 0.01$) more than the 0.15% found in fetuses from dams receiving no carrier. The carrier-free levels of $^{144}$Ce may be absorbed by surface sites on the various tissues and thus leave the blood rapidly. Dilution by greater quantities of stable cerium may saturate these sites resulting in a slower removal of $^{144}$Ce from the blood. The longer circulating time of cerium results in deeper deposition in organs having an abundance of reticuloendothelial elements. Presumably the longer circulation times also result in greater opportunity for placental transport and greater deposition in the fetus. This movement is intensified by the rapid and increased blood production of the 17- to 18-day-old fetus.

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## GASTROINTESTINAL ABSORPTION OF CERIUM-144 IN THE NEWBORN MOUSE, RAT, AND PIG


G. R. Eisele and F. R. Mraz


Recent studies have shown that the relationship between age and gastrointestinal absorption is age dependent. The "open gut" phenomenon associated with perinatal animals permits increased absorption by the neonate and leads to significantly higher internal levels than those found in adults. We investigated specifically the quantitative variations in the absorption of cerium ions from the gastrointestinal tract of neonates of several species.

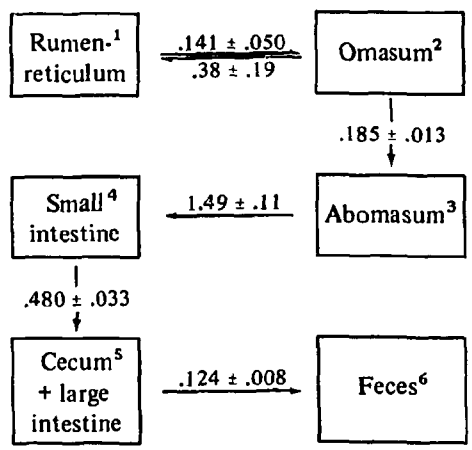
Mice, rats, and pigs less than 24 hr old received $^{144}$Ce-$^{144}$Pr by gavage and were sacrificed at periodic intervals. Neonatal rat pups and piglets absorbed 9.4 and 2.5%, respectively, 21 days after an oral dose, whereas the mouse pups absorbed about 24%. In the newborn pig, the major site of cerium deposition was in the skeleton (59% of that absorbed). At 12 days of age approximately 1.4, 7.3, and 11% of the dose was still in the digestive tract and contents of the pig, mouse, and rat, respectively. Liver concentrations remained constant in the mouse and rat from 12 days to 21 days but decreased in the pig.

These data confirm that intestinal absorption by the neonate is several orders of magnitude greater than that reported for the adult. We have also found that species differences exist in absorption during this early period. The exact reason for this is unclear, and studies are being initiated to clarify the mechanism.

## TRANSPORT OF FEED RESIDUES IN THE CALF GASTROINTESTINAL TRACT


G. E. Spalding and J. K. Miller


Radiocerium, which absorbs onto and moves with feed residues (Ellis and Huston, 1968), was used to label material in the rumen-reticulum of the calf and to measure its presence in the remainder of the GI tract at subsequent times. Data were collected from five divisions of the tract and from feces by serially sacrificing 120-kg calves at 4-hr intervals to 72 hr after oral dosing with radiocerium (Miller et al., 1971). Since the original publication of these data (Miller et al., 1971) did not include a mathematical treatment of the GI transport, and since such treatment is potentially useful to the development of whole-body transport models of important environmental pollutants, we attempted to model the data. The compartmental model was developed with three criteria in mind: (a) it should describe the essential features of the data; (b) it should correspond biologically to the calf digestive tract; and (c) it should be as simple as possible after meeting criteria (a) and (b). The model arrived at was:




where values shown are fractional transfer coefficients in $(hr)^{-1}$ with their estimated standard errors.

Fig. 8 shows the observations and the curves computed from the model for the six compartments. Mean residence times of Ce in compartments 1 through 6 were calculated from the transfer coefficients to be 21.9, 5.4, 0.7, 2.1, and 8.1 hr, respectively; this sums to a mean residence time of 38.2 hr for the GI tract as a whole.

That the transfer coefficient from omasum to rumen-reticulum is nonzero may be surprising to some. It was included because it significantly improved the fit of the model to the data and was estimated with a reasonably small ratio of standard error to estimated value (0.5). A more sensitive test for the presence and value of this coefficient would be to analyze data similar to that of this study, but where the dose was administered to the omasum.

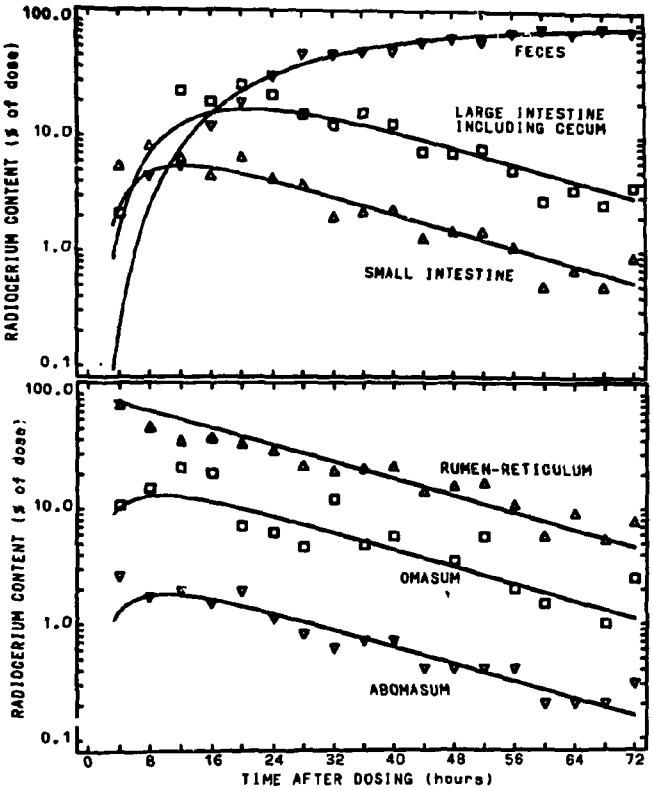



Fig. 8 Retention of radiocerium in the calf digestive tract following single oral administration. Points are observed values; curves are computed from the best least-square fit of the model to the data.

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## SOIL INGESTION AND RATION UTILIZATION BY CATTLE AND SWINE


J. K. Miller, E. W. Swanson,* and S. L. Hansard


Dairy cattle confined in concrete lots and fed corn silage as the primary roughage commonly will ingest large amounts of soil when given an opportunity. Unconfined cattle and swine may also consume considerable quantities of soil as a natural consequence of feeding. Ingested soil could affect animal health by providing a source of essential elements, affecting availability of dietary elements in the digestive tract, and providing a source of undesirable materials such as pesticide residues, heavy metals, and radionuclides. In our experiments, effects on utilization of organic and inorganic ration components were investigated in Holstein cows fed 0, 450, and 900 g red clay subsoil daily and in feeder pigs fed diets containing 0, 3, 6, and 9% added soil.

Each soil addition further decreased apparent digestibility of total dry matter in both cattle and swine but did not affect voluntary intake or digestibility of organic matter. In cattle, apparent digestibilities of crude fiber, nitrogen-free extract, energy, calcium, magnesium, and phosphorus were unaffected, protein and potassium digestibilities were decreased, and ether extract digestibility was increased by added soil. Although analyses are incomplete, soil added to swine rations did not appreciably affect excretions or apparent absorptions of magnesium, calcium, sodium, or potassium; but apparent absorptions of copper and manganese were linearly increased.

These results indicate either that copper and manganese in the soil type fed were in an almost completely absorbable form or that the soil influenced mineral utilization by swine in ways other than addition of minerals to the diet. Red clay soil, fed at levels up to 8% of total dry matter intake, did not adversely affect utilization of major dietary nutrients by dairy cows. It is recognized that different results might have been obtained with different soil types.


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## GASTROINTESTINAL ABSORPTION OF NIOBIUM-95 BY RATS OF DIFFERENT AGES


F. R. Mraz and G. R. Eisele


Niobium radionuclides constitute a substantial percentage of the total activity of fission products during the first year and may be formed by neutron-activation of structural materials in fusion reactors. Since the data of $^{95}Nb$ absorption from the gastrointestinal (GI) tract for suckling animals are not readily available, our experiments were undertaken to compare the gastrointestinal absorption of $^{95}Nb$ by juvenile and adult rats and to determine residence times in the digestive tract.

Carrier-free $^{95}Nb$ in oxalic acid was administered by gavage to rats 6 hr, 7, 21, or 129 days of age; these were sacrificed four days later. Rats less than 6 hr old were similarly dosed and sacrificed 1, 4, 5, 7, 9, 12, 15, 17, 19, or 21 days after dosing. The experiments used eight to 11 rats per point.

After four days, 5.54 and 3.86% of the $^{95}Nb$ administered orally was found in the bodies of rats dosed at 6 hr and 7 days of age, respectively. This was significantly higher ($P < 0.01$) than the 0.088 and 0.091% found in the bodies of the 21- and 129-day-old rats. As much as 47.5 and 21.8% of the $^{95}Nb$ was found in the GI tract and contents of rats dosed at 6 hr and 7 days of age while only about 0.015% was still in the tracts of the older rats. Some of the $^{95}Nb$ in the digestive tracts of the suckling pups must have been available for absorption since an elevation ($P < 0.01$) of $^{95}Nb$ in the whole body was observed as late as nine days after dosing when 20% of the dose was still in the GI tract. Although no

measurements of excretion of absorbed $^{95}$Nb by the suckling pups were made, it most likely occurred throughout the course of the 21-day experiment since there was a significant decline in body $^{95}$Nb from the maximums experienced at 9 to 12 days. Excretion was probably masked in the earlier periods of $^{95}$Nb absorption from the GI tract. No other data were found on neonatal absorption of $^{95}$Nb in the literature, but Inaba and Lengemann (1972) reported a similar retention of $^{141}$Ce in the suckling rat intestinal epithelial cells and suggested this would likely be a general phenomenon for elements that formed colloids at intestinal pH's. The relatively high (6.6% of the dose) absorption of $^{95}$Nb by the suckling pup and the rather long residence time in the GI tract suggests that $^{95}$Nb is more hazardous for the juvenile than the adult.

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## DISTRIBUTION OF NIOBIUM-95 AFTER ORAL OR INTRAVENOUS ADMINISTRATION TO THE NEWBORN, WEANLING, OR ADULT SHEEP, PIG, RAT, GUINEA PIG, OR MOUSE


G. R. Eisele and F. R. Mraz


In predicting the fate of any radionuclide, especially in man, it is advantageous to have comparative animal metabolic data. Unfortunately, only limited data exist on $^{95}$Nb for this type of comparative evaluation. To develop data which can be extrapolated directly to man, experiments on localization, redistribution, and excretion of $^{95}$Nb in a variety of animal species were undertaken. These data will be incorporated into the development of an interspecies compartmentalized model.

Comparative studies on newborn, weanling, and adult sheep have been completed and the data are being analyzed. In the newborn lamb an esophageal groove is present which permits milk to pass directly to the abomasum, bypassing the immature rumen. Placing the dose in the rumen rather than the true stomach resulted in a 3-fold increase in the absorption of $^{95}$Nb. There was a 1,000-fold decrease in absorption from the newborn lamb to the weanling lamb and approximately a 2-fold decrease from the weanling to the adult. The difference in absorption by newborn pigs as contrasted with weanling pigs showed a similar but less sizable response. Preliminary values indicate that absorption by the newborn pig is comparable to that obtained by newborn sheep, but weanling pigs absorb about five times more than comparable sheep. In animals injected intravenously, no age-related differences in retention were observed; however, significant differences were observed in skeletal retention, which decreased with age. The liver of the newborn or weanling pig absorbed a significantly greater percent of dose than did the liver of comparable sheep. Comparative studies utilizing mice, rats, and guinea pigs are currently underway.

## ABSORPTION, EXCRETION, AND TISSUE DEPOSITION OF TITANIUM IN SHEEP


J. K. Miller, F. C. Madsen,* and S. L. Hansard


Titanium is extremely abundant in soils but is poorly absorbed and retained by plants and animals (Underwood, 1971). More than 96% of orally administered $^{44}$Ti was recovered in feces plus digestive tract contents of lambs 24 hr later (Miller et al., 1976), but measurement of extremely low absorption by difference from fecal excretion is likely to be inaccurate. More reliable estimates could be obtained by comparing $^{44}$Ti contents of individual organs (excluding the digestive tract) or total tissue $^{44}$Ti accumulation in orally and intravenously dosed animals (Table 14). These comparisons indicate that $^{44}$Ti absorption averaged less than 0.5% of oral intake. Despite the low titanium content of uncontaminated herbage, considerable titanium could be ingested with soil during grazing (Healy, 1968). Its almost complete recovery in feces indicates that fecal titanium could be used as a satisfactory index of amounts of soil ingested by grazing animals.

**Table 14 Total Contents of $^{44}$Ti in Tissues, Organs and Digestive Tracts, and Excretions in Feces and Urine Following Oral or Intravenous Administration to Lambs**

| Item | Method of dosing | |
|---|---|---|
| | Oral | Intravenous |
| | (% of dose) | |
| Internal organs | 0.07 | 10.6 |
| Blood | –* | 18.4 |
| Skeleton and cartilage | 0.03 | 24.8 |
| Skeletal muscle | 0.03 | 13.3 |
| Hide plus fleece | 0.01 | 5.0 |
| Digestive tract tissue | 0.28 | 4.3 |
| Digestive tract contents | 12.18 | 0.7 |
| Feces | 83.91 | 0.9 |
| Urine | –* | 3.0 |
| Miscellaneous, fluids, scraps, etc. | –* | 10.5 |

*Activity below limits for measurement.

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*Syntex Agri Business, Inc., Springfield, MO.

## MAGNESIUM TRANSPORT IN SHEEP

G. E. Spalding and J. K. Miller

CARL is interested in the use of whole-body compartmental models to describe the transport of energy-related environmental pollutants in various animal species. It is thought that species differences as well as those caused by factors such as physiological state, age, and diet can be best understood by determining how these factors affect the detail of transport models. With this in mind, we attempted to model available data on the transport of Mg in normal and in hypothyroid sheep (Madsen et al., 1975).

$^{28}$Mg was orally administered to five hypothyroid and five control sheep at 11 months of age. Serial blood samples were taken and urine and feces were measured regularly to 106 hr after dosing. A month later, the experiment was repeated, except that the dose was intravenously administered.

The compartmental model developed for these data is represented by Fig. 9. The model was regressed simultaneously to the blood, feces, and urine data collected from the oral and intravenous experiments. The fit of the model to the data is presented in Fig. 10 and the transfer coefficients of the model in Table 15. An examination of Table 15 shows that four of the transfer coefficients appear to be affected by the hypothyroid condition while six do not.

Although data were collected from only three of the model's compartments, the model can be used to estimate the percentage of the $^{28}$Mg dose in all eight compartments as a function of time after dosing. These estimations are, of course, less reliable than estimations for compartments containing data. They are of great interest in suggesting tentative conclusions, however, and aid in designing experiments which can more directly test these conclusions. The results of the current analysis indicate that the $^{28}$Mg contents of the bone and GI tract were greater for hypothyroid than for control sheep under both oral and intravenous administration. The reverse was true for the outer pool (soft tissue).

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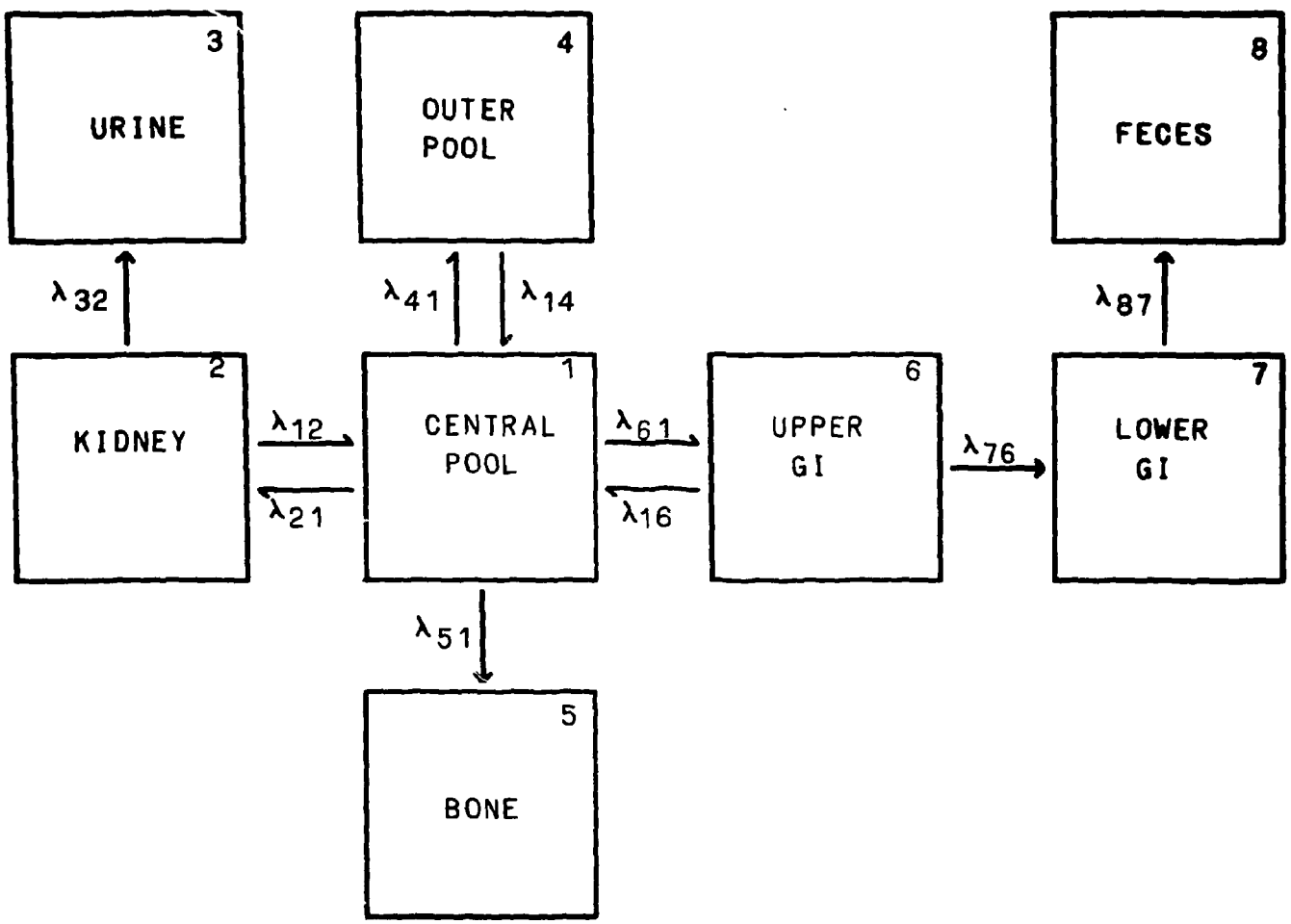


Fig. 9 Compartmental model for Mg transport in sheep.

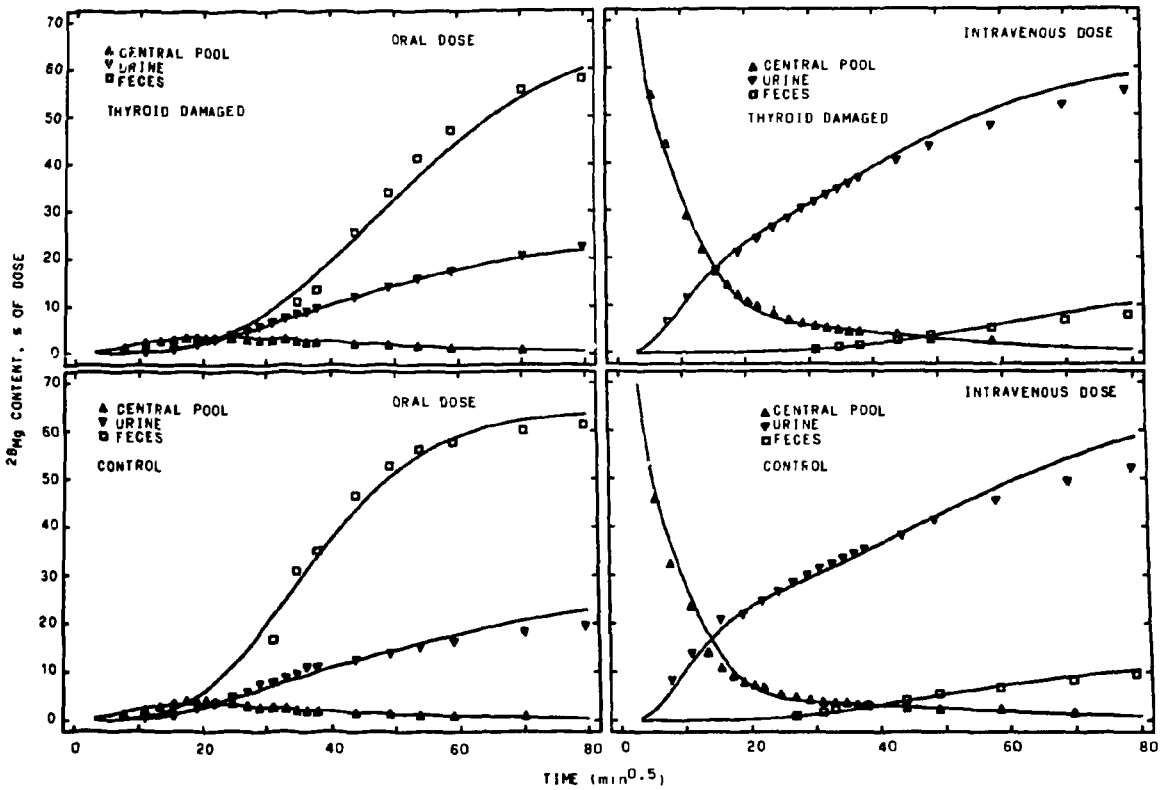


Fig. 10 Content of $^{28}$Mg in central pool and in cumulative excretions in urine and feces of thyroid-damaged and control sheep during 6400 min after an oral or intravenous dose.

**Table 15 Transfer Coefficients for $^{28}$Mg Transport in Thyroid-Damaged and Control Sheep, $Min^{-1} \times 10^3$**

| Compartments and direction | Thyroid damaged | Control |
|---|---|---|
| $\lambda_{61}$ Center pool to upper GI | 0.805 ± 0.04† | 0.881 ± 0.06 |
| $\lambda_{16}$* Upper GI to central pool | 0.434 ± 0.01 | 0.544 ± 0.03 |
| $\lambda_{76}$ Upper GI to lower GI | 0.705 ± 0.04 | 0.802 ± 0.07 |
| $\lambda_{87}$* Lower GI to feces | 0.459 ± 0.03 | 1.259 ± 0.16 |
| $\lambda_{21}$ Central pool to kidney | 32.450 ± 0.79 | 35.300 ± 0.97 |
| $\lambda_{12}$ Kidney to central pool | 50.920 ± 1.07 | 46.280 ± 0.63 |
| $\lambda_{32}$ Kidney to urine | 3.747 ± 0.17 | 3.813 ± 0.14 |
| $\lambda_{41}$* Central pool to outer pool | 5.691 ± 0.38 | 7.238 ± 0.36 |
| $\lambda_{14}$* Outer pool to central pool | 1.266 ± 0.10 | 0.781 ± 0.07 |
| $\lambda_{51}$ Central pool to bone | 0.895 ± 0.13 | 0.717 ± 0.21 |

*Treatment means differ ($P < 0.01$).
†Means ± SEM.

## MAGNESIUM METABOLISM IN THE DEVELOPING OVINE

S. L. Hansard, F. C. Madsen,*
and J. K. Miller

As gestation advances in the ovine, dietary magnesium may become more critical for maintaining magnesium homeostasis (Hansard et al., 1975). Parameters calculated from changes in maternal blood plasma magnesium specific activity during 72 hr after intravenous $^{28}$Mg administration between the 6th and 20th wk of gestation indicated 18% decreases in total exchangeable body magnesium but 15% increases in the plasma magnesium pool size. Concentrations of $^{28}$Mg in maternal organs decreased as corresponding fetal concentrations increased to 58 hr after dosing, but both stable and radio magnesium were lower in fetal than in maternal tissues. Fetal stable magnesium concentrations increased much more in bone than in soft tissue with advancing development (Table 16).

During the times sampled, fetal stable magnesium accounted for a higher percentage of the magnesium in total products of conception and increased faster than $^{28}$Mg as development progressed. Although $^{28}$Mg freely crossed the ovine placental membranes during the three stages of gestation, the greater part of that retained was deposited in the maternal body. Measurement of magnesium during advancing gestation can provide data needed to evaluate metabolic patterns during pregnancy (Hansard and Berry, 1969).

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Table 16 Magnesium in Maternal and Fetal Tissues During Advancing Gestation

| Item | Measurement and week of gestation | | | | | |
|---|---|---|---|---|---|---|
| | Magnesium-28 | | | Stable magnesium | | |
| | 6 | 12 | 20 | 6 | 12 | 20 |
| | (% of dose/kg) | | | (mg/100 g) | | |
| Plasma | | | | | | |
| Maternal | 0.3 | 0.4 | 0.1 | 2.0 | 2.0 | 2.0 |
| Fetal | – | 0.3 | 0.2 | 4.4 | 3.5 | 3.0 |
| Skeletal muscle | | | | | | |
| Maternal | 1.3 | 1.4 | 0.4 | 27.7 | 25.9 | 26.5 |
| Fetal | – | 0.9 | 0.3 | 12.7 | 12.3 | 20.4 |
| Liver | | | | | | |
| Maternal | 1.8 | 2.7 | 1.6 | 19.3 | 19.6 | 21.0 |
| Fetal | 4.0 | 1.8 | 1.9 | 10.6 | 17.5 | 18.3 |
| Bone | | | | | | |
| Maternal | 3.4 | 3.9 | 1.6 | 268.0 | 255.0 | 309.0 |
| Fetal | – | 2.6 | 0.8 | 7.2 | 73.6 | 128.0 |
| | (% of dose) | | | (total mg) | | |
| Products of conception | 0.4 | 2.0 | 3.0 | 121.0 | 234.0 | 2912.0 |
| Placenta | 0.4 | 1.4 | 1.3 | 35.0 | 71.0 | 147.0 |
| Fluids | 0.02 | 0.05 | 0.1 | 10.0 | 13.0 | 45.0 |
| Fetus | 0.01 | 0.6 | 1.6 | 76.0 | 150.0 | 2720.0 |

## MAGNESIUM-POTASSIUM-CARBOHYDRATE-HORMONAL INTERRELATIONSHIPS IN RUMINANTS

S. L. Hansard, F. C. Madsen,* D. E. Lentz,† and J. K. Miller

Utilization of magnesium by the ruminant is greatly influenced by interrelationships among dietary magnesium, potassium, and readily available carbohydrate. These interrelationships were investigated in a series of studies using sheep and cattle fed different amounts of soluble carbohydrate (Madsen et al., 1976) and magnesium (Lentz et al., 1976) and/or infused intravenously or intraruminally with potassium (Lentz et al., 1976) (Fig. 11). The absorption of magnesium by sheep was increased, as indicated by higher plasma magnesium and lower fecal loss, when soluble carbohydrate contents of hay or early spring forage were elevated by additions of glucose.

Elevation of plasma potassium by intravenous or intraruminal infusion increased circulating insulin and reduced glucose concentrations in plasma as has been demonstrated in other species. Plasma potassium levels peaked at lower levels in magnesium-deficient calves than in normal calves during infusion. This may have resulted from higher insulin levels in magnesium-deficient calves, since insulin is involved in a mechanism which increases removal of potassium during hyperkalemia (Hiatt et al., 1974).

Fig. 11 Calves in metabolism stalls for intravenous infusion with potassium chloride solutions to study effects of hyperkalemia on plasma changes in magnesium, insulin, and glucose.

Blood glucose reached higher levels in magnesium-deficient calves, however, despite the higher insulin. This response may have been due to glucagon release stimulated by high

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insulin levels, since intravenously infused glucagon increased plasma levels of both insulin and glucose in calves (Madsen et al., 1976a).

These interrelationships were investigated further in lactating cows abruptly moved to early spring pasture with a high potassium content. Within 4 days, plasma insulin increased 50% and magnesium decreased 24% in unsupplemented cows (Fig. 12). Daily supplements of 30 g magnesium oxide or 5 lb corn meal greatly reduced or prevented these changes in other cows. The high potassium, low magnesium, and low readily available carbohydrate contents of early spring forage could initiate a series of metabolic disturbances in cattle which may be involved in clinical grass tetany.

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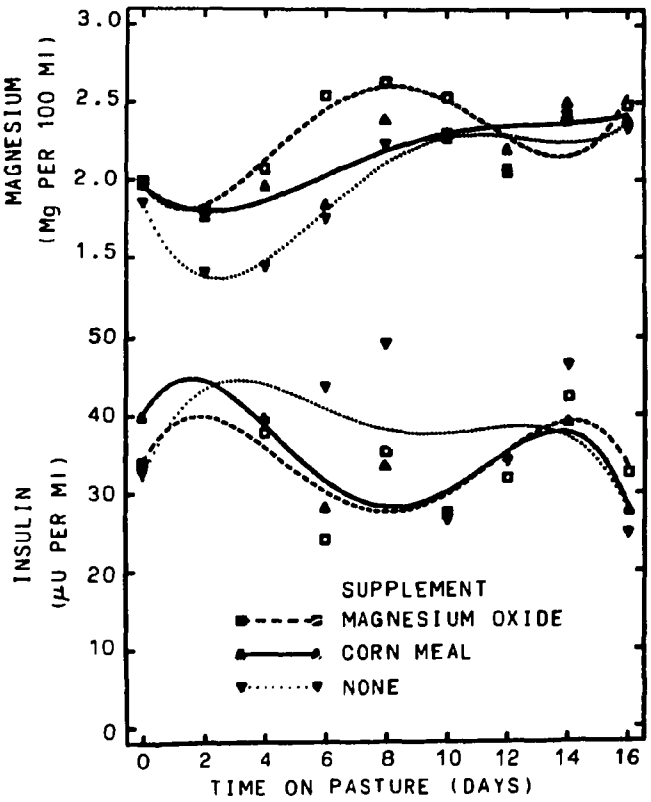
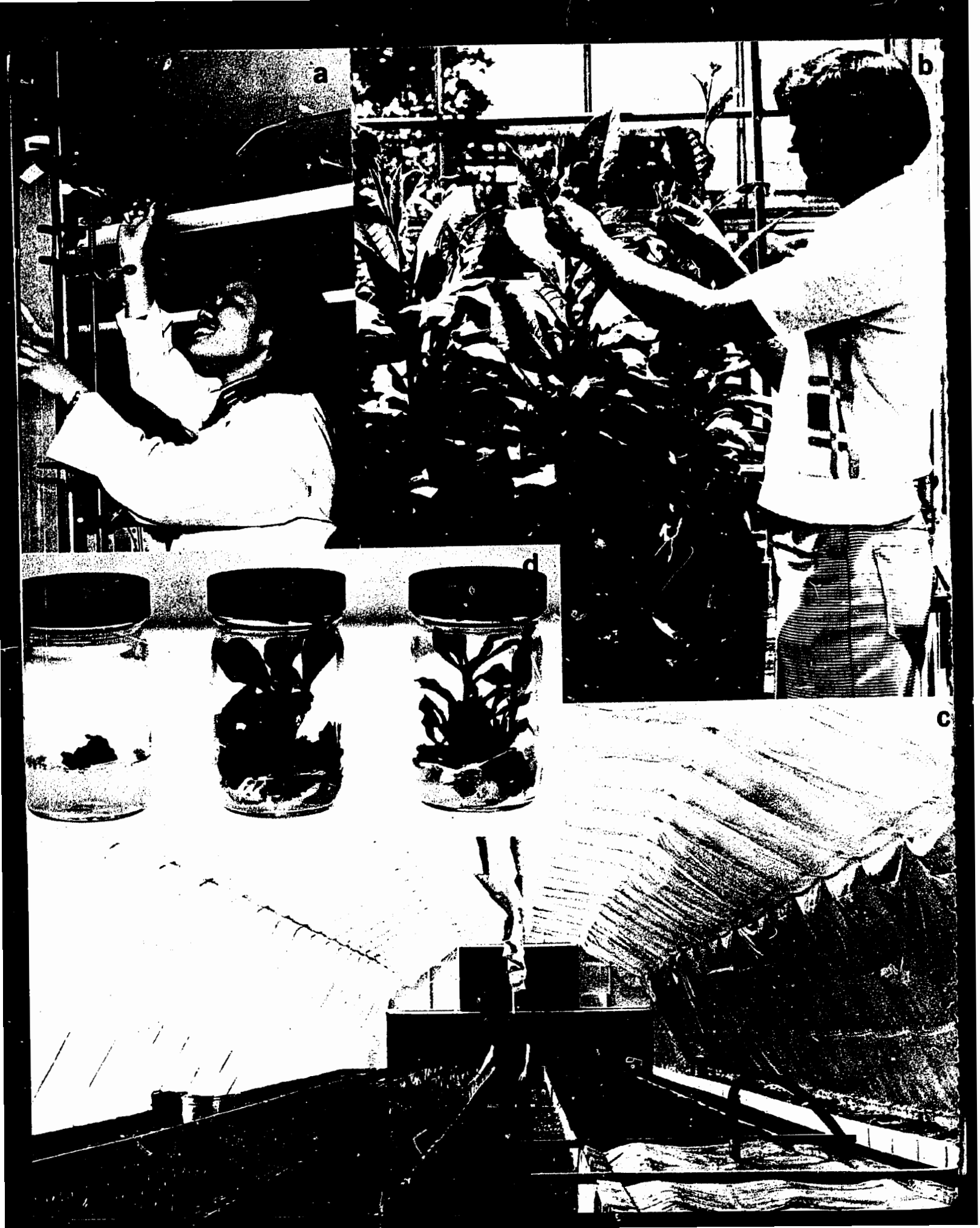



Fig. 12 Changes in blood plasma magnesium and insulin in lactating cows abruptly changed from barn feeding to early spring pasture with high potassium content.


a
b
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# Plant Research

M. J. Constantin, Head

Mutagenesis for Crop Improvement M.J. Constantin, Group Leader
Amino Acid Regulation and Synthesis R. R. Henke, Group Leader
Mutagenesis for Increased Methionine Content D. E. Foard, Group Leader
Biological Effects of Energy Related Pollutants F. J. Ryan, Group Leader

## EFFECTS OF S-2-AMINOETHYLCYSTEINE ON THE GROWTH OF BARLEY SEEDLINGS

R. R. Henke, M. J. Constantin,
L. K. West, and G. S. Boone

S-2-aminoethylcysteine (Aec) competes for lysine (Lys) at several metabolic sites [e.g., amino acid permease(s), end-product control of Lys biosynthetic enzymes, and in protein synthesis and function]. Amino acid analogs, including Aec, have been successfully used as selective agents in mutation screens designed to obtain mutants with altered amino acid metabolism. We are characterizing several metabolic and growth effects of Aec in seedlings derived from excised embryos and from whole seeds.

An initial effort was made to determine the phenotypic response to Aec, as measured by several growth parameters, over a range of concentrations. Intact barley seeds (*Hordeum vulgare*, variety Atlas-57) were germinated in the presence of Aec (0.1–0.5 mM) on germination paper and agarized media containing either water, 2% sucrose, or Murashige and Skoog basal medium (1962). Aec inhibited fresh weight (root and shoot) and primary leaf height over 8 days of growth. Each of the growth conditions and parameters measured resulted in similar dose responses. Generally, Aec inhibition was linear from 0.5 mM–5.0 mM with 5.0 mM resulting in growth which was 20% of the control level. This Aec inhibition could be partially alleviated by supplementing the media with Lys at equal molar concentrations. Higher levels of Lys did little to further relieve the Aec inhibition.

Similar experiments were carried out with seedlings derived from excised embryos of Atlas-57. These embryos (plus scutellum) were excised free of endosperm and other associated tissues of the seed and cultured aseptically on agarized MS medium plus appropriate Aec and/or Lys supplements. The same growth parameters were measured over 8 days. Aec inhibition of growth was linear from about $1 \times 10^{-5}$ M to $1 \times 10^{-4}$ M, approximately 10-fold more inhibitory than in seedlings derived from whole seeds. Lys successfully competed for Aec inhibition, but unlike the whole seed experiments complete elimination of the inhibition required supplementing Lys at a concentration 10 times that of Aec. For example, 0.1 mM Aec inhibited shoot height by 70% while embryos grown on 0.1 mM Aec + 1.0 mM Lys were 98% of normal growth. Lys itself linearly inhibited growth over a narrow concentration range (i.e., 1.25 to 3.0 mM, 74 to 25% of control, respectively) in seedlings derived from excised embryos while no significant inhibition of growth was observed in seedlings grown from the intact seed, even when Lys was supplemented at 5.0 mM.

Since barley seedlings derived from intact seeds (endosperm present) were about 10 times more resistant to Aec, this response could be due to the Lys supplied to the growing seedling by the endosperm and associated tissues. If this correlation exists it may then be conceivable to expect barley varieties differing in their total Lys content to respond accordingly to the Aec and thus possibly provide a "bioassay" for Lys content. To accomplish this 115 varieties of barley were obtained from the Northeastern Regional Agricultural Research Station at Beltsville, Maryland. To date, total protein and total Lys have been calculated for all varieties. A total amino acid analysis has been completed for eight varieties. At present a method to uniformly grow these diverse varieties in aseptic culture is being devised. As a further test of this "bioassay" hypothesis various amounts of Lys were injected into the endosperm of germinating (1–3 days after planting) barley

(a) Use of gel-filtration in purification of nutritionally important proteins of soybean. (b) Wisconsin 38 tobacco plants serve as the source of pith cells for in vitro culture studies. (c) $M_2$ generation seedlings of barley growing in the greenhouse for the detection of chlorophyll-deficient mutants induced by chronic gamma irradiation. (d) The effects of plating pith-derived callus (third subculture) of tobacco on Murashige and Skoog medium without hormones (left), with $N^6$-($\Delta^2$ isopentenyl) adenine (center), and 6-benzylaminopurine (right) on shoot organogenesis.

seeds. This injected Lys partially repressed the Aec inhibition, but completely normal growth was not observed.

Results indicate that the mode(s) of Aec inhibition may differ in seedlings derived from excised embryos vs. those derived from whole seeds, that Lys is antagonistic to this inhibition, and that the endosperm and associated tissues present in the intact seed play an important role in contributing to a degree of Aec-resistance.

### Reference

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## EFFECTS OF S-2-AMINOETHYLCYSTEINE ON THE METABOLISM OF LYSINE IN BARLEY

R. R. Henke and G. S. Boone

Recently the selection of specific biochemical mutants in higher plants has proven successful when employing amino acid analogs as selective agents. Adapting this approach of mutation selection, we have begun identifying the metabolic sites which may be perturbed by the lysine (Lys) analog, S-2-aminoethylcysteine (Aec). These studies have been carried out in young barley seedlings (*Hordeum vulgare*, variety Atlas-57) derived from excised embryos grown in culture or from intact seeds grown on germination paper or in culture with and without nutrient.

Aec was previously shown to inhibit barley seedling growth; Lys was antagonistic to these effects. Evidence was also found which suggested that the mode(s) of Aec inhibition may differ between seedlings derived from excised embryos and those grown from whole seeds.

In whole seed experiments there was a noticeable reduction in endosperm liquification at concentrations of Aec that caused the greatest growth inhibitions. The growth inhibition could not be attributed to a starvation for nutrients supplied through the hydrolysis of the endosperm food reserve since inhibition also occurred in seedlings grown on medium containing all essential nutrients. A preliminary experiment designed to further characterize this Aec-reduced endosperm hydrolysis demonstrated a reduction in α-amylase activity as assayed by the production of reducing sugars. Amylase activity was not inhibited when equimolar amounts of Aec and Lys were present in the growth medium. In barley, amylase is synthesized de novo during germination, suggesting that Aec either may be inhibiting protein synthesis or is being incorporated into proteins and rendering them nonfunctional.

In an attempt to further understand the Aec inhibition of barley, isotope competition experiments using $^{14}$C-Lys have been initiated. Embryos were excised from Atlas-57 seeds and cultured aseptically for 7 days in MS medium. At the end of 7 days' growth the root systems were harvested, placed in fresh medium containing $^{14}$C-Lys with varying amounts of Aec, and incubated from 15 min to 4 hr. After incubation total radioactivity was determined in the free amino acid pool, in the soluble and insoluble proteins, and as total $^{14}$C-Lys taken up by the root tissue. Data indicate a competitive inhibition of Lys uptake in the roots by increasing amounts of Aec. These preliminary results suggest the presence of a carrier-mediated, energy-requiring uptake system that transports only basic amino acids. The determination of $^{14}$C-Lys (or $^{14}$C leucine) in the soluble and insoluble pool after a 4-hr incubation indicated a reduction in label found in the insoluble (protein) fraction. Since the competition by Aec for $^{14}$C leucine in the insoluble fraction was slight compared to insoluble $^{14}$C-Lys the possibility exists that Aec affects protein synthesis and/or degradation as well as competing for Lys residues in protein. A clear interpretation requires further investigation.

These results have provided a preliminary picture of how Aec affects the metabolism of barley. It also indicates possible sites of Aec function and thus possible mutable sites in mutation screens employing Aec as a selective agent. Further investigation is under way to more precisely characterize these Aec effects.

## ISOLATION, PARTIAL PURIFICATION, AND CHARACTERIZATION OF ASPARTOKINASE FROM HIGHER PLANTS

R. R. Henke and Rosemarie Wahnbaeck*

The enzyme aspartokinase (ATP:L-aspartate 4-phosphotransferase, EC 2.7.2.4) (AK) catalyzes the first step in the biosynthesis of lysine, methionine, threonine, and isoleucine in higher plants. In keeping with our program on the elucidation of the biology related to modifying the regulation and synthesis of aspartate-derived amino acids that are essential in animal nutrition, we have isolated, partially purified, and characterized AK from corn (Pioneer, 3145).

The enzyme was isolated from 4–6-day-old etiolated roots and shoots and mature and immature (30 days post anthesis) endosperm using an extraction buffer of 0.2 M tris • HCl (pH 8.5), 1.0 mM EDTA, 1.4 mM mercaptoethanol, 30% glycerol, 0.1 M KCl, 1.0 mM lysine, and 1.0 mM threonine. AK was partially purified with a 0–40% cut of $(NH_4)_2SO_4$ with the resulting pellet resuspended in 0.05 M phosphate buffer (pH 7.5) containing 1.4 mM mercaptoethanol, 1.0 mM EDTA, 20% glycerol, and 1.0 mM threonine, and either dialyzed against an excess of the same buffer or desalted with G-25 gel filtration chromatography. The enzyme could be stored in this buffer frozen for several weeks without significant loss of activity. Enzyme from this preparation was used in the enzyme assay or for further purification using Sephacryl S-200 gel filtration chromatography.

The enzyme was assayed according to the method of Black and Wright (1955) where the product of the AK catalyzed reaction, β-aspartylphosphate, in the presence of hydroxylamine is converted into a hydroxyamic acid which forms a colored complex with trivalent iron and can be

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detected spectrophotometrically at 505 nm. The standard assay mixture contained 50 mM L-aspartate, 25 mM ATP, 25 mM MgCl, and 400 mM hydroxylamine in 0.1 M tris • HCl (pH 8.0). AK activity was dependent upon aspartate, ATP, $Mg^{++}$ or $Mn^{++}$, and enzyme concentration. The reaction rate was linear for about 75 min and dependent upon enzyme concentration under standard assay conditions. Specific activities have been observed ranging from 3.2 to 4.5 nm/mg protein/min. The pH optimum was found to be between 8.0 and 8.5. Two apparent Kms for aspartate ranging from 1.5–3.0 and 7.0–8.5 were estimated from double reciprocal plots of velocity vs. aspartate concentration. AK activity was inhibited by lysine and the lysine analog, S-2-aminoethylcysteine.

The enzyme was reproducibly isolated from the tissues described above. The presence of AK in developing corn endosperm has not been previously reported and supports recent evidence (Sodek, 1976) that the biosynthetic pathway for the aspartate family of amino acids may function in developing endosperm.

Further investigations are designed to elucidate the nature of end product regulation of AK and to identify other possible effectors; to attempt to resolve the complex relationship between activity and aspartate concentration; and to compare the AKs isolated from different tissues of corn and in other plants.

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## THE ISOLATION OF PUTATIVE MUTANTS WITH RESISTANCE TO S-AMINOETHYLCYSTEINE IN CULTURED CELLS OF WISCONSIN-38 TOBACCO


M. J. Constantin, R. R. Henke, and L. K. West


Techniques of culturing plant cells and tissues are being used extensively in pursuing a wide range of fundamental and applied problems. One reason for the rapid increase in popularity of in vitro culture techniques is that they make possible the use of microbial genetic technology for plant improvement. The genetic regulatory mechanism in microbes has been partially elucidated through the characterization of mutants that are resistant to metabolite analogs. A similar approach in higher plants using in vitro-cultured cells has yielded results which indicate that by using analogs of amino acids essential to human nutrition, crop cultivars that produce increased amounts of these essential metabolites can be developed. Such improvements can result from mutations that alter various processes (i.e., uptake, transport, feedback control, etc.) affecting metabolic pathways.

The objective of this study was to develop techniques that permit the use of antimetabolites to isolate resistant cells from which whole plants can be regenerated for biochemical, genetic, and agronomic characterization. The characterization efforts will indicate the nature of the mutations and which aspects of the metabolic pathway were subject to mutagenesis. Initial efforts are focused on using S-aminoethylcysteine (Aec), an analog of lysine, in *Nicotiana tabacum* cv. Wisconsin-38, a model system. Results of this study will have important implications on other research efforts involving the improvements of protein quality in other food crops.

The experimental approach involved the planting of pith explants onto Murashige and Skoog medium containing auxin and cytokinin. Induced callus was cultured 3 wk at 28°C in continuous darkness after which time the callus was suspended in liquid medium and cultured for 9–10 days on a gyro-rotary shaker. Suspension cultures were then filtered and cell aggregates (1–20 cells) that passed through a 503 $\mu$ but collected onto a 53 $\mu$ pore-size filter were suspended in molten agar and poured into petri plates. For those studies that involved induced mutations, the callus was irradiated (0, 2, 4, 6 kr) with $^{60}$Co gamma radiation prior to being transferred to liquid medium. The entire procedure, i.e., beginning with a different new population of plants in the greenhouse, was repeated for each experiment.

The selection screen was based on colony formation in the presence of 50 $\mu$M of Aec in culture medium. Colonies were isolated one month after planting; culture was in continuous darkness at 28°C. In repeated trials, we have observed that the effect of Aec concentration on colony formation is dependent on cell density; however, with the exception of putative mutants, colony formation has not been observed at $\geq$60 $\mu$M Aec. Putative mutant colonies were transferred individually to Aec-containing medium to verify their ability to survive and proliferate in the presence of the selective agent. Callus from each putative mutant (i.e., survivor on Aec-containing medium) was increased sufficiently to permit plant regeneration trials in the presence and absence of Aec and to do Aec dose-response trials based on callus growth. Eighteen putative mutants have been isolated to date, and these are in various stages of evaluation at the phenotypic level. We do not have proof that irradiation of the callus increases the frequency of putative mutants.

## HIGH-METHIONINE, LOW MOLECULAR WEIGHT PROTEINS IN SOYBEAN


D. E. Foard and David Hwang*


Legumes constitute one of the main hopes for long-term progress in meeting world demands for more concentrated sources of dietary protein. As a source for man or other monogastric mammals, legume seed proteins char-


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acteristically show a significant deficiency in the sulfur-containing essential amino acid methionine and in cysteine, which can partially replace dietary requirements for methionine. The goals of legume breeding in general are to increase yield potential, to increase relative protein content, and to improve the sulfur–amino acid composition of the seed protein. To these ends we have begun exploratory studies to determine the feasibility of genetically enhancing the methionine content of soybean protein. A basic step in this approach is the isolation of a protein or proteins with the highest percentage of methionine. Genetic variants with elevated levels of these "high-methionine proteins" might then be detected by a quantitative immunological assay.

In our procedure we have purposely looked for proteins other than the two major globulin fractions, the 7S vicilin and 11S glycinin (legumin). Although these constitute approximately 65% of the seed protein, each contains a smaller percentage of methionine (0.4 and 1.0%, respectively) than the percentage in the total protein (1.5%). The high-methionine proteins have been found among five low molecular weight proteins (7000–8000 MW) isolated by our procedures. These low molecular weight proteins contain a large amount of half-cystine and lack tryptophan, a characteristic of all previously described legume trypsin inhibitors. All five proteins tested have in fact been proven to be trypsin inhibitors. One of the five is also a strong inhibitor of α-chymotrypsin; this characteristic and others indicate that it is identical to the well-known Bowman-Birk inhibitor. The other four are the richest proteins in methionine thus far described for soybean and contain the following percentages (W/W) of the amino acid: Inhibitor I, 1.9; II, 5.0; III, 3.5; IV, 3.2. Inhibitor V (Bowman-Birk) contains about 1.5% methionine. Antisera against each of the five inhibitors have been prepared, and immunological tests show that the content of inhibitor I–IV type is 2.3% and inhibitor V type is 4% of the total protein in Tracy soybean seeds. We are presently using the antisera to assay the amount of these high-methionine, low molecular weight proteins in seeds of genetically diverse populations.

## SOYBEAN LECTIN IN VIVO

D. E. Foard, D. W. Fountain*, Wen-Kuang Yang†, and Wendy Replogle‡

Although much is known about the lectins in leguminous seeds because they can agglutinate erythrocytes, their physiological significance in the seed and growing plant is still largely speculative. Lectins may provide the means by which the appropriate strains of *Rhizobium* are recognized and bound to the external surfaces of the plant root. It has also been suggested that lectins have a protective (antibiotic) role as inhibitors of fungal polysaccharases, hyphal cell wall synthesis, and spore germination. The role of lectins as either a recognition-binding mechanism for *Rhizobium* or an antibiotic mechanism presupposes the presence of free lectin in the cellulosic cell wall or in the rhizosphere (or potential rhizosphere). Consistent with this supposition are our observations that lectin is released from soybean seeds during water uptake.

The presence of hemagglutination activity in the water is detectable as early as 4 hr after the start of imbibition and reaches a peak at 8 hr, declining thereafter up to 48 hr. This pattern of agglutination activity suggests that soybean lectin is a preferential export product within the first 8 hr of germination and that it is subsequently subject to dilution by additional nonlectin protein. The mechanism of lectin release is presently unknown, although it is known that nonviable seeds and those moistened with sodium azide, a potent metabolic inhibitor, show the early release of lectin. Lectin may be localized in cell walls, and its release upon seed hydration may be a result of simple diffusion of the protein. We are currently engaged in studies using an antiserum against the lectin to detect and localize its presence in developing seeds and seedlings.

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## INDUCTION OF CALLUS AND ORGANOGENESIS IN RICE *ORYZA SATIVA* L.

R. R. Henke, M. A. Mansur*, and M. J. Constantin

As part of our program on the application of plant cell and tissue culture in mutation selection for crop improvement, we began an evaluation of the ability to culture rice in vitro and to subsequently induce regeneration of a whole plant. Our initial objectives were to test different explants for their ability to form rapidly growing callus and to optimize culture conditions and medium for growth rate.

We have obtained callus induction in *Oryza sativa* L. (variety, C. I. 8970-8) root and leaf explants of 4-day-old seedlings and from whole seeds in the region of the scutellum. The callus initiation media were Murashige and Skoog (1962) medium (MS) supplemented with the appropriate amount of 2,4-dichlorophenoxyacetic acid (2,4-D). Root-derived callus could be induced with 0.5 mg/l 2,4-D and required 2.0–10.0 mg/l for optimal growth. The leaf-derived callus required a higher 2,4-D concentration of 4.0 mg/l for callus initiation while at least 6.0 mg/l was required for optimal growth. The callus derived from the whole seed required 1.0 mg/l for induction and 2.0–1.0 mg/l for optimal growth and was slower growing, much more heterogeneous in appearance, and less friable than either the root- or leaf-derived callus.

Few reports exist on shoot and root organogenesis in rice and data concerning the frequency of these events are unclear. An attempt was made to determine and possibly increase the frequency of organogenesis and plantlet formation. Root and shoot organogenesis could be induced in

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callus derived from the three explants by subculturing the callus on MS without 2,4-D in the light at 27°C. The frequency of the callus exhibiting root and shoot organogenesis is shown in Table 17. Callus from each explant source showed organogenesis at high frequencies with seedling-derived callus exhibiting approximately half the root and shoot formation as that of the root- and leaf-derived callus. In all cases, root organogenesis occurred more often than shoot organogenesis. Several plantlets obtained from each explant source were successfully grown to maturity in the greenhouse. Root- and leaf-derived callus maintained in culture for five months showed a large decrease in the capacity to differentiate shoots and a lesser decrease for roots (Table 17). A preliminary experiment using the cytokinin 6-γγ-dimethylallylamino purine (2iP) resulted in a partial restoration of the lost organogenesis in these older cultures.

**Table 17 Organogenesis in Root, Leaf, and Seedling Derived Callus of Rice**

| Explant tissue | Subculture* | No. of callus | % Callus with Differentiated: Root | Shoot | Both | Total |
|---|---|---|---|---|---|---|
| Root | 3 | 46 | 100 | 61 | 61 | 100 |
| | 5 | 190 | 40 | 8 | 7 | 41 |
| +2iP† | 5 | 207 | 72 | 40 | 38 | 73 |
| Leaf | 3 | 77 | 89 | 50 | 47 | 91 |
| | 5 | 110 | 74 | 1 | 1 | 74 |
| +2iP† | 5 | 107 | 79 | 15 | 13 | 81 |
| Seedling | 3 | 54 | 56 | 26 | 26 | 56 |

*Callus were subdivided and subcultured into fresh medium every 4 wks. Organogenesis was scored at the end of the third or fifth subculture.

†2iP, 6-γγ-dimethylallylamino purine was added to MS medium at the fifth subculture. Data were combined from several experiments employing 2iP from 0.05 to 4.0 mg/l.

These investigations have demonstrated successful induction of callus, organogenesis, and subsequent plantlet formation in rice. The results may also implicate 2iP in the control of shoot organogenesis and indicate that the endogenous hormone levels in callus derived from different organs may differ. This problem is still under investigation.

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## ORGANOGENESIS IN *IPOMOEA BATATAS*

K. Samphantharak*, M. J. Constantin, and R. R. Henke

The sweet potato, an important crop of the South and Southeastern United States, is potentially a staple food crop in tropical countries. Induced mutations offer advantages for cultivar improvement because the sweet potato is vegetatively propagated, is highly heterozygous, and exhibits genetic incompatibilities. The in vitro cell and tissue culture approach offers a potential for the propagation of planting material as well as for improvement by either genetic engineering or mutagenesis.

Since limited information exists concerning cell and tissue culture, and no procedure is available to routinely regenerate sweet potato plants from either callus or suspended cells, research was initiated to (1) find a suitable medium for inducing and maintaining callus and (2) establish the requirements for shoot and root organogenesis. Accomplishment of these goals is a prerequisite for any practical application of mutagenesis and/or genetic engineering with sweet potato cells.

Several factors, including explant source (mature roots, stems, petioles, and leaves), basic media composition (Murashige and Skoog, White, and $B_5$) supplemented with vitamins and organics, and auxins (IAA, NAA, and 2,4-D) and cytokinins (2iP, kinetin, and BAP) alone and in combinations at various concentrations, are being investigated systematically with six leading varieties of sweet potatoes. Culture conditions involved include solid media (0.8% DiFCO agar) in petri plates under either continuous or periodic low-level light at either 25° or 28°C.

Callus induction and growth were very dependent on the source of explants (root > stems > petiole > leaves; in fact, the leaf disks necrosed) when IAA was used as the sole source of hormone. Performance was less on White's medium than on either Murashige and Skoog or $B_5$, and the effective range of concentration for IAA was from 0.1 to 5.0 mg/l. Root organogenesis was observed in root, stem, and petiole primary explants; however, shoot organogenesis was not observed. Differences were observed among the six genotypes.

Callus induction and growth occurred when 2,4-D (2 mg/l) was used as the sole source of hormone; however, chlorophyll pigment did not develop and the callus necrosed within a few weeks. Prolific callus growth was maintained, however, when the primary callus was transferred to Murashige and Skoog medium containing various combinations of IAA and kinetin. Optimum callus growth was observed when IAA and kinetin were combined in the medium at a concentration of 1 mg/l each. Chlorophyll and carotenoid pigments developed in most of the calli which could be maintained for months without subculturing. Organogenesis was not observed.

The cultivars Centennial and Jewel were selected for continued research involving the effects of auxins and cytokinins on callus induction and growth and plant regeneration. This material is still in culture, and only preliminary qualitative observations have been made. In the absence of hormones, for example, stem segments remain green but do not show callus growth. The highest concentration of IAA and kinetin used (2 mg/l of each) has induced the greatest amount of callus growth. Root organogenesis has occurred at various levels of IAA and

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kinetin. One apparently complete plant has been obtained from stem primary callus cultured on medium containing 1 mg/l of kinetin as the sole source of hormone.

Research efforts in the immediate future will focus on subculturing callus, establishing cell suspension cultures, and regenerating plants.

## EFFECTS OF GAMMA IRRADIATION ON THE PRODUCTIVITY OF CULTURED TOBACCO ANTHERS

M. J. Constantin and L. K. West

Severe limitations exist in adapting the anther culture procedure to a large-scale production (e.g., $10^4$–$10^6$ plantlets per wk or month) for mutation induction and recovery. The present study was conducted to determine another productivity (i.e., percent productive anthers and the mean number of plantlets per cultured anther) as affected by the mutagen, gamma radiation. Information concerning the dose response for microspore embryogenesis is needed for the proper application of mutagenesis. Gamma radiation from $^{60}$Co was selected because of its known mutagenicity and its penetration power. The high penetrability of gamma rays eliminates the problem of determining the mutagenic dose to the target cells, in this case, the uninucleate microspores. Results of previous experiments indicated to us that the culture of anthers in a liquid medium might improve the percentage of productive anthers and the number of plantlets per anther. Anthers were cultured individually to remove the possibility that a productive anther could release either an activator or an inhibitor that would influence other anthers in the same culture vessel.

Flower buds at the stage of the first pollen mitosis were collected from Wisconsin-38 tobacco plants growing under greenhouse conditions. Buds were collected from the first inflorescence of several plants, bulked, stored for 3–4 days at 0–5°C, randomly subdivided into groups, irradiated, surface sterilized according to standard procedure, and placed into culture. Gamma radiation exposures were 0, 1, 2, 3, and 4 kr at 178 R/min. Test tubes containing individual anthers were arranged in racks so that all anthers within a bud could be identified at the time of scoring. Each anther was cultured in 5 ml of filter-sterilized Nitsch's medium H at 28°C in continuous darkness for 2 wk and then in continuous light at 1000 lux for an additional 3 wk.

One limitation in the use of anther culture on a large scale is that many anthers are nonproductive even in the solanaceous plants such as tobacco. A common practice is to culture four anthers and use the fifth one to determine the microspore d velopmental stage. This assumes that the one anther used for staging is representative of the regenerative capability of the other four. Since in this experiment each anther was cultured individually, the fallacy of the above assumption can be seen readily. Results across all radiation treatments were as follows: 41.5% of the buds had zero productive anthers; 28.5% had one, 20.5% had two, 8.5% had three, 1.0% had four, and 0.0% had five. Thus in a group of 200 buds collected at the recommended developmental stage, one would have at least a 40% chance of selecting a nonproductive anther for analysis of developmental stage in 99% of the buds.

Regarding the effects of gamma irradiation on the percentage of productive anthers, the results were as follows: 21.5% for the nonirradiated buds, 24.0% for 1000 R, 33.8% for 2000 R, 14.0% for 3000 R, and 5.5% for 4000 R. Since only 40 buds represented each treatment, it is questionable whether the 1000-R and 2000-R treatments significantly increased the percentage of productive anthers; however, little doubt exists that the 4000-R treatment reduced it appreciably.

In terms of plantlets per cultured anther, the results were as follows: 2.02 for the nonirradiated buds, 2.46 for 1000 R, 2.73 for 2000 R, 0.92 for 3000 R, and 0.19 for 4000 R. The three most productive anthers produced 60, 59, and 55 plantlets each; these occurred in the 1000-R, 0-R, and 2000-R treatments, respectively.

In conclusion, the culture of individual anthers in liquid medium does not appear to offer an advantage over the use of agar medium. For the purpose of mutation induction, an exposure of 2000 R to the cold-treated tobacco bud can be used without sacrificing anther productivity.

## EFFECT OF ACTIVATED CHARCOAL ON GROWTH AND SHOOT DEVELOPMENT IN TOBACCO PITH-DERIVED CALLUS

M. J. Constantin, R. R. Henke
and M. A. Mansur*

Activated charcoal (AC) reportedly either stimulates growth, organogenesis, and embryogenesis in a relatively wide range of higher plants, differentiation in lower plants, growth of *Ustilago maydis* dikaryons and heterokaryon formation in *Rhizoctonia solani*, or completely inhibits growth of in vitro-cultured *Glycine max* and *Haplopappus gracilis*. The observed effects of AC in culture media have been attributed to the adsorption of toxic metabolites, the removal of toxic material present in the medium or originating in cultured anthers, and the removal of substances from the medium which promote unorganized growth, inhibition of embryogenesis, and root formation and elongation.

Since we were seeking ways to improve planting efficiency and plantlet regeneration using tobacco somatic cells, we decided to test the effect of AC in the experimental system. We asked the following questions: (1) What is the effect of AC on callus growth and shoot development in Wisconsin-38 tobacco? (2) Does AC remove from the

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medium auxin (indoleacetic acid, IAA) and cytokinin [$N^6$-($\Delta^2$-isopentenyl)adenosine,2iP] required for callus growth and shoot development in Wisconsin-38 and sucrose required as an energy source?

Tobacco pith explants were callused on Murashige and Skoog (MS) medium containing 1 mg/l of IAA, 0.1 mg/l of 2,4-D, and 0.01 mg/l of 2iP plus 30 g/l of sucrose, and 8 g/l of DIFCO agar. Pith explants were cultured for 3 wk on the above medium in complete darkness at 28°C. Callus was subcultured every 2 wk thereafter under the same environmental conditions and medium composition except for the deletion of 2,4-D.

The experimental media used to determine callus growth and shoot development in either the presence or absence of 10 mg/l of AC consisted of the stock medium with modifications of the hormonal constituents. For shoot development experiments, 2iP was the only hormone used and experiments were conducted at 25°C in continuous light (1000 lux, cool-white fluorescent). In all cases, the MS medium ingredients were sterilized using a 0.22 $\mu$ pore size filter at a 2x concentration and then mixed in equal proportions with autoclaved 2x concentration of agar (and AC when required) in double-distilled water.

Laboratory spectrophotometric analyses showed that AC adsorbed IAA and 2iP which are required for callus growth and shoot development by Wisconsin-38 tobacco. Sucrose, as expected, was unaffected by AC in the medium. The removal of IAA and 2iP plus the probable removal of several other substances from the medium inhibited callus growth and shoot development. These effects were observed even when AC was removed from the medium by filtration prior to culturing the callus.

We maintain that the adsorptive properties of AC will cause drastic alterations in the medium to which AC is added. The degree to which the addition of AC is either beneficial or detrimental depends on the requirements of the cultured material for those substances removed.

## INDUCTION, ISOLATION, AND CHARACTERIZATION OF ATRAZINE-RESISTANT MUTANTS OF SOYBEANS

M. J. Constantin, W. D. Klobe*, and D. S. Walker

The chlorotriazine herbicides including Atrazine, Simazine, and Propazine are commonly used to control effectively a wide range of weed species, especially in corn fields. In some cases, the residue from these herbicides is injurious to other crop plants that follow in the rotation plan. Such is the case with soybeans. Thus the grower who wishes to plant soybeans following corn is faced with a problem if chlorotriazine herbicides were used for weed control in producing the corn crop.

Chlorotriazines are powerful inhibitors of photosynthesis; they interfere in the Hill Reaction in the chloroplastids. Chlorotriazines can enter the plant through either the roots or the leaves depending on the mode of application. The key to whether a plant is resistant to chlorotriazines is the rate of detoxification relative to the rate of uptake, transport, and accumulation in the chloroplastids. In corn, sorghum, and sugarcane, $\geq$60% of the Atrazine introduced into excised leaves is detoxified within 6 hr; for soybeans the amount detoxified is $\geq$4% within 20 hr. Yet soybean plants exhibit two of the three known pathways of detoxification.

A screening of the world collection of soybean germplasm failed to isolate any strain possessing resistance to Atrazine. This means that genetic diversity for resistance to Atrazine is not available to the soybean breeder. Evidence in the literature, however, indicates that susceptibility to Atrazine in corn (one isogenic line is known to exist) is controlled by a single recessive gene located on the long arm of chromosome 8. Resistance to Atrazine in flax is apparently the result of additive gene action with a low heritability. Appreciable variability in the degree of resistance is known to exist in *Setaria*, *Panicum*, and *Cruciferae*. Thus evidence exists in favor of a genetic control for resistance to Atrazine in several plant genera and species.

The objective of this study was to induce, isolate, and characterize soybean mutants that are resistant to Atrazine. An Atrazine-resistant mutant could result from an increase in the detoxification rate by either or both of the pathways known to exist in the soybean plant, or by the induction of the third pathway which is not known to exist in the soybean plant. In fact, an Atrazine-resistant strain has been isolated from diploid soybean cells in suspension culture (Zenk, 1974).

Seeds of a sib-line of the soybean cultivar, Forrest, were treated with physical and chemical mutagens, the $M_1$ generation plants grown in the field, and $M_2$ seeds harvested in bulk within treatments. Different screening procedures were evaluated in the greenhouse and in field plots. Both $M_2$ and $M_3$ generation seedlings were screened for resistant mutants. Atrazine does not impair germination; the seedling dies when the supply of stored food in the cotyledons is exhausted (this occurs within 2–3 wk).

A concentration of 0.5 ppm of Atrazine is lethal to soybean plants in solution culture; however, this technique limits severely the population size for screening purposes. For our initial efforts, 3 lb/acre of Atrazine (based on surface area) were applied to a peat : perlite : sand growing medium in greenhouse beds. For screening during the summer, 5 lb of Atrazine were applied to a one-acre field plot in which soybeans were seeded in rows at a high density. Planting was done at monthly intervals from May through August.

Over 4 million seedlings were screened during a 3-yr period. Seventy-four (74) putative mutants were isolated for further studies. Two of these putative mutants yielded

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offspring that survived and set a few seeds in 'Atrazine-treated growing medium. At that time, we felt that these offspring were really resistant; therefore, all seeds were planted in a field plot without Atrazine in order to get maximum seed yield. Sixteen plants survived which when progeny-tested failed to produce any resistant offspring. Thus if resistance was actually induced via seed mutagenic treatments it was either not stable or not readily transmitted via sexual reproduction.

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## CHLOROPHYLL-DEFICIENT MUTATION FREQUENCY IN BARLEY FOLLOWING CONTINUOUS GAMMA IRRADIATION OVER A WIDE RANGE OF EXPOSURES THROUGHOUT THE LIFE CYCLE

B. V. Conger*, M. J. Constantin, and P. J. Bottino†

Higher plants, eukaryotic organisms whose chromosome structure and biochemistry are similar to those of humans, offer the advantage of population size to study the genetic effects of low levels of ionizing radiation. Barley is a self-fertilized diploid ($2n = 14$ chromosomes) plant for which much information pertaining to mutagenesis exists in the literature. The chlorophyll-deficient mutation system in barley has been used to evaluate mutagenic effectiveness and efficiency of both physical and chemical agents. Objectives of this study were to determine (1) the nature of the dose-response curve for mutation frequency, (2) the mutation spectrum, and (3) the exposure required to double the spontaneous mutation frequency.

A preliminary experiment was conducted using the gamma field at Brookhaven National Laboratory in which exposure rate varied from 0.17 to 23.2 R/day (20 hr per day from the time of emergence until harvest, i.e., 96 days). The apical spike was harvested from each surviving barley plant (200 seeds were planted per radiation treatment) and bulked within each treatment. Twenty seeds from each spike were used to produce the $M_1$ generation. Five spikes were harvested from surviving $M_1$ plants and planted in greenhouse beds; resulting seedlings were scored for mutations.

Results showed that mutation frequency per 10,000 $M_2$ seedlings increased from 1.3 for the nonirradiated population to 4.3 at 0.17 R/day. The frequency of mutants remained about the same through 1.45 R/day; however, the frequency increased to 7.0 at 3.17 R/day and reached 45.4 at 23.2 R/day. Mutation spectrum expressed as a percentage value for each class of mutants summarized over all exposures was as follows: albino = 70.1%, viridis = 18.3%, xantha = 7.3%, tigrina = 2.5%, and striata = 1.9%. Although mutant spectrum varied among treatments, this variability was judged to be insignificant. It should be noted that the frequency of albino mutants is higher than that observed following mutagenic treatments of dormant seeds. The doubling exposure was calculated to be approximately 18 R (i.e., 0.1875 R/day) for barley compared to 1 rad for somatic mutations in *Tradescantia* and approximately 90 R for specific locus mutations in the mouse.

Several models were utilized with both transformed and nontransformed data to describe the observed mutation frequency dose-response curve. All models tested were deficient in one or more aspects. The model $y = Be^{CX^2}$ with Poisson weighting summarized the data best in the experimental region. However, this model is also deficient in that it predicts a mutation frequency for the nonirradiated population that is higher than observed. Thus the exact nature of the dose-response curve cannot be resolved with the data in hand.

A follow-up experiment is presently being conducted in which a similar design has been followed except that population size has been adjusted based on expected mutation frequency (i.e., nonirradiated and lowest exposures have $n = 1600$, intermediate exposures have $n = 800$, and highest exposures have $n = 200$ plants), and exposure rate varies from 0.01 to 24.2 R/day. Results of this experiment will permit a more accurate determination of the exact nature of the dose-response curve, and of the gamma radiation exposure that doubles the spontaneous mutation frequency.

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## EFFECTS OF PHYSICAL AND CHEMICAL MUTAGENS ON SURVIVAL, GROWTH, AND SEED YIELD OF SOYBEANS

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A knowledge of the dose response in terms of survival and various other performance endpoints is required for organisms treated with different mutagens to develop a sound mutation induction program. Although information exists in the literature concerning the response of soybean seeds to ionizing radiation, this information is limited primarily to x-rays and thermal neutrons.

Seeds of a sib-line of the soybean cultivar Forrest were treated with $^{60}Co$ $\gamma$ rays, fission spectrum neutrons, ethylmethane sulfonate, and diethyl sulfate. Plants of the

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$M_1$ generation were grown in replicated plots over a 3-yr period to provide dose response data and $M_2$ seeds to be used to isolate mutants. Seed water content was adjusted to 13% prior to mutagenic treatment and planting was done immediately after irradiation. For chemical treatments, seeds were presoaked 16 hr in air-bubbled water at 22°C, and then soaked aerobically either for 8 hr in various concentrations of ethylmethane sulfonate or for various time periods in 0.15% by volume of diethyl sulfate. Chemical solutions were adjusted to pH 7.0 with a phosphate buffer. Following chemical treatments, the seeds were rinsed thoroughly and planted immediately. The field was irrigated to prevent seed desiccation.

Survival dose-response curves show the typical shoulder region at low doses of γ-rays and neutrons; this is less evident for the chemical mutagens. Survival at the highest level of each mutagen used is as follows: 20% of control at 3.5 krad of neutrons, 55% at 40 krad of γ rays, 0% at 0.10 M of ethylmethane sulfonate, and 10% at 180 min soaking in diethyl sulfate.

Plant height was reduced to ≈15% of control at 3.5 krad of neutrons, ~35% at 40 krad of γ rays, 0% at 0.10 M of ethylmethane sulfonate, and ~35% at 180 min soaking in diethyl sulfate. Yield of beans per $M_1$ plant was 0% of control at 3.5 krad of neutrons and 0.10 M of ethylmethane sulfonate, 10% of control at 40 krad of γ rays, and 55% of control at 180 min soaking in diethyl sulfate.

Our recommendations for soybean seed treatment, based solely on the production of adequate quantities of $M_2$ seed for mutation selection, are as follows:

1. $N_f$—1.5 to 2.5 krad to seeds with 13% water.
2. γ—20 to 30 krad to seeds with 13% water.
3. EMS—8 hr of aerobic soaking in 0.025 to 0.050 M solutions using 16-hr presoaked seeds, thoroughly rinsed in running tap water and planted wet.
4. DES—90- to 150-min aerobic soaking in 0.15% by volume solution using 16-hr presoaked seeds, thoroughly rinsed in running tap water and planted wet.

## EFFECT OF GAMMA RAYS AND FISSION NEUTRONS ON DORMANT SEEDS OF LEGUME FORAGE CROPS

Ahmad Mokhtarzadeh* and M. J. Constantin

Natural and induced genetic diversity has been the primary source of raw material for plant breeding. High-energy ionizing radiations have been used successfully to induce genetic diversity in a large number of higher plant species. For such a mutation induction program it is essential to understand the biological effects of the mutagens. Radiosensitivity studies provide information which is valuable for plant breeders using ionizing radiations to induce genetic variability in their crops.

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Extensive literature exists on the biological effects of ionizing radiation on the majority of agricultural crops. Little is known, however, about the response of legume forage crops to gamma and neutron ionizing radiations. The present study was designed to quantify the effects of increasing doses of gamma radiation and fission neutrons on dry-dormant seeds of three selected species of legume forage crops. Seeds of three Iranian-bred forage legume species, namely, berseem clover (*Trifolium alexandrinum* L.), red clover (*T. pratense* L.), and alfalfa (*Medicago sativa* L.), were adjusted to 10% water content and exposed to either 20, 40, 60, 80, or 120 Kr of $^{60}$Co gamma rays at 850 R/min or 1.0, 2.0, 3.0, 4.0, or 5.0 krad, tissue equivalent in air, of fission neutrons. Treated and nontreated seeds were planted in a greenhouse bench containing a sterilized 1 : 1 peat-perlite mixture. The experimental design for each species was a randomized complete block with three replications. Each replication consisted of an 80-cm row with 35 seeds.

Data were collected for the following characteristics in each of the three species: germination, number of plants with simple leaf, number of plants with the first trifoliate leaf, number of plants with abnormal leaflets, plant height, number of trifoliate-leaf per plant, dry weight per plant, biological yield (number of trifoliate-leaf × plant height × dry weight per plant in grams), and survival. In addition, data on stem height, internode length, and coefficient of number of trifoliate-leaf per plant × height of stem × dry weight per plant in grams were collected for alfalfa and berseem clover. Statistical analyses are not completed as yet. The results thus far indicate that:

1. Differences exist in response of species to the morpho-physiologic effects of gamma and fission neutron irradiations.
2. Direct correlation exists between radiation exposure levels and response of a majority of traits in this experiment.
3. The highest exposure of radiation produced more damaging effects than the highest exposure of fission neutrons.
4. Some traits, such as percent germination, number of trifoliate-leaf per plant, and survival after a short period of time (1—2 mo after germination) are not good indices of seed response to fission neutrons.
5. Alfalfa, which was the only polyploid species in this experiment, tolerated higher exposures of gamma radiations than the diploid species.
6. Leaf morphological abnormalities were very frequent in both types of radiation treatments.

In conclusion, it appears that seed irradiation for a mutation induction program should not exceed the following:

| | Berseem clover | Red clover | Alfalfa |
|---|---|---|---|
| Fission neutrons | 4 krad | 5 krad | 3 krad |
| Cobalt-60 gamma | 60 Kr | 40 Kr | 80 Kr |

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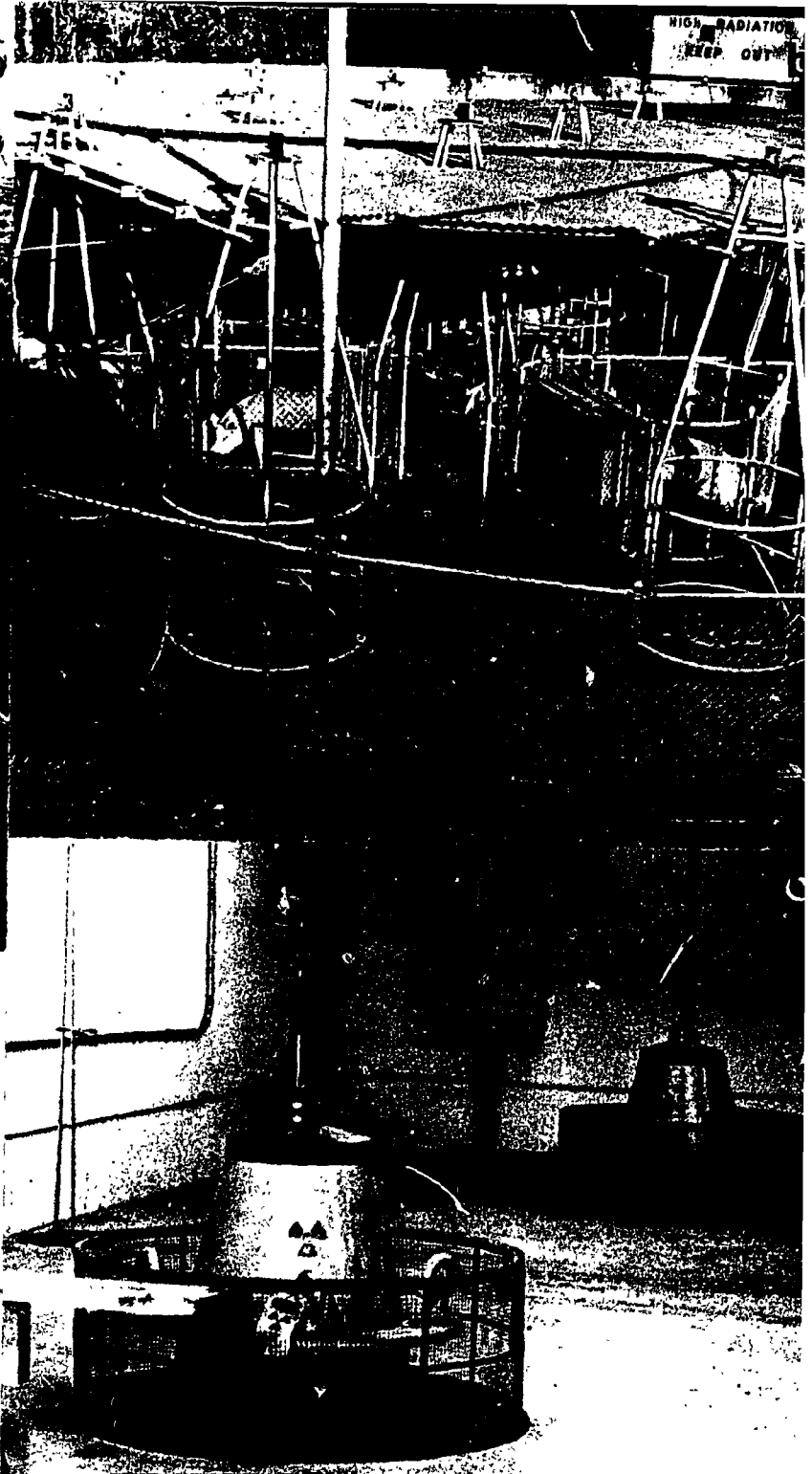


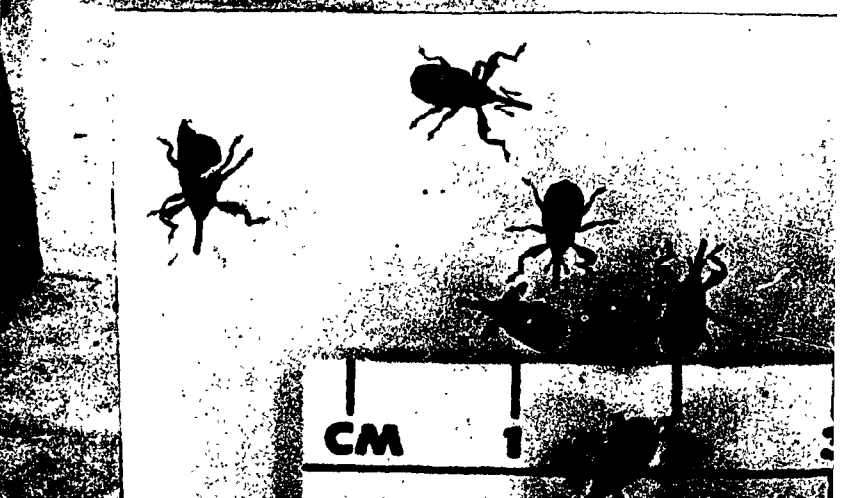

# Animal Effects

T. R. Noonan, Head

Mammalian Genetics and Reproduction B. H. Erickson, Group Leader
Mammalian Cytogenetics A. F. McFee, Group Leader
Late Somatic Effects T. R. Noonan, Group Leader

## RADIATION STERILIZATION EXPERIMENTS ON THE BOLL WEEVIL

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Cooperative studies were begun in the summer of 1975, at the request of the Agricultural Research Service of the USDA, to investigate the feasibility of using the variable dose rate irradiation facility (VDRIF) for mass radiation sterilization of the cotton boll weevil, *Anthonomus grandis* Boheman, for projected large-scale field studies.

In experiments at the Boll Weevil Research Laboratory (BWRL) small samples of the insects have been sterilized successfully in an enclosed irradiator using a dose of 6-7 kilorads of $^{60}$Co gamma radiation delivered at a high dose rate in 25 fractions spread over a 4-day period. The treatment is begun at a particular time after the eggs are hatched and carried out under closely controlled conditions, particularly regarding diet, humidity, and temperature. Similar satisfactory results have now been repeatedly demonstrated in pilot experiments at the VDRIF in which large sample sizes of weevils from the BWRL production facility were transported at the proper point in the life cycle to Oak Ridge, irradiated under a similar fractionation schedule while being maintained under the appropriate controlled conditions, and then returned to the BWRL for evaluation of the treatment. Feasibility studies are continuing with the immediate objective of scaling up operations to irradiate 5 million weevils/wk for pilot field demonstrations to be conducted by the ARS in the near future.

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## RADIATION-INDUCED OPACIFICATION OF THE LENS IN MICE: EFFECTS OF RADIATION QUALITY AND RATE OF DELIVERY

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These studies were initiated as part of the comprehensive project conducted at the Oak Ridge National Laboratory Biology Division on late somatic effects of ionizing radiation in mice. The mouse lens experiments were carried out to obtain a better understanding of the influence of radiological variables such as the ionizing quality and dose rate of the radiations used on the endpoint. The mouse lens is particularly suitable for this type of study because it is highly vulnerable to the induction of opacification by ionizing radiation at low doses and level of effect may be conveniently and nondestructively scored.

In these experiments replicate groups of mice received graded doses of fission neutrons either in the ORNL Health Physics Reactor at dose rates on the order of rad per min or in a $^{252}$Cf facility at a level of ~1 rad per day. Comparable groups of mice were exposed to graded doses of x-rays or to $^{137}$Cs gamma radiation at a dose rate of either ~45 rad/min or about 8 rad/day. Sham irradiated mice subjected to the same handling, transportation, and restraining procedures as the corresponding irradiated animals served as controls. The eyes of the mice were examined periodically with the slit lamp through periods of at least one year after

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(a) Survivors of a band of burros experimentally exposed to radiation in the 1950s. Some of the burros are now over 25 years old. (b) Pregnant sows receiving continuous doses of $^{60}$Co $\gamma$ radiation in low dose rate irradiation facility to study effects of external radiation on the developing fetus. (c) CARL's irradiation facility currently used in a joint research project with the Boll Weevil Radiation Laboratory for eradication of the boll weevil. (d) Sister chromatid exchanges in a bovine lymphocyte visualized by the fluorescence-plus-Giemso technique. (e) Photomicrograph of normal blood platelets in citrated platelet-rich-plasma (PRP).

treatment, and the degree of opacification observed scored according to accepted ophthalmological criteria.

The results are as yet tentative but are consistent with the well-known observation that the murine lens has a high radiosensitivity to fast neutrons with a relatively small, possibly marginal, influence of dose rate as compared to sparsely ionizing radiation. The dose response for both types of radiation is nonlinear so that for acute radiation the RBE appears to vary as a small ($\leq$0.5) negative power of the neutron dose. This has been reported in a variety of other biological systems in both animals and plants. The RBE is consequently very high at the lowest doses ($\leq$0.5 rad) at which our system can detect a positive effect. At lower doses the normal background of spontaneous opacification effectively sets a lower limit to the range over which estimates of RBE can be made within reasonable limits.

## EFFECTS OF FISSION NEUTRONS AND X-RAYS ON THE MOUSE EMBRYO

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This project was developed because of the scarcity of adequate data on effects of prenatal exposure to densely ionizing radiation in mammals. For example, data were particularly lacking for very early postconception exposure in humans when pregnancy may be unsuspected. One of the important practical concerns is the possible risk to occupants of commercial aircraft since at cruising altitudes, for both conventional jet airliners and SST's, fast neutron levels are many times higher than normally encountered on the ground. In our studies the fast neutron irradiations were done in the ORNL Health Physics Research Reactor with the cooperation of personnel in the DOSAR facility. The x-ray experiments were done at the ORNL Biology Division and at the Civil AeroMedical Institute.

Our earlier studies (Friedberg et al., 1973) on the mouse pronuclear zygote stage suggested that inactivation of the zygote with either neutrons or x-rays is predominantly a single-hit process with a relative biological effectiveness (RBE) of 4.5 for fast neutrons, close to the low dose limiting value. Furthermore, we observed that irradiation early in that stage appears to impede progression to a less radiosensitive phase of the same stage.

A manuscript is in preparation on the subject of risk estimates of prenatal death in mice irradiated with neutrons or x-rays during the pronuclear zygote stage, based on the survival curve parameter reported by Friedberg et al. (1973). This stage of early gestation is possibly the most sensitive period to lethal injury from ionizing radiation during the entire life of a mammal. We know of no comparable human or other animal data on this very early stage. This information has provided risk estimates of injury to humans in the aviation environment.

We have recently published an account (Friedberg et al., 1976) of negative findings in regard to late effects on survivors of mice irradiated with a single 15-rad dose of fast neutrons in the early pronuclear zygote stage, a dose, according to our earlier studies, sufficient to produce a substantial mortality in utero. For the two major endpoints, effect on longevity and the incidence of neoplasms, no significant increase in risk resulted from this treatment.

We have subsequently irradiated the embryo at the two-cell stage and found it to be many times more radioresistant in terms of prenatal survival than the pronuclear zygote. At low doses, results were consistent with the assumption that lethal injury leading to prenatal death is the direct result of the passage of a single ionizing particle through a sensitive region in each of the two blastomeres. At higher doses indirect radiation effects on the surrounding placental and uterine tissues appear to contribute additionally to prenatal deaths. Microscopic as well as gross examinations are being conducted to pinpoint more accurately the nature and development of the underlying changes.

Present studies are also concerned with induction of congenital lesions by low doses of radiation during periods of embryogenesis critical for the development of various organ systems. Despite the abundant literature on the subject, no satisfactory estimates exist concerning the risk of congenital lesion induction at low dose levels. Ongoing work is concentrating on effects of irradiation during the second week of gestation in the mouse (corresponding to the second through sixth weeks in humans), when the embryonic brain is highly susceptible to radiation-induced malformation. Results for irradiation at one critical time, gestation day 8.5, show a relatively high RBE of ~4.5 for late prenatal mortality (at day 16). In addition, at various postirradiation intervals survivors are being examined for gross anomalies.

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## RESPONSE OF THE DEVELOPING RAT BRAIN TO VARYING DOSES AND DOSE RATES OF RADIATION

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The effects of prenatal radiation on the brain can be extrapolated from an experimental species to man only if

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studies involve the same developmental event. Cortical neuron accumulation occurs between days 16 and 19 in the rat. At this time the brain contains a heterogenous cell population which includes neurons (mature, migrating, differentiating, and stem cell) as well as neuroglial precursors with a threshold for cell death, recovery, or compensatory response which varies at each stage of cell maturation. A comparable stage of cortical neuron development in man is between weeks 10 and 18, a critical period previously observed in offspring irradiated at Hiroshima and Nagasaki where mental retardation and decreased head size occurred.

To study the effect of dose and dose rate on cortical development, pregnant rats were irradiated on day 18 of gestation at doses of 40, 80, 160, 200, and 240 rad and at dose rates of 1, 3, 10, and 47 rad/min. Two sections of the brain-cerebral cortex and rest-of-brain were analyzed for DNA (cell numbers), RNA, and protein at birth, 21, 63, and 160 days of age. The cortex was affected to a greater degree than the rest-of-brain by dose and dose rate with weight and DNA values reduced to 43% of control levels after receiving 240 rad. In the rest-of-brain by 160 days, compensatory cell division after birth produced DNA values and weight not different from controls in rats which had received 40, 80, and 160 rad at all rates, 200 rad at 1, 3, and 10 rad/min, and 240 rad at 1 and 3 rad/min.

From the data accumulated on man and rat irradiated during comparable developmental stages (i.e., cortical neuron multiplication) it would appear that a reduction in cortical weight and DNA would be affected by dose but not rate at 40 and 80 rad but that at higher doses weight and DNA would decrease as dose rate increased. The rest-of-brain is affected by dose or rate only at higher doses.

## EFFECT OF $^{60}$Co $\gamma$ RADIATION ON THE STEM AND DIFFERENTIATING SPERMATOGONIA OF THE POSTPUBERAL RAT

B. H. Erickson

Previous descriptions of the radioresponse of rodent spermatogonia have relied on schemes of spermatogonial proliferation and differentiation that were either incomplete or inaccurate. This study was therefore undertaken to interpret the radioresponse of rat spermatogonia after a currently accepted pattern for their renewal and evolution (Fig. 13).

Rats were irradiated with $^{60}$Co $\gamma$ radiation at doses varying from 12 to 600 rad at 30 rad/min. Tubules were isolated from Zenker-fixed testes and evaluated according to the method of Huckins (1971).

Stem spermatogonial number was little affected by doses up to 200 rad, and the dose-response curve for this criterion yielded a $D_0$ of 373 ± 37 rad and an extrapolation number (n) of 2.1 ± 0.3. Stem-cell mitosis (as reflected by counts of type $A_1$ spermatogonia) was, however, substantially affected by a dose of 12 rad and was characterized by a $D_0$ of 148 ± 8 rad and an n of 0.5 ± 0.1. The dose required to produce a measurable effect increased as the spermatogonium attained higher states of differentiation. $D_0$ and n varied from 139 ± 24 rad and 1.2 ± 0.9, respectively, for type $A_1$ to 215 ± 10 rad and 2 ± 0.4 for type $A_4$. Due to the extreme lability of its mitosis, the stem spermatogonium appeared to be the weakest link in the chain leading to the spermatozoon.

At doses of 100 rad and below stem-cell mitotic delay appeared to be the principal cause for the diminution in the population of differentiating spermatogonia. Decreases in the elaboration of differentiating spermatogonia at higher doses were due to a combination of cell-killing and mitotic delay.

Giant cells (Fig. 13D) appeared in the stem cell population at approximately 4 days after irradiation. Their number peaked at 7 to 9 days and declined thereafter, reaching 40% of the preirradiation value by 13 days after irradiation. Assuming that giant stem cells arise as a consequence of mitotic delay and disappear as a consequence of a subsequent mitosis, the difference in time between the 50% points on the ascending and descending portions of a curve describing the rise and fall of the giant cell population should provide a rough estimate of the average length of the stem-cell mitotic cycle. The estimated length of the average intermitotic interval for the rat stem spermatogonium resulting from the application of this method was 7 days. The average duration of stem-cell mitotic delay following a dose of 100 rad was also 7 days.

Since the stem-cell mitotic index is low (0.10-0.30, depending on researcher and stage of the spermatogenic cycle; Huckins, 1971 and Clermont and Hermo, 1975), it is likely that the stem cell is only under an intermittent stimulus to divide. Thus if a few hours of mitotic delay were coupled with a normal $G_1$ of several days' duration, what is apparently mitotic delay could also be a reflection of length of mitotic cycle. And, in light of the estimate of cycle time based on the giant cell, this is quite likely the case.

Since cells with a protracted $G_1$ have been noted to be especially refractory to the cell-killing effects of ionizing radiation (Sinclair and Morton, 1966), the ascription of an extended $G_1$ to the stem spermatogonium could explain its unusual survivability.

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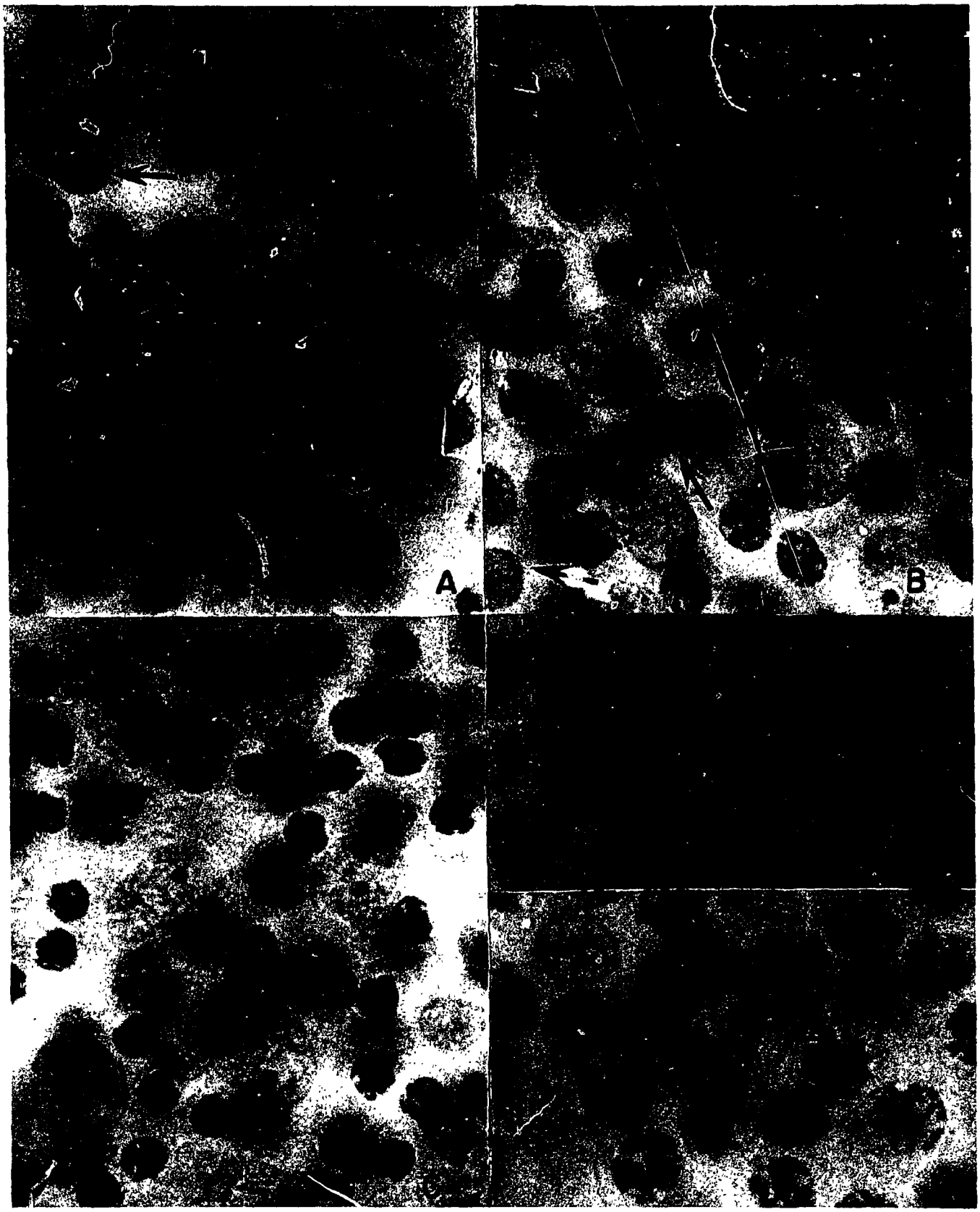


Fig. 13 A. Chain of undifferentiated $A_1$ spermatogonia in a Stage II tubule. Note irregular shape of $A_1$ nuclei (x910). B. Chain of $A_1$ spermatogonia and stem cell (S) in a Stage VIII tubule characterized by preleptotene spermatocytes. Note typical oblong shape of stem cell and coarse deposition of chromatin; contrasted with globular shape and fine chromatin deposition of $A_1$ spermatogonia (x910). C. Stem spermatogonium in Stage VIII tubule. Note state of isolation with respect to $A_1$ spermatogonia. Adjoining cells are either preleptotene spermatocytes (small and deeply stained) or Sertoli cells (large cells with single prominent nucleolus) (x910). D. Giant stem cell. Contrast size with stem cells of B and C. Note also prominent nucleoli of giant stem cell (x910). E. Stem cell in advanced stage of necrosis. Contrast size with giant cell of D (x910).

## RESPONSE OF THE SERTOLI CELL AND STEM GERM CELL TO $^{60}$Co $\gamma$ RADIATION (DOSE AND DOSE RATE) IN TESTES OF IMMATURE RATS

B. H. Erickson and M. J. Blend*

Regaud and Dubreuil (1907) established that spermatogonia of the immature rabbit testis were more adversely affected by ionizing radiation than those of the adult testis. Shaver (1953) found that rats exposed to 300 R of x-rays when 1 to 5 days old were essentially sterilized, but that rats exposed to 400 R at 10 to 15 days of age apparently regained full fertility. In another analysis of germ-cell radioresponse in the neonatal rat testis, Hughes (1962) found that after either 150 or 460 R of x-rays, germ-cell survival was almost zero in testes irradiated prior to the third postnatal day. At day 3, however, survival increased markedly. These findings were related to gonocyte transformation to spermatogonia—a process that begins at about the third postnatal day in the rat (Clermont and Perey, 1957).

In a sole report on x-ray effects on the undifferentiated Sertoli cell, Courot (1963) reported that in the testes of 18- and 93-day-old lambs the number of Sertoli cells per tubule cross section at 3 days after irradiation was reduced by 50% or more by doses of 500 to 2000 R. Since doses of such magnitude were either sterilizing or near sterilizing in their effect, a meaningful analysis of the impact of apparent Sertoli cell loss on testicular function was not possible.

Except for the Sertoli cell, the pattern of the radioresponse of the immature rat testis is therefore unknown. Dose effects have not been adequately covered, however, and the influence of age on the spermatogonial response to change in dose rate has not been tested. These deficiencies provided the impetus for this study.

Rats in age groups varying from 2 to 60 days were irradiated ($^{60}$Co, $\gamma$) with doses from 50 to 400 rad at rates of either 1 or 40 rad/min. Effects were assayed at 5 and 80 days after irradiation in either testicular cross sections or tubule whole mounts.

Prior to day 4 of postnatal development, the gonocyte or primitive stem cell was the most radiosensitive cell in the testis. From day 4 to approximately day 15 the Sertoli cell was the most critical element, and thereafter the definitive stem cell was of first importance. A dose of 100 rad irreversibly reduced the number of Sertoli cells to 63% of control. Of the ages tested beyond day 2, the 9-day testis was most severely affected. We estimated that a dose of 400 rad would reduce sperm output of the 9-day testis to 21% of control. After day 4 and prior to day 20, 300 rad produced a permanent decrement in the stem-cell population. Six hundred rad are required to produce this effect in the adult. Dose rate was an important mediator of the radioresponse of both Sertoli and germ cells.

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## SISTER CHROMATID EXCHANGES IN LARGE ANIMAL LEUKOCYTES

A. F. McFee and M. N. Sherrill

At some point during or after the DNA replication process, the chromatids of a chromosome are capable of undergoing a reciprocal exchange of material. The mechanisr s underlying these sister chromatid exchanges (SCE) are not presently understood, although it is generally accepted that they do occur spontaneously at a low level. Relatively simple procedures have recently been developed for treating cells so that SCEs can be microscopically visualized. The procedures are based on the incorporation of 5-bromodeoxyuridine (BRdU) into the DNA strands during the S phases of two successive cell cycles. The semiconservative nature of DNA replication causes such cells at the second metaphase to contain one doubly-substituted and one singly-substituted chromatid. These chromosome arms then differentially absorb fluorescent dyes and/or Giemsa stain. Sites of material exchange between the two arms are thus readily identified. The rate of SCE induction is known to be increased by a variety of chemicals and is becoming widely accepted as a very sensitive indicator of the mutagenicity of test substances.

Most works thus far reported in which SCE evaluations were performed have utilized either cultured human or Chinese hamster cells. We feel that the application of this technique to large-animal cells can contribute to an understanding of the mechanism of SCE induction through comparisons in species with varying numbers and morphology of chromosomes and can broaden the mutagenicity testing through the utilization of species with widely varying physiological differences. From the practical point of view, mutagenicity data are needed for species of economic importance as well as for man.

Since BRdU itself is known to induce SCEs, we have undertaken initially to establish the dose-response relationship in large-animal species by evaluating SCEs in leukocytes cultured in the presence of varying levels of the chemical. We have seen that the number of exchanges in bovine leukocytes (Fig. 14) increases linearly in a significant relationship to BRdU concentration between I

Fig. 14 Bovine leukocyte metaphase with several sister chromatid exchanges.

and 20 μg/ml. The exchange level in our bovine cells was only about 30% of that reported for comparable BRdU levels in Chinese hamster cells (Wolff, 1974) and approximately half that for human leukocytes (Lambert et al., 1976). These species comparisons have not yet been examined on the basis of relative chromosome lengths or differences in morphology. Data are being collected for similar dose-response evaluations in other species.

A preliminary examination of only two pigs at two BRdU levels strongly suggests that a significant difference in sensitivity may exist among individual animals within a species.

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## CHROMOSOME ABERRATIONS IN SWINE LEUKOCYTES INDUCED BY HALF-BODY AND WHOLE-BODY IRRADIATION


A. F. McFee, M. N. Sherrill and M. W. Banner


Estimation of the dose received in cases of accidental exposure to ionizing radiation can be accomplished through the scoring of chromosome aberrations in the peripheral blood lymphocytes. One shortcoming of this system is that few accidental cases involve whole-body exposures which deliver relatively uniform doses to the circulating leukocytes. The possibility thus exists that cells in that part of the body receiving the higher radiation dose might be selectively eliminated due to a higher rate of interphase death or to mitotic delay, thereby biasing downward the chromosome aberration rate scored at metaphase. Partial-body exposures have been simulated by the culture of 50:50 mixtures of normal and in vitro-irradiated cells; however, these results have not been adequately related to the in vivo situation. We have irradiated groups of pigs at different levels to establish the in vivo-in vitro relationship and to determine whether it varies with exposure level.

Chromosome aberrations were scored in 48-hr leukocyte cultures from pigs subjected to whole-body or half-body gamma irradiation with 100, 150, 200, 300, or 400 R. Half-body (anterior) irradiation resulted in the recovery of approximately half as many aberrations as did equivalent whole-body exposures at levels of 200 R or less. Higher exposures yielded proportionally fewer anomalies in half-body irradiated subjects. These lower levels apparently resulted from the selective disadvantage of irradiated cells in coming to mitosis but did not seem to be related to the amount of chromosome damage sustained by the cell. When adjustments were made for effective dose to the in vivo cells, the dose-response pattern showed good agreement with published values for mixtures of normal and in vitro-irradiated human lymphocytes.

## RADIATION-INDUCED ABERRATION RATES IN ANEUPLOID VS. DIPLOID LEUKOCYTES


A. F. McFee, M. N. Sherrill, and M. W. Banner


Cytological preparations always contain a percentage of cells which are deficient for one or more chromosomes of the normal complement and a smaller number which contain an extra chromosome. Although many of these aneuploid cells are presumed to be preparational artifacts, at least a portion of them are evidently native to the peripheral leukocyte population. In scoring aberrations, some workers select only those cells with the modal chromosome number while others accept limited departure from modality. Since irradiation increases aneuploid frequency among leukocytes, bias would be introduced into aberration coefficients by selecting for scoring only those cells with the modal centromere number if aberrations were either more or less frequent in aneuploid cells.

Chromosome aberrations were scored in 48-hr cultures of pig blood which had received 0, 100, 200, 300, or 400 R of gamma irradiation. Exposure to all four levels of irradiation significantly increased aneuploidy levels but not in a dose-related manner. Aberration coefficients for hypodiploid cells were consistently higher than those for diploid metaphases for both 1-hit and 2-hit aberrations. Insufficient numbers of hyperdiploid metaphases were seen among the 5072 cells scored for an adequate evaluation. The results indicate that aneuploid cells do in fact have higher aberration levels and that their elimination at scoring results in artificially lowered aberration coefficients.

## BLOOD CELL DAMAGE AND RECOVERY IN PIGS CHRONICALLY IRRADIATED THROUGHOUT PRENATAL DEVELOPMENT

A. F. McFee, M. N. Sherrill, and M. W. Banner

Various organ systems of the developing embryo pass through stages during which they are particularly sensitive to irradiation damage. The rather extended period over which the hemopoietic system develops makes it a particular candidate for damage as a result of chronic irradiation. In these studies swine embryos were continuously irradiated during essentially all of their developmental period. Relatively low levels of exposure resulted in lowered numbers of lymphocytes in the blood of newborn piglets. The neonatal animals, however, had a capability for recovery from this lymphocytopenia which far exceeded that of adult animals.

Pregnant gilts were maintained on irradiation fields which delivered either 19 or 3.3 R/day through the first 108 days of gestation (av. length 112 days). Body shielding reduced the mean exposure rate for the fetuses to 37% of that received by the dam. Gilts exposed to the two rates thus gave birth to pigs which had accumulated 731 and 127 R. Routine analyses were performed on blood samples from these pigs on the day of birth and at biweekly intervals through 22 wk of age.

Leukocyte numbers in newborn pigs were suppressed by both irradiation rates to approximately 70% of the level in accompanying control pigs. The most significant depression was among the segmented neutrophils, although lymphocytes were also noticeably affected by the higher dose rate. Unlike adult animals, which did not fully recover their blood cell numbers during a 22-wk postirradiation period, young pigs had regained the control level of leukocytes within 2 wk and showed no further effect of the irradiation on these elements.

The hemopoietic system of the fetal pig exhibits most of the physical characteristics of the adult system during the last trimester of gestation. The rapid recovery capability of the newborn suggests, however, that the system is still immature at birth and capable of rapid dedifferentiation to increase cellular production at the stem cell level.

## CHROMOSOME ABERRATIONS IN SWINE LEUKOCYTES INDUCED BY CHRONIC IRRADIATION

A. F. McFee, M. N. Sherrill, and M. W. Banner

Chromosome aberration levels in peripheral blood lymphocytes have been utilized for dosimetry estimates following acute radiation exposures and are known to be well related to the dose level. The general body distribution and ready accessibility of lymphocytes suit them for use as a standard, and several investigators are relating their sensitivity to chromosome damage to that of the more critical germ cell lines.

Whereas aberration induction by acute exposures has been adequately defined, the levels produced by chronic irradiation have received very little attention. Because relatively large segments of human population could be exposed to additional levels of chronic irradiation as a result of increased industrialization, the quantitative determination of the effects of such exposure is necessary. Our results indicate that even though repair processes and a definitive chromosome rejoining time act to minimize their levels, chromosome aberrations nevertheless do accumulate with continued irradiation.

We scored chromosome aberrations in blood samples taken at various intervals from pigs which were receiving continuous exposure to 19 or 3.3 R/day of gamma radiation. Terminal deletions among the 19 R/day animals were elevated significantly above the preirradiation level by accumulated exposures of 400 R or more. These 1-hit anomalies continued to increase throughout the 108-day exposure period at a rate which was significantly related to the accumulated dose. The rate of production of 2-break aberrations (rings and dicentrics) is normally reduced by protraction of the exposure. At the relatively low level of 19 R/day, these aberrations accumulated rather slowly but at a rate which was related to accumulated exposure in a highly significant manner.

Aberration rates were somewhat more erratic among samples from animals receiving only 3.3 R/day. Both 1-hit and 2-hit anomalies, however, again showed a definite relationship to the cumulative exposure although only in the 1-hit category was it statistically significant. Cumulative exposures of 150 R or more at this rate consistently produced levels of both aberration types which were above the preirradiation level. There was no indication that aberration rates plateau during the irradiation period even though the 19 R/day animals accumulated total exposures of more than 2200 R.

## PERIPHERAL BLOOD CELL DAMAGE AND RECOVERY IN CHRONICALLY IRRADIATED SWINE

A. F. McFee and M. N. Sherrill

The pattern of dose-dependent depression and gradual recovery of white blood cells is well defined as a consequence of acute radiation exposure. While it is accepted that chronic irradiation is less damaging, insufficient information is available to adequately relate damage and repair following chronic exposure to that resulting from acute doses or to evaluate differences in the rate at which chronic exposure might be accumulated. Complete evaluations have been performed on weekly blood samples taken from mature swine during and after 108-day periods in which they were chronically irradiated at rates of 19 or 3.3 R per day (administered over 23 or 22 hr per day).

Continuous irradiation at both rates produced a measurable depression of the number of lymphocytes in the peripheral circulation during the second week of exposure. The 19 R/day rate reduced lymphocyte numbers to 50% of their preirradiation level by the third week. From the third week through the remainder of the 16-wk irradiation period, lymphocyte levels in irradiated sows remained at about 50% of their accompanying control animals. When the exposure rate was reduced to 3.3 R/day, circulating lymphocytes were depressed to only about 80% of control values. This effect was again expressed by the third week and the relationship to control animals did not change appreciably during the remainder of the exposure period.

The greater rate of delivery of the irradiation thus did not appreciably affect the time required for the expression of damage but did increase the severity of damage. This relationship seems compatible with the theory that peripheral lymphocytes are a population with a continuously variable radiosensitivity and that with increased irradiation rate more cells will be killed but within a similar time frame. Among the animals receiving the lower dose rate, lymphocyte depression was detected after average accumulations of only 33 R. This level is only slightly higher than the minimum acute exposure required to produce a similar effect.

The 19 R/day exposure rate reduced the average level of circulating platelets to approximately 75% of the control level after 4 wk. This general level of suppression continued throughout the experiment. Red cell numbers in irradiated animals showed a very slight but consistent depression below the control level.

Some recovery of lymphocyte numbers occurred shortly after the irradiation ended, but they continued at a significantly depressed level throughout the 22-wk post-irradiation sampling period.

## THE EFFECT OF CONTINUOUS PRENATAL IRRADIATION ON THE IMMUNE RESPONSE IN SWINE

L. B. Sasser and G. E. Jarboe

Little is known of the effects of continuous low-level irradiation on the immune response of long-lived mammals, especially when exposure occurs during the prenatal period. If prenatal exposure of the population to low levels of radiation causes impairment of immune competence, then significant health problems could arise many years after exposure. In this study pregnant sows were irradiated continuously for the first 108 days of pregnancy at rates of 20 or 9 R per day, which exposed the fetus to approximately 7 or 3 R per day. The Jerne hemolytic plaque technique was used to study the immune response at 10, 70, and 150 days of age. Spleens were removed and homogenized 6 days after pigs were injected with $10^9$ to $10^{10}$ sheep red blood cells. Spleen cells were incubated on an agar medium for proper plaque development, and the hemolytic plaque-forming cells (PFC) were counted.

Prenatal irradiation did not appear to affect the number of PFC in the spleens at either dose studied. The average number of PFC was 80, 350, and 910 PFC/$10^7$ spleen cells at 10, 70, and 150 days of age, respectively. Spleens from the group receiving 7 R per day weighed (as a percentage of body weight) significantly less ($P < 0.05$) than that of control spleens at all ages studied, whereas no change in spleen weight was found in the group receiving 3 R per day. The significance of spleen weight loss is not known, but it does not appear to be related to a loss of immunologically competent cells.

## RADIATION DOSIMETRY IN THE DEVELOPING PIG FETUS

A. F. McFee, Leo Wade, Jr.*, and J. A. Bacon†

The quantitative assessment of radiation effects on the unborn fetus is hampered by a lack of knowledge of the relationship between dose administered to the pregnant animal and that actually absorbed by the fetus. The extent of body shielding afforded the unborn would be expected to vary with such factors as stage of gestation, position of the fetus, and size of the dam. We have made a series of dosimetric measurements which indicate that body size of the dam is the factor having the most significant influence of the dose received by the pig fetus.

Six lithium fluoride glass-rod dosimeters were surgically attached at random locations on the outer surface of each uterine horn of 15 gilts. Animals were then bred and groups of five gilts were exposed to chronic irradiation during 21-day periods representing the first, middle, and last trimester of gestation. Irradiation was at the rate of 19 R/day spread uniformly over a 23-hr day. At the end of the exposure periods, hysterectomies were performed to recover the dosimeters. The cumulative doses thus measured at the uterine surface was assumed to adequately represent those received by the developing fetuses.

The position of the fetus within the uterus seemed to have little bearing on the dose received since the standard deviations for the 12 dosimeters in each gilt averaged only 5.4% of their mean. A significant negative correlation was found between body weight of the dam and the proportion of her midline air dose received by the fetuses (Fig. 15). This relationship was defined as: % of air dose = 52.82 − 0.118 W, where W is body weight (in kg) of the pregnant gilt. Since increased body weight accompanied advancing pregnancy, it was not possible to separately evaluate the effects of the two factors. It seems logical, however, that body size of the dam would be the predominant factor.

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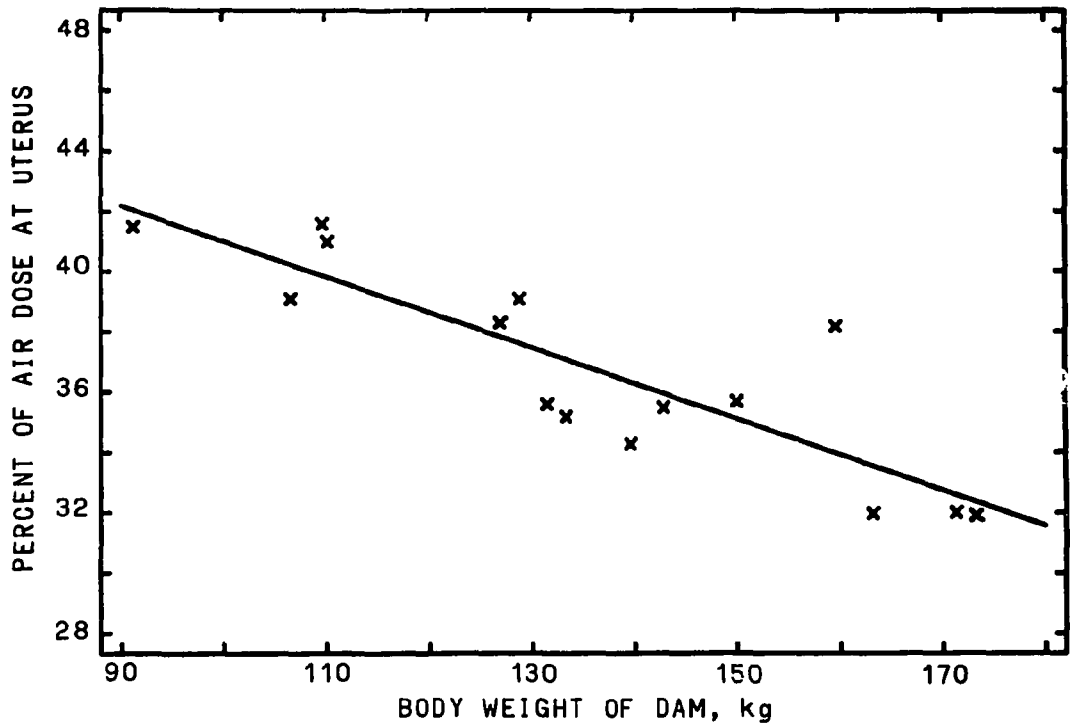


Fig. 15 Relationship between body weight of dam and percent of air dose of chronic irradiation received by fetuses.

## THE EFFECT OF CONTINUOUS PRENATAL IRRADIATION OF THE PIG UPON SELECTED HEPATIC ENZYMES AT DIFFERENT PERIODS AFTER BIRTH

Frank R. Mraz

The formation of new fundamental proteins which have enzymatic activity is a necessary capability of developing tissues and a part of the process of development. Irradiation of the developing embryo kills some cells, causes mitotic delay in others, and may alter the developmental capabilities of surviving cells. Changes that occur days after irradiation might be important manifestations of injurious action on enzyme synthesis mechanisms. Using appropriate techniques, measurements were made of the development of the hepatic microsomal system that catalyzes the detoxification of hexobarbital and the enzymes involved in gluconeogenesis in the liver: glycerol-phosphate dehydrogenase (GPD), glucose-6-phosphatase (GP), and glucose-l-phosphate uridyl transferase (GPUT).

The offspring of sows continuously irradiated at 20 R/day from $^{60}Co$ sources and those of control sows were sacrificed 5 hr to 62 days after birth. Their livers were removed, chilled, and portions homogenized. The activity of the four selected enzymes was determined on suitable substitutes. No irradiation effect was observed upon the hepatic enzymes (GPD, GP, GPUT, or the microsomal oxidase that detoxifies hexobarbital).

## EFFECTS OF CONTINUOUS PRENATAL $\gamma$ RADIATION ON THE PIG AND RAT

B. H. Erickson and P. G. Martin

Little is known of the effects of continuous low-level irradiation applied prenatally to the long-lived mammal. As compared with the rodent, developmental events are protracted in long-lived species and consequently are at risk longer. Estimation of radiation risk to man therefore requires data from animals in which developmental events are similarly protracted.

Pigs were irradiated continuously for the first 108 days of their 112-day gestation period at rates of 20, 9, 3, and 1.5 R per 22-hr day. Six pregnant gilts and six controls were employed at each dose rate. Fetal doses were 7, 3, 1, and 0.5 rad/day. Neither the health of the gilt nor the number of live births was affected by any exposure. Postnatal viability was also unaffected. Radiation effects on growth and organ development were assayed at birth, 70, and 150 days of age. Body weight and growth were unaffected by dose rates of 3 rad/day or less; in addition to the gonad, only the weight of the brain was affected by 3 rad/day. At 1 rad/day or less only gonadal weight was reduced. The most spectacular finding at doses of 7 and 3 rad/day was sterility in both sexes. Following 1 rad/day, germ-cell number was reduced to 5% and 2% of control in the female and male, respectively. At 0.5 rad/day, germ cells were reduced to 43% of control in the female and 11% of control in the male. In contrast to the pig, 7 rad/day

reduced the germ-cell population of male and female rats to only 49% and 35% of control, respectively, and 1 rad/day produced no apparent effect in either sex. It therefore appears that interspecific differences in the response to continuous $\gamma$ radiation are large and that the germ cell is the most labile cell type.

The cell types in the gametogenic pathway found most vulnerable to the destructive effects of ionizing radiation were the oogonium in the female and primitive stem cell (gonocyte) in the male.

The reason for the interspecific difference hence resides in the characteristics of these cell types. Factors such as length of mitotic cycle and time required for an oogonium to evolve to an oocyte may be involved, but the most obvious explanation for the difference is the time allotted to oogonial mitosis. Before being transformed to oocytes, rat oogonia are mitotically active for only 5 days, whereas in the pig, oogonia undergo mitosis for approximately 60 days. Thus at a dose rate of 1 rad/day, the oogonia of the rat would absorb only 5 rads versus 60 rads for the pig. The primitive stem cell of the male is at risk for 13 days in the rat and approximately 140 days in the boar. This is then the probable basis for both the intersexual and interspecific differences in the response to continuous irradiation. Oogonia are mitotically active in the human female for approximately 150 days, and the primitive stem germ cell of the male is at risk for approximately 11 yr; therefore, if length of stage is a limiting criterion, it is likely that the germ cell of the developing human will be more susceptible to the adverse effects of continuous irradiation than any other mammalian species.

## EFFECTS OF CONTINUOUS PRENATAL IRRADIATION ON THE BRAIN OF THE PIG AND RAT

Polly Martin and Joyce King

To predict the effects of continuous low dose irradiation on the human fetus, two experimental species with different gestational periods, the pig and the rat, were selected for study. The pig's developmental sequences take place over a protracted period (112 days) while the rat's developmental pattern is compressed (22 days). Pigs were irradiated at 7, 3, and 1 rad/day, and rats received 7 and 3 rad/day for a 21-day period. DNA (an indication of cell numbers), RNA, and protein were analyzed on three sections from the pig brain (cerebral cortex, cerebellum, and rest-of-brain) and two from the rat (cerebral cortex and rest-of-brain) to evaluate postnatal growth patterns (initial effects and possible recovery). Based on the difference in stage of prenatal brain maturation in the two species, the greater reduction in cells and weight in all parts of the brain would be expected in piglets which received 7 and 3 rad/day in utero (83 to 89% of controls) than in rat brains (90 to 103%). Brains from piglets receiving 1 rad/day did not differ from controls. No compensatory cell division was apparent postnatally in piglets that received 7 rad/day or in rats at 7 and 3 rad/day that were evaluated at birth and near adulthood. Growth in weight, DNA, RNA, and protein was similar to that of controls. Although the three sections of the piglet brain were proportionately reduced, the cortex in the rat group receiving 3 rad/day was depressed to 94% of control weight and DNA ($P < 0.05$), whereas the rest-of-brain was 101% of control values. This rate appeared to be the threshold for cell killing of cortical neurons, but in the rest-of-brain, neurons and neuroglial cells required a greater dose for cell death and permanent damage. At 7 rad/day, cortical and rest-of-brain weight and DNA were equally depressed to 90-94% of control values.

In the rat, only cortical and cerebella neurons have been accumulated at birth with 87% of the brain weight and approximately 75% of its cells to be added postnatally. The cortex and rest-of-brain contain 43 and 17%, respectively, of adult cell numbers at birth. In this respect the rat brain more closely parallels the developmental pattern of the human brain prenatally and postnatally. The human brain has 16% of its adult weight at birth with 25% of its cortical cells and 15% of the cerebella and rest-of-brain cells accumulated. The pig brain, on the other hand, weighs $\frac{1}{3}$ of its adult weight at birth but the cortex and cerebellum contain 60% each and the rest-of-brain 42% of adult levels of DNA. Although the time at risk is longer in the pig than the rat, the prenatal period also crosses a more complete developmental period with a more mature brain at birth in the pig than the rat. Consequently, more cells are at risk. The damage appears to be additive with cell death resulting from 3 rad/day but not at 1 rad/day. In the rat, the threshold dose for cell death is lower for cortical neurons than cells in the rest of the brain with reductions in weight and cell numbers apparent at 3 rad/day.

## OOGENESIS AND FOLLICULAR DEVELOPMENT IN THE PRENATALLY IRRADIATED BOVINE

B. H. Erickson

The physical and temporal attributes of the succession of developmental stages that characterize oogenesis in the prenatal human female are mimicked almost precisely by the bovine. It should follow, therefore, that what we learn about the radioresponse of the developing bovine germ cell will be of great value in our attempts to predict how the germ cell of man will respond at similar stages of development.

To determine the radioresponse of the various developmental stages, cows bearing fetuses varying in age from 40 ± 5 (point of gonadal sex differentiation) to 270 ± 10 (approximately 13 days prior to parturition) days of gestation were irradiated with 300 R of $\gamma$ radiation at 30 R/min. Exposures greater than 300 R will result in severe decrements in fetal growth and viability as well as gross abnormalities at some developmental stages (Erickson and Reynolds, 1969). Dose to the fetal gonad was approximately 100 rad. Twenty cows were irradiated at

each 10-day interval between 40 and 90. A like number were irradiated at each 20-day interval between 90 and 150 and at each 30-day interval between 150 and 270 days of gestation. When the prenatally irradiated heifers were approximately 10 mo old their ovaries were recovered at slaughter, serially sectioned, and prepared for microscopic analysis. A complete quantitative analysis of oocytes in primordial, growing, and vesicular follicles was effected.

Follicular development, as reflected by counts of growing and vesicular follicles, was apparently unaffected at all ages tested ($P > 0.05$). Oogenesis, as reflected by counts of oocytes in primordial follicles (these representing approximately 95% of the total germ cell population), was significantly impeded (60% of control) only between 70 and 90 days of gestation ($P < 0.05$). This period in oogenesis is marked by a high rate of oogonial mitosis and only marginal meiotic activity; hence it appears that the oogonium is the most vulnerable cell type. The effect of prenatal irradiation and a 40% decrement in the germ cell population on the reproductive capacity of the bovine is now under study.

## LATE EFFECTS OF $^{60}$Co $\gamma$ RADIATION ON THE BOVINE OOCYTE AS REFLECTED BY OOCYTE SURVIVAL, FOLLICULAR DEVELOPMENT, AND REPRODUCTIVE PERFORMANCE

B. H. Erickson, R. A. Reynolds, and R. L. Murphree*

The immediate effects of $\gamma$ radiation on the germ cell of the female mammal have been described in general, and results of these studies have shown that oocytes in the primordial follicles of mice and rats are much more susceptible to the cell-killing effects of irradiation than are their counterparts in the long-lived mammal. Oakberg (1962) has shown, for example, that 50% of the primordial follicles of the 10-day-old mouse are destroyed by as little as 8 R, and Mole and Papworth (1966) found that the $D_{37}$ for the primordial follicle of the postpuberal rat is 91 R. In contrast, Baker (1966) has shown that the oocyte of the monkey is little affected by a dose as high as 1000 rad, and estimates of Lindop (1969) and Baker (1971) would set the $LD_{50}$ for the human oocyte at least as high as 600 rad. Erickson's studies revealed that the $LD_{50}$'s for the oocytes of swine (1957) and cattle (1967) are approximately 500 and 900 rad, respectively.

It thus appears that a sublethal dose of $\gamma$ radiation administered to the postpuberal long-lived mammal would have little effect on reproductive capacity, but to date this assumption has not been adequately tested. In addition, the long-term effects of ionizing radiation on the germ cell of a long-lived mammal have not been quantified, nor has the effect of irradiation on the reproductive capacity of a monotocous species been determined. This study was performed to fill these voids, and the species to be studied was dictated by the fact that of those readily available only the cow, like man, is long-lived, nonseasonal and spontaneous with ovulation, and monotocous.

Two groups of 140 grade Herefords, 15 to 18 months of age, were irradiated in a multipoint $^{60}$Co field at levels varying from 200 to 600 R. Dose rate was 0.69 to 0.78 R/min. The 600-R exposure was delivered in two 300-R fractions with 55 days intervening between exposures. No deaths occurred within the first 2 months after the 200-R exposure, but two of the 40 heifers exposed to 300 R died during this interval as did 32 of the 80 irradiated with 400 R and 29 of the 80 irradiated twice with 300 R. The ovarian, or midline, dose averaged 37 ± 3% of the air dose. The study was terminated when over 50% of the herd was infertile or when the survivors were 14 to 15 yr of age. Cows gave birth to their first calf when approximately 2 yr of age and the more productive cows produced a calf during each of the succeeding 13 yr.

Ovarian effects were assessed in the survivors through (1) counts of germ cells and follicles in serially-sectioned ovaries, (2) incidence of abnormalities, and (3) reproductive performance.

Neither germ cell nor follicular counts were significantly affected by irradiation ($P > 0.05$). Incidence of ovarian abnormalities was not altered nor was reproductive performance or quality of offspring. It was therefore concluded that a sublethal postpuberally applied dose of $\gamma$ radiation would not affect the reproductive capacity of the bovine female.

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## OVARIAN CHARACTERISTICS AND REPRODUCTIVE PERFORMANCE OF THE AGED COW

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Although published reports provide some insight into what may be the upper limit of the lifespan and repro-

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ductive capacity of the bovine female, to our knowledge data that would provide a reasonable estimate of mean age at infertility and mean reproductive capacity have not been published. Reports contrasting the ovarian characteristics of the fertile and infertile aged bovine are also not extant.

Data essential to these needs have been made available through a study designed to determine the long-term effects of $\gamma$ radiation on reproductive performance and germ-cell survival. Results of that study did not reveal an irradiation effect on these criteria or on any other criterion tested ($P > 0.05$). It therefore seemed reasonable to conclude that results of analyses reported herein are a close approximation of what could be expected from similar analyses of a nonirradiated population.

The reproductive performance of 152 grade Hereford cows that were 14 to 15 yr of age at either death or slaughter resulted in an observed mean of 9.3 ± 0.3 calves and a calculated mean lifetime reproductive capacity of 10.4 calves. The median was 10 calves and the modal number was 12. As dictated by the criterion "failure to bear a calf during two successive years," 15 yr was the age at which over 50% of the herd became infertile.

Ovaries from 69 fertile and 78 infertile cows were serially sectioned and microscopically examined; the groups did not differ in total germ-cell endowment ($P > 0.05$), and fertile significantly exceeded infertile only in number of growing follicles ($P < 0.01$). No intergroup difference occurred in either number or quality of vesicular follicles. The mean germ-cell endowment of 14- to 15-yr-old cows was 24,000 ± 3,000 (N = 147). Cystic corpus luteum was apparently the principal cause of infertility.

## LATE EFFECTS OF WHOLE-BODY GAMMA IRRADIATION ON HEMOGRAMS OF FEMALE HEREFORD CATTLE

T. R. Noonan, R. A. Reynolds, and R. L. Murphree*

As part of an experiment to investigate the late effects of whole-body irradiation on the reproductive activity of female cattle, hemograms of irradiated or control animals were determined at least semiannually for 11 or 12 yr. Three groups of Hereford heifers were exposed, at 18 mo, to 200, 300, or 400 R; a fourth group received two 300-R exposures 8 wk apart; and the fifth group were sham-irradiated to serve as controls. One month after the last irradiation, groups numbered between 40 and 51. Animals were exposed to $^{60}$Co gamma rays in a multisource field at rates of 0.78 or 0.69 R/min. All blood samples, obtained by jugular venipuncture, were drawn at the same time of day (7–8 a.m.). Standard hematological procedures were used, and all determinations were made by or under the direct supervision of the same technician.

For all groups, we determined hemograms twice before real or sham exposure and used the means of these two determinations as the zero time values for all measurements. The groups of animals were irradiated only once and the control group were bled 3, 8, 26, and 52 wk after the first exposure. The group which received two exposures were studied at 3, 7, 11, 16, 34, and 52 wk after the first irradiation. Subsequently, all groups were bled semiannually (April and October) within a 2-wk period.

Erythrocyte counts and other measurements associated with red cell mass were not affected by radiation at the times when blood was collected. At three weeks after irradiation, the granulocyte, agranulocyte, and platelet counts were decreased in a dose-dependent fashion. A second exposure to 300 R reduced further the level of agranulocytes to that produced by a single 400-R exposure. Granulocyte and platelet counts also were reduced further by the second 300-R exposure but remained above the counts measured at 3 wk after a single exposure to 400 R.

From measurements made during the first year, exposure-response curves for granulocytes, agranulocytes, and platelets have been constructed and are shown in Fig. 16. To facilitate comparison among the three cell types, the results are expressed as percent of simultaneously determined values of the control group. At 3 wk after a single exposure, granulocyte and platelet levels were slightly more depressed than agranulocytes. By 8 wk postirradiation, recovery of platelet counts was nearly complete, granulocyte levels showed substantial recovery, and agranulocyte recovery was least rapid. One year after exposure, granulocyte and platelet counts of irradiated animals did not differ from those of controls. Agranulocyte levels in irradiated animals, however, still showed a highly significant ($P < 0.01$) depression. Mean agranulocyte counts from the first through the fifth postirradiation years are shown in Fig. 17. Through the first 3 yr, the irradiation effect is highly significant ($P < 0.01$). The effect remains significant ($P < 0.05$) through 4.5 yr. The disappearance of a radiation effect on agranulocyte level is due to a decrease with age in counts of the control group rather than an increase with time in the irradiated groups.

At 5 yr after exposure, hemograms of irradiated animals did not differ from those of controls. No case of bovine lymphosarcoma or other late manifestations of a radiation effect on the hematopoietic system was seen.

## EFFECTS OF AGE, SEASON, AND REPRODUCTIVE ACTIVITY ON HEMOGRAMS OF FEMALE HEREFORD CATTLE

T. R. Noonan, F. H. Cross, R. A. Reynolds, and R. L. Murphree*

In addition to providing data on late hematological effects of irradiation, our long-term study of female Herefords revealed changes in hemograms associated with

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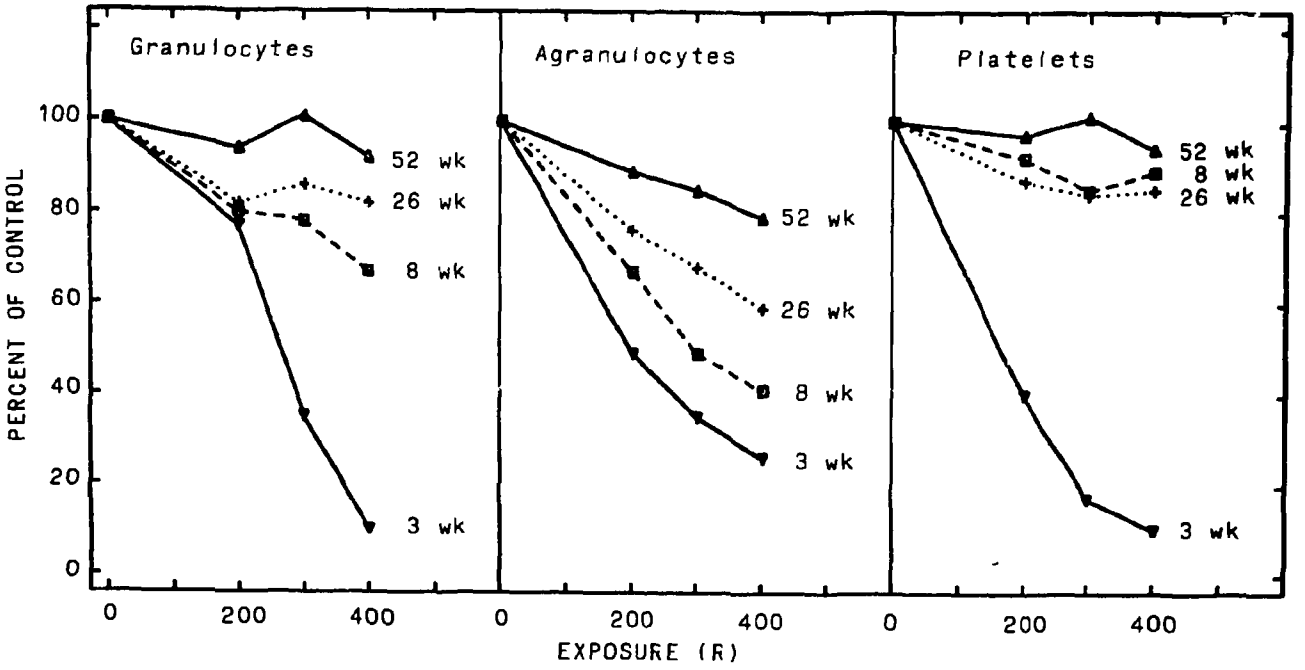



Fig. 16 Exposure-response curves for granulocytes, agranulocytes, and platelets at four different times after irradiation.

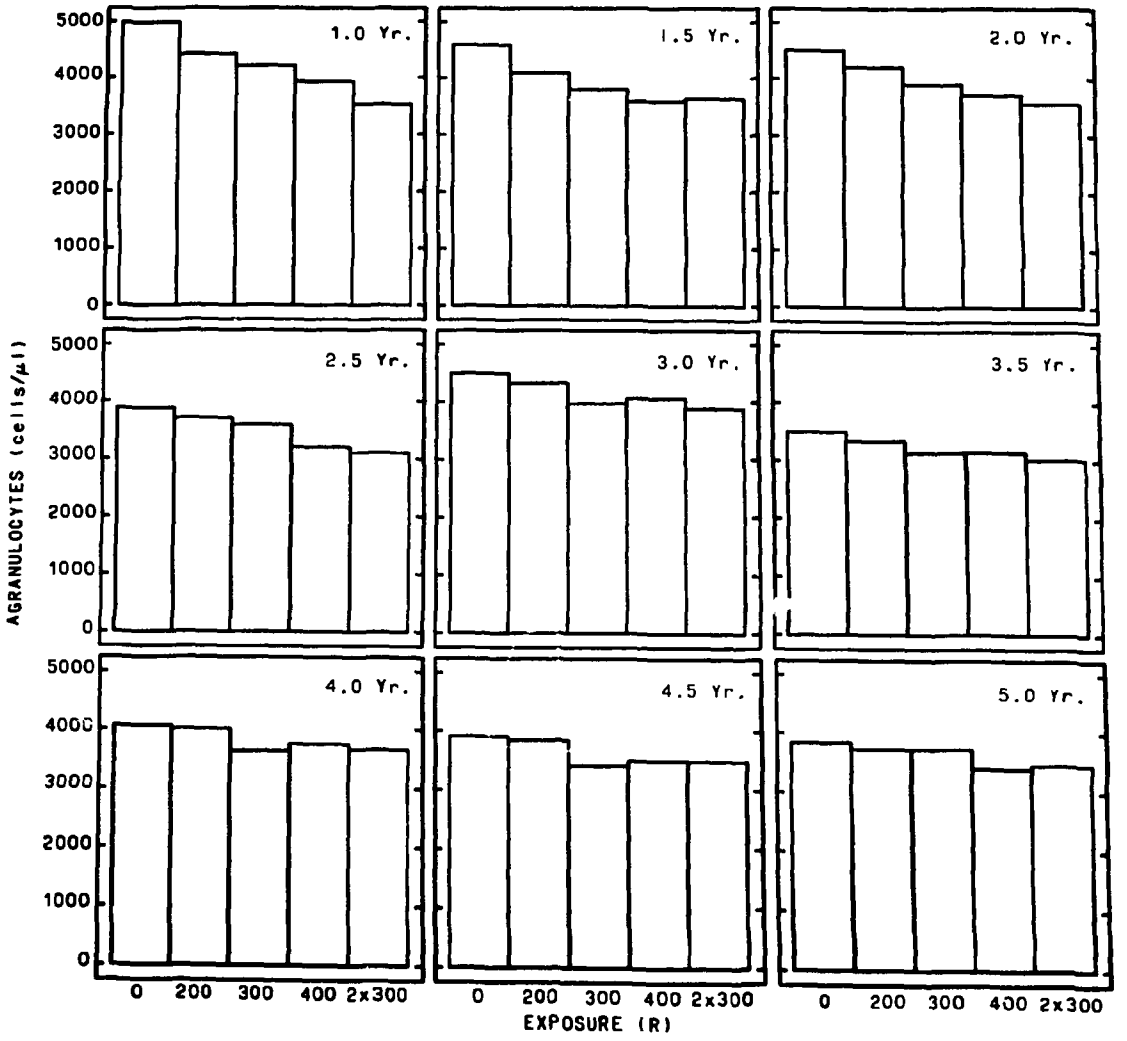



Fig. 17 Changes in mean agranulocyte counts from the first through the fifth year after irradiation.

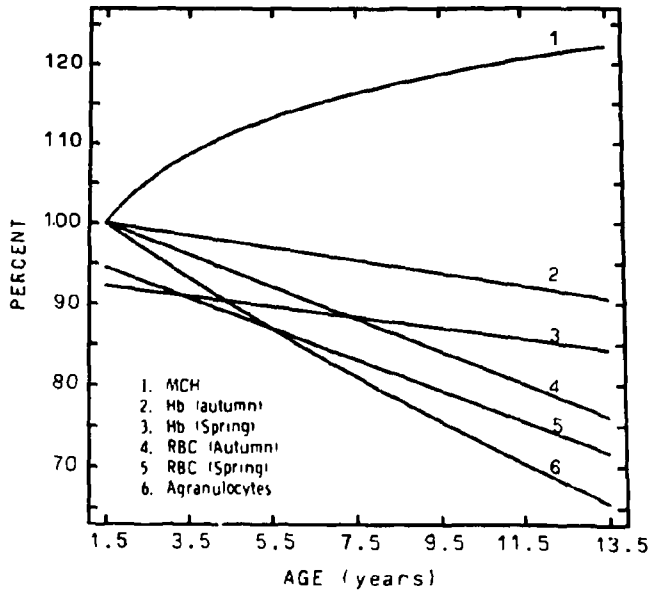
age, season, and reproductive activity. Data from control cattle which were bled semiannually (in April and October) for at least 11 yr have been analyzed. The original group numbered 40 animals and decreased to 28 at the end of the experiment. From these data, best-fit equations relating measurement to age of animal have been computed for each component of the hemogram. For measured variables with consistent seasonal differences, separate equations have been computed from autumn or spring determinations. To facilitate comparisons between different components of the hemograms, the values were computed from the regression curves and expressed as a percent of the value at age 1.5 yr (an autumn measurement). These data are shown in Figs. 18 and 19.

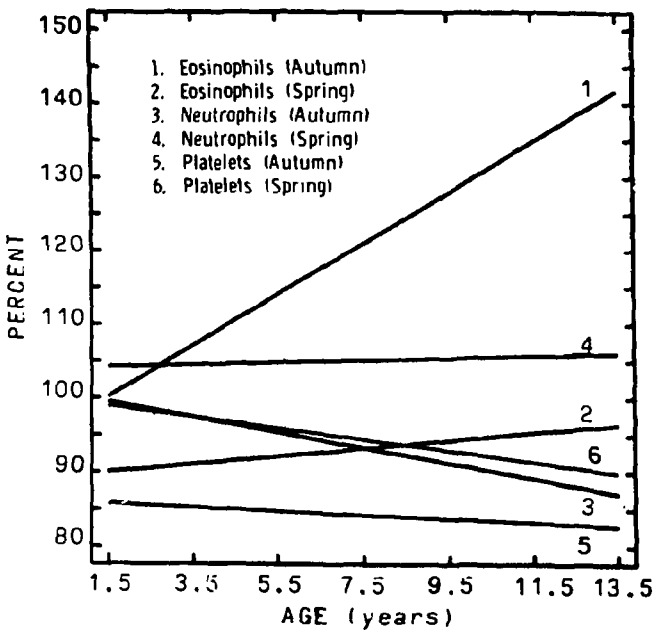
Erythrocyte counts and packed cell volumes declined with age in practically an identical manner; only changes in red cell count are shown in Fig. 18 (curves 4 and 5). Hemoglobin concentrations also decreased with age but less rapidly (curves 2 and 3, Fig. 18). All three measurements were higher in autumn, and the relationship with age seems to be linear. Since the hemoglobin concentration decreases less rapidly with age than erythrocyte count or packed cell volume the mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) values increase nonlinearly with age. Curve 1 (Fig. 18) represents MCH and the curve for MCHC could practically be superimposed. Mean corpuscular volume (not graphed) does not change with age, but values are consistently higher (about 2.5%) in autumn. As shown in Fig. 18, agranulocyte counts decline nonlinearly with age. Although autumn values were generally higher than those in spring the effect was inconsistent and not statistically significant.

Changes of neutrophils, eosinophils, and platelets with age are shown in Fig. 19. All of these measurements show relatively poor correlation with age, but the increase of eosinophils with age is significant ($P < 0.05$). Eosinophil counts were markedly higher in autumn in some years, but the apparent differences in slopes of autumn versus spring curves cannot be established as statistically significant. Neither the tendency of neutrophils and platelets to decrease with age nor the seasonal differences (spring-autumn) are significant at the 5% level.

Some seasonal changes were noted early in the experiment and were thought to be due to the effects of parturition and lactation. At the end of the study, the data were analyzed retrospectively. For each calendar year, both control and irradiated animals were divided into two groups. Cows which did not give birth during the year were classed as reproductively inactive and were compared to the rest of the herd. Seasonal changes in erythrocyte counts, hemoglobin concentrations, and packed cell volumes were seen in both groups. The changes with season were slightly smaller, but the general levels of these measurements were higher in the reproductively inactive group. The results for packed cell volumes are shown in Fig. 20.

Mean agranulocyte counts were, with only two exceptions in 11 yr, higher in the reproductively inactive group. The differences in the measurements made in the spring




Figs. 18 and 19 Curves derived from regression equations relating various components of the hemogram to age. For each component, the values have been expressed as percent of that at age 1.5 yr (an autumn determination).

were significant ($P < 0.05$) in 8 of 11 years. For the autumn determination, however, differences were significant in only 2 yr. In contrast, platelet counts in reproductively inactive animals were lower than those in the group producing calves. This effect was seen in 19 of 22 determinations. The differences, however, were significant at the 5% level on only seven determinations (three autumn, four spring) made during the 11 yr of the study.

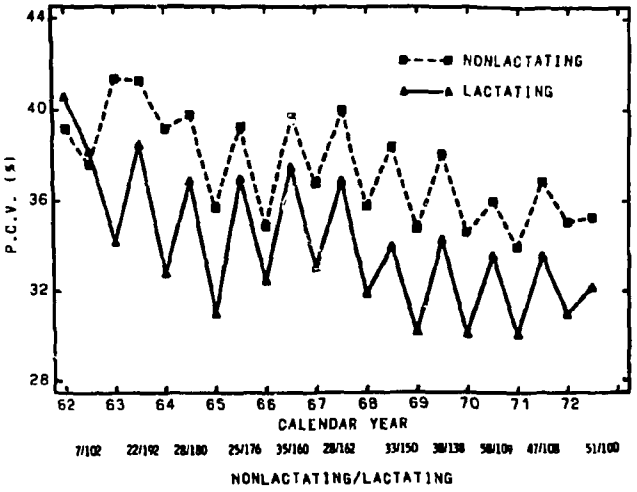



Fig. 20 Mean packed cell volumes of reproductively inactive (nonlactating) and reproductively active (lactating) cows in each calendar year. First point in each year represents a spring determination.

## EFFECTS OF GAMMA RADIATION ON LIFESPAN AND DISEASE IN CATTLE

T. R. Noonan, R. A. Reynolds, and R. L. Murphree*

To study the effect of whole body irradiation on lifetime fertility of cattle, Hereford heifers were exposed to $^{60}$Co gamma irradiation in 1960 or 1961. The experiment was ended in 1973, due to lack of funds, with over half of the experimental animals alive. The limited information obtained on survival and causes of death is presented here.

In April 1960, 140 female Herefords 18 ± 0.5 months of age were assigned to the study; in April 1961, 141 similar animals were added. In each year, animals were randomly assigned to one of five groups. Control cattle were sham-irradiated in September of each year. Three other groups were exposed to gamma radiation from multiple $^{60}$Co sources in September. The fifth group received one exposure in September and a second equal exposure 8 wk later. Table 18 shows initial group size, numbers, and percent surviving at 30 days after irradiation, and numbers and percent alive at the end of the experiment. All animals were managed as a single herd on pasture and were artificially inseminated and exposed to fertile males for a 6-wk period each spring. Five cows were removed from study in 1965 because they had failed to give birth to a calf. Shortly after, it was decided that sterility would not be a cause for removal. Most deaths were spontaneous, but in a few instances animals were killed when it was decided that further maintenance would be inhumane. Post mortem examinations were done in all but a few animals. The cause of death was determined in all but two cows.

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Causes of removal of animals from the study were varied. Uterine prolapse occurred in 18 cows, and five other animals died because of dystocia. Ocular squamous cell carcinoma was the cause of removal of 13 cows, and two additional animals died from other types of neoplasia. Metabolic disorders (grass tetany, rumen atony, and acetonemia) caused the deaths of 13 cows. The causes of death in the other animals removed from the study included infection, crippling arthritis, and ingestion of metallic objects.

As shown in Table 18, survival in three of four groups of irradiated animals was equal to that of the controls. The reduced survival of the 300-R groups is not believed due to a radiation-induced condition since uterine prolapse and rumen atony are unlikely to be the result of irradiation and since increase in these diseases was not seen at higher or lower radiation doses.

**Table 18 Survival Rates of Control and Irradiated Cows**

| Group | Number assigned | Survivors at 30 days | | Survivors at end of experiment | |
|---|---|---|---|---|---|
| | | No. | % | No. | %* |
| Control | 40 | 40 | 100 | 28 | 70 |
| 200 R | 40 | 40 | 100 | 27 | 68 |
| 300 R | 42 | 40 | 95 | 22 | 55 |
| 400 R | 79 | 51 | 65 | 37 | 73 |
| 2 X 300 R† | 80 | 48 | 60 | 34 | 71 |

*Computed on basis of survivors at 30 days.
†Two exposures 8 wks apart.

## PHYSIOLOGIC RESPONSES TO EXERCISE OF IRRADIATED AND NONIRRADIATED PONIES: A FIVE-YEAR STUDY

D. G. Brown*

Physiologic responses of irradiated and nonirradiated Shetland Ponies to controlled exercise were measured over a period of 5 yr. The 5-yr test began when the ponies were 3 yr old and 5 months after they were exposed to 650 R of $^{60}$Co gamma radiation.

Significant differences in heart rates, respiratory rates, and rectal temperatures were demonstrated between irradiated and nonirradiated ponies when subjected to exercise and high ambient temperatures. In the irradiated group, heart rates were usually slower, especially during recovery immediately after exercise, and respiratory rates and rectal temperatures were higher than these rates were in the nonirradiated group when exercising in ambient temperature at 29.5°C. Exhaustive exercise did not amplify any of the differences which were apparent with moderate exercise. From a general viewpoint, the irradiated ponies performed work as efficiently as did the nonirradiated ponies.

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Early changes in blood-cell concentrations after irradiation were similar to those which have been observed in other large animal species. Time required for the various types of blood cells to return to base line values ranged between 3 months and 3 yr.

## PLATELET AFFINITY FOR BURRO AORTA COLLAGEN

M. D. Schneider

Purified vascular components with high affinity for platelets have not been available, creating a major obstacle to a more basic understanding of the biological roles of various constituents present in the arterial wall. This is of considerable importance in human medical and surgical applications because of the known involvement of platelet deposits on subendothelial connective tissues in the early pathogenesis of thromboatheromatous lesions.

Our study focused on the extraction, partial purification, and characterization of a potent collagen-active stimulator from the aortas of aged burros (*Equus asinus*). All measurements were obtained by platelet aggregometry.

The stimulator invariably had a higher affinity for platelets in citrated platelet-rich-plasma (PRP) of humans than for homologous burro platelets. In interspecies comparisons of induced platelet aggregation in PRP of farm animals, it was noted that sheep platelets exhibited a slightly lesser affinity for the collagen-active stimulator than burro platelets. Platelets from cattle had the least affinity for the aorta collagen.

The stimulator for platelets in the aorta extract was retained by incubation with α-chymotrypsin. Platelet-aggregating activity was rapidly and totally abolished subsequent to incubation with purified (bacterial) collagenase. Evidence presented (based on light microscope study of polysaccharide histochemical staining reactions) indicated that the amorphous matrix (which retained periodic acid-Schiff coloration) and the myofilamentous elements within the smooth muscle cell (which retained coloration by Mallory's phosphotungstic acid-hematoxylin staining) very likely were the principal sources of the potent inducer of platelet aggregation.

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## FUNCTIONAL ASPECTS OF PLATELETS IN IRRADIATED BURROS

M. D. Schneider

The effects of total-body exposure to gamma irradiation on the hematopoietic system in living mammalian species, which include a depletion of thrombocytes and a depression of thrombopoiesis, are well known. There are gaps, however, in the present knowledge of the precise causal connection between induced whole-body γ radiation and the radiation sensitivity of the functional ability of the blood platelet.

The objective of this study was to determine the functional status of platelets in collections of platelet-rich-plasma from a group of seven surviving irradiated and three control unirradiated burros (*Equus asinus*). These animals were all > 22 yr old and were between 18 and 24 yr in experiment (subsequent to the induced irradiation). Burro platelet aggregation was induced by chemical stimuli (adenosine diphosphate and thrombin) and measurements of responsiveness obtained using a self-calibrating platelet aggregometer with an integrated recorder. A complex biomaterial was extracted from homogenates of aortas of two experimental burros dying during 1975. One of the burros had a lifespan > 24 yr after exposure to a whole-body dose of 545 roentgens $^{182}$Ta γ radiation. The second animal, an unirradiated control, was suffering from a severe maxillary sinus infection that had spread to the brain. It did not respond to therapy and was therefore killed and immediately exsanguinated.

Aggregometry data obtained indicated that the aggregation responsiveness to the chemical stimuli of adenosine diphosphate and thrombin was essentially the same for platelets from the surviving irradiated animals and unirradiated controls. A potent inducer of platelet aggregation was discovered, however, in the homogenates of the aortas of the dead burros. Moreover, the platelet-aggregating potency of the stimulator extracted from the aorta of the irradiated burro was nearly four-fold higher than that obtained from the aorta of the unirradiated control. A tentative suggestion was made that a delayed radiation effect could be the cause of the vascular component's high platelet-aggregating ability in the aorta of the irradiated animal. Presence of such a potent inducer of platelet aggregation in the arterial wall could (if exposed to flowing blood) produce a clinical syndrome marked by a depletion of megakaryocytes and circulating platelets.

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## LATE SOMATIC EFFECTS OF WHOLE-BODY GAMMA RADIATION ON BURROS

D. G. Brown* and T. R. Noonan

A study of the effects of whole-body gamma radiation on burros began at this laboratory in 1951. In an experiment designed to determine the $LD_{50}$, groups of 10 burros were exposed in a multipoint field to graded amounts of gamma radiation from $^{182}$Ta sources. The mortalities in groups exposed to 320, 425, and 545 R were 0, 10, and 0%, respectively. These 29 animals and 10

unirradiated burros which had served as controls in hematological studies were assigned to a long-term study. In 1954, we added to the experiment 20 burros which had been exposed to gamma radiation from a multipoint field with $^{60}Co$ sources. The pattern of exposure used, 15 exposures to 25 R at weekly intervals, did not cause any acute mortality. Finally, in 1957, 33 burros which had been exposed to mixed neutron-gamma radiation from the detonation of a nuclear weapon and 11 unexposed animals of similar age were added. The original numbers of animals in the groups and the survivors on 15 February 1977 are shown in Table 19.

*Table 19 Burro Survival by Experimental Groups*

| Designation | Date of entry into experiment | Original number | Alive 15 Feb. 1977 |
|---|---|---|---|
| Control | Sept. 1951 | 10 | 5 |
| 320 R gamma | Sept. 1951 | 10 | 6 |
| 425 R gamma | Sept. 1951 | 9 | 3 |
| 540 R gamma | Sept. 1951 | 10 | 1 |
| 15 x 25 R gamma | Dec. 1953* | 20 | 10 |
| Control | Sept. 1957 | 11 | 7 |
| 230–260 R bomb | Sept. 1957 | 15 | 6 |
| 290–325 R bomb | Sept. 1957 | 12 | 2 |
| 375–510 R bomb | Sept. 1957 | 6 | 3 |

*Irradiation weekly from 8 Dec. 1953 to 15 Mar. 1954.

All of the animals have been maintained on pasture with supplementary grain, have been observed daily, and have received semiannual physical and laboratory examinations. Burros which died were given complete post mortem examinations.

The cumulative mortality curves for gamma-irradiated animals are shown in Fig. 21. The first death of an unirradiated burro occurred 17.1 yr from the start of the experiment. Four more controls died during the next 8 yr, bringing the mortality in the unirradiated group to 50%. In the groups exposed to 320 or 425 R, three deaths occurred before the first death occurred in the controls. At 25.4 yr after the start of the experiment, however, mortalities were not different among the two lowest exposed and the control groups. In contrast, mortality of the group exposed to 545 R was 70% at the time of the first control death. The mean survival time of these burros was 15.1 yr and was significantly less ($P < 0.01$) than the control mean of 23.6 yr. Half of the group of burros which received a total

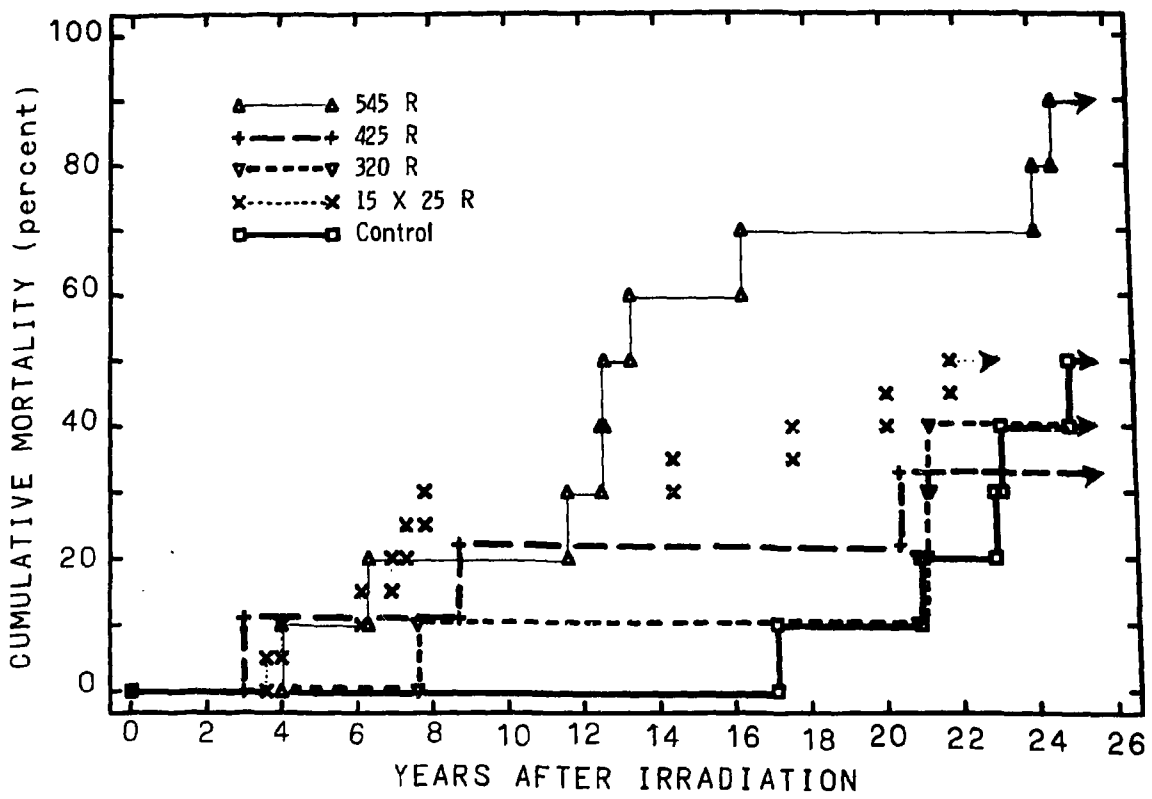


Fig. 21 Cumulative mortality of burros exposed to gamma irradiation. Single exposures were to $^{182}Ta$ in September 1952. Multiple exposures were to $^{60}Co$ ending in March 1954.

*Professor of Biochemistry, The University of Tennessee, Knoxville.

exposure of 375 R in 15 equal fractions were dead in 22.9 yr after the end of the irradiation. Control mortality 22.9 yr after the start of the study was 20%. The difference between mean survival times (measured at 22.9 yr) of exposed (16.9 yr) and control (22.1 yr) was significant at the 5% level.

Cumulative mortality curves for groups exposed to a nuclear weapon and the group of controls are presented in Fig. 22. One of 11 control burros died about 6 months after the start of the experiment. The second death of an unirradiated animal did not occur until 17.2 yr. At present, seven control burros are alive. In the first four years after exposure, 13 of 33 irradiated animals died. Between the 4th and 17th yr after exposure, when no deaths occurred in the control group, six of the remaining 20 burros died. The mean survival time of each of the three exposed groups was less than that of the controls. For all animals receiving radiation the mean survival time at present is just under 11 yr. This is less than the comparable value for the control group (17.3 yr), and the difference is significant at the 5% level.

Causes of death have been varied. No control animal has died as a result of a benign or malignant tumor. Malignant tumors have not been seen in any of the exposed burros, but one of them died with an intussusception probably due to the presence of a benign tumor of the mucosa. The very low incidence of tumors in this species is in contrast to a high incidence of tumors in irradiated swine and a substantial number of ocular squamous carcinomas seen in control and irradiated cattle. During the first four years after irradiation, almost half of the animals which died had low platelet counts. In some cases leukopenia was also present and in a few instances pancytopenia was seen. Infestation with a lungworm (*Dictyocaulus arnfieldi*) was very common and could have been at least a significant contributory cause of death.

## A COMPARATIVE STUDY OF PLATELET AGGREGATION IN DOMESTIC FARM ANIMALS AND MAN

M. D. Schneider

The use of artificial prostheses for successful medical and surgical applications is repeatedly hampered by a lack of knowledge concerning the hemostatic mechanisms and especially the functional characteristics of platelets in large animals. Information about the ultrastructural and functional peculiarities of blood platelets in large animals compared to human platelets is fragmentary.

In the present study, quantitative measurements of experimentally-induced platelet aggregability by turbidimetry in a self-calibrating aggregometer with an integrated recorder were employed. Biogenic stimuli which were utilized included adenosine diphosphate (ADP), thrombin, epinephrine, and stimuli present in dialyzed extracts of upper thoracic aortas of large animals (burro, pig, and bovine). The affinity of different stimuli for platelets in platelet-rich-plasma (PRP) collections was invariably highest for platelets from volunteer human subjects and greater for sheep than for bovine or burro platelets. The action on platelets of a collagen stimulator from the aortas of three species of farm animals invariably was the

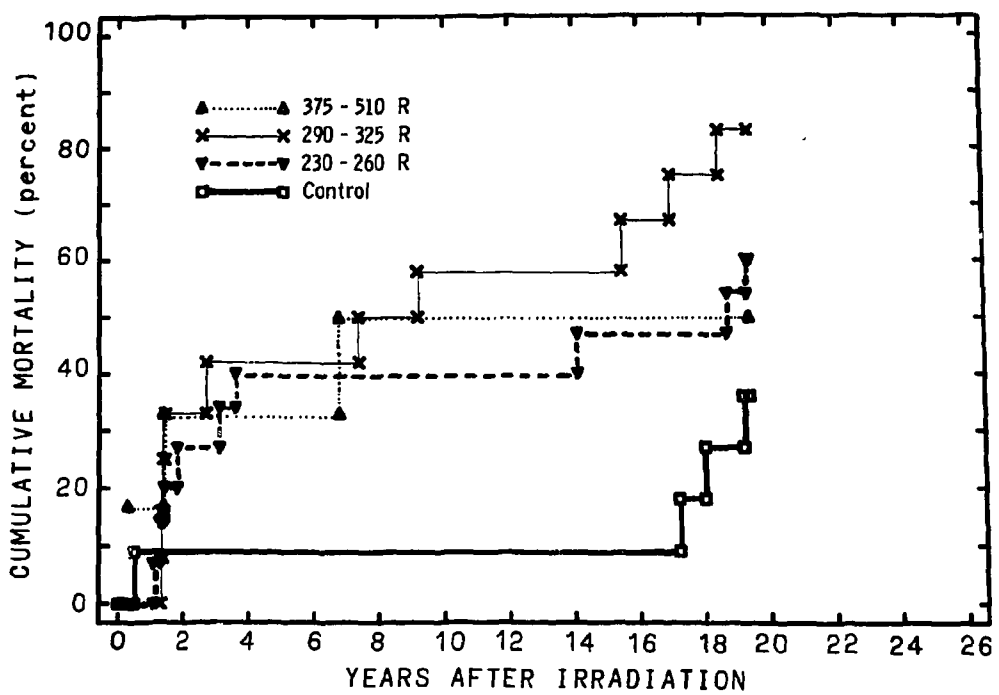


Fig. 22 Cumulative mortality of burros exposed to mixed neutron-gamma radiation from a nuclear weapon on 14 September 1954.

most potent for that obtained from the burro aorta > the pig > the bovine aorta. In particular, the bovine platelet: bovine aorta stimulator interaction was characterized by a long delay time from the addition of the stimulator to PRP to the onset of aggregation.

A functional strangeness of platelets of both large (equine, bovine, pig, and sheep) and laboratory animals (guinea pig and rat) is their inability to respond to an epinephrine stimulus. Adrenalin, however, is a powerful inducer of human platelet aggregation even in extremely low amounts, producing a characteristic secondary aggregation responsiveness as determined by platelet aggregometry. The present study indicates that the adsorption of platelets at the blood/artificial materials interface and the subsequent aggregation responsiveness in living animals will not necessarily be the same as in man. Great care needs to be taken in interpreting the results of test and evaluation experiments of blood compatibility of artificial prostheses in living large animals.

## ACTION OF INTRAVENOUSLY INJECTED AORTA COLLAGEN ON SUBSEQUENT PLATELET FUNCTION AND PULMONARY THROMBOSIS

M. D. Schneider

Results of in vitro experiments to investigate the mechanisms of collagen-induced platelet adhesion and aggregation indicate this reaction may be mediated by a complex formed between a specific plasma membrane-bound enzyme (platelet:glycosyltransferase) and a carbohydrate-protein residue of collagen (collagen:galactosylhydroxylysine).

Research was initiated for two primary purposes. The first involved determining the influence of an extractable arterial collagen-active material (from a burro aorta) on the hematological profile (particularly the platelet levels, subsequent platelet functional capacity, and pulmonary thrombotic events) in guinea pigs intravenously infused with a bolus of the aorta collagen material. A second goal was the development of a model animal system to interpret possible platelet function impairment or irreversible injury that might result from activation of the specific platelet plasma-membrane-bound glycosyltransferase by an environmental pollutant (nonnuclear cadmium, methylmercury, lead) or depression produced by a blocking of the specific glycosyltransferase action by a therapeutic chemical agent such as aspirin or clofibrate.

Preliminary experiments were done in a series of guinea pigs. A rapid depletion of platelets and white blood cells was produced within 5 minutes after infusion via ear vein of a bolus of the fibrillar aorta collagen. Platelets rebounded back in the peripheral circulation of the guinea pigs by 150 minutes after the collagen injection. White blood cells, however, were retained in in vivo location. Rebound platelets retained their ability to respond to the same collagen stimulus, as measured by turbidimetry in the platelet aggregation profiler. Lungs of guinea pigs were dissected at postmortem. Light microscope examination of cut sections of preserved lung showed a multitude of granular cellular elements retained in the tissues (in the alveolar walls), including a noticeable discharge of numerous eosinophils. Microthrombi, mainly composed of sphered mononuclear agranulocytes, were observed lodged in pulmonary veins. A significant discharge of large mast cells containing basophil granules was observed in the mucosal layer of the walls of bronchus. No cellular accumulations were seen in pulmonary arterioles.

## SHAPE TRANSFORMATION OF PLATELET AGGREGATION INFLUENCED BY AORTA COLLAGEN SPECIES

M. D. Schneider and J. M. Dumont*

A study of the nature and pattern of platelet interaction with constituents from a major arterial wall is of fundamental importance. There is little doubt that platelet adhesion to subendothelial connective tissues and microthrombus formation play a role in the pathogenesis of early thromboatheromatous lesions. An appreciation of platelet shape transformation and of fine details of the platelet surface architecture closely resembling the changes produced in a living location (as at the blood/interior vessel wall interface) is important to hematologists, cardiologists, nephrologists, cardiovascular and pulmonary surgeons, and biomaterials engineers.

Shape transformations in platelets lead to a cell disintegration process once referred to as "viscous metamorphosis". Embolizing platelet microthrombi are believed involved in myocardial depression which sometimes follows successful open heart surgery. Shape transformation from stimuli produced in a living location may lead to embolic phenomena and substantial complications. Such complications may nullify benefits that can be gained from improved surgical procedures, hemodialysis, or the construction of blood biocompatible prosthetic devices (oxygenators, artificial heart valves, vascular heterografts, cardiac assist devices, etc.). Platelets exposed to some well-defined chemical stimuli (adenosine diphosphate, thrombin, epinephrine, serotonin, etc.) rapidly transform from disc forms with smooth surfaces to spheres or spindly and tapering forms with projecting pseudopodia, finally changing into an amorphous mass. It is presently not known what platelet shape transformations result from stimulation by a collagen species obtained from aortas.

The objective of this study is to obtain insights using scanning electron microscope images of progressive shape transformations in platelets induced by stimulation with an aorta collagen species. Perhaps this type of reaction is involved in the induction of platelet aggregation, mural thrombus formation, or embolic phenomena which may be a key factor in the pathogenesis of early arteriosclerotic lesions or thrombotic events.

*Biology Division, Oak Ridge National Laboratory.


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# Interactions

## RESEARCH STAFF MEMBERSHIPS in ASSOCIATIONS AND SOCIETIES

American Association for the Advancement of Science
American Association for Cancer Research
American Association for Laboratory Animal Science
American Chemical Society
American Dairy Science Association
American Genetic Association
American Institute of Biological Sciences
American Institute of Nutrition
American Physical Society
American Physiological Society
American Society of Animal Science
American Society for Artificial Internal Organs
American Society of Laboratory Animal Practitioners
American Society for Microbiology
American Society of Nephrology
American Society of Plant Physiologists
American Veterinary Medical Association
Association of Southeastern Biologists
Council for Agricultural Science and Technology
Crop Science Society of America
Genetics Society of America
Gerontological Society
Health Physics Society
International Association for Plant Tissue Culture
Poultry Science Association
Radiation Research Society
Society of American Parasitologists
Society of Environmental Geochemistry and Health
Society for Experimental Biology and Medicine
Society for the Study of Fertility
Society for the Study of Reproduction
Tennessee Veterinary Medical Association
Tissue Culture Association
United States Animal Health Association
Wildlife Disease Association

(a) ORAU Undergraduate Research Participants Timothy Silvis, Emory University, Atlanta, GA; Samuel Steinmetz, Eastern Illinois University, Charleston, IL; Lynn Godsey, University of Tennessee, Knoxville, TN; and Charles Tysinger, Campbell College, Buies Creek, NC. (b) CARL visitor V. E. Sokolov, Director, Institute of Evolutionary Morphology and Ecology of Animals, Moscow (left), with CARL Director H. E. Walburg. (c) Foreign travel funds are hard to obtain. (d) Dr. Sam L. Hansard (left) is presented the American Society of Animal Science's prestigious Morrison Award by President Tom Marlow at the 68th Annual Meeting at College Station, TX, for signal service to animal science through teaching and research. (e) Because of CARL's outstanding safety record, William H. Travis (left) of ERDA-ORO presented this award to D. M. Robie, Head of Industrial Safety and Fire Protection.

## RESEARCH STAFF HONORS AND AWARDS

### Honorary Societies

*Alpha Xi Sigma*
J. F. Weiss

*Gamma Sigma Delta*
G. R. Eisele
S. L. Hansard
P. G. Martin
A. F. McFee

*Omicron Delta Kappa*
S. L. Hansard

*Phi Beta Kappa*
E. B. Darden, Jr.

*Phi Kappa Phi*
P. J. Bredderman
S. L. Hansard

*Sigma Xi*
M. J. Constantin
E. B. Darden, Jr.
G. R. Eisele
D. E. Foard
S. L. Hansard
J. K. Miller
F. J. Ryan
J. F. Weiss

### Honorary Activities

M. J. Constantin
Regional Editorial Board of *Environmental and Experimental Botany*

G. R. Eisele
American Registry of Certified Animal Physiologists

S. L. Hansard
Director, American Society of Animal Science
Recipient, American Society of Animal Science Morrison Award, 1976
Recipient, Southern Section, American Society of Animal Science Distinguished Service Award, 1977
Director, Tennessee Cattlemen's Association
American Registry of Certified Animal Scientists

J. K. Miller
Recipient, American Society of Animal Science Gustav Bohstedt Award in Minerals Research, 1974

H. E. Walburg
Director, American Institute of Biological Sciences

# TRAVEL, TALKS, AND PARTICIPATION IN NATIONAL AND INTERNATIONAL MEETINGS

## 1973

M. J. Constantin

Consultation with personnel of the Defense Civilian Preparedness Agency, Stanford Research Institute, and United States Department of Agriculture, Washington, DC, July 18-19.

Regional Project S-9 Meeting, University of Kentucky, Lexington, KY, July 22-24.

Reassignment of Duty Post as a Visiting Professor in Harold Smith's laboratory, Brookhaven National Laboratory, Upton, NY, August, 1973-August, 1974.

A. F. McFee

ORAU Traveling Lecture Series, Department of Biology, Murray State University, Murray, KY, "Radiation Effects at the Chromosome Level," November 1-2.

J. K. Miller

University of Tennessee Institute of Agriculture Conference, Crossville, TN, September 12-14.

Joint Regional Meetings on Magnesium Tetany Research, St. Louis, MO, October 14-15.

H. E. Walburg

University of Tennessee Institute of Agriculture Conference, Crossville, TN, September 12-14.

Semiannual Meeting of the Association for Gnotobiotics, Chicago, IL, October 2.

## 1974

M. J. Constantin

International Symposium on Haploids in Higher Plants—Advances and Potential, Guelph, Canada, June 8-16.

NATO Advanced Study Institute on Genetic Manipulations with Plant Material, Liege, Belgium, July 29-August 23.

S-9 New Crops Technical Committee Meeting, El Paso, TX, October 29-November 1.

ORAU Traveling Lecture Series, Knoxville College, Knoxville, TN, "Mutagenesis via Cell and Tissue Culture," December 4.

E. B. Darden, Jr.

Second AEC Pollution Control Conference, Albuquerque, NM; Respirator Training Course, Los Alamos, NM, April 14-28.

G. R. Eisele

Fifth International Congress of Radiation Research, Seattle, WA, to present paper entitled "Internal Emitter Data Retrieval Systems/A New Approach," July 14-18.

Plutonium Information Conference, Nevada Applied Ecology Group, Las Vegas, NV, October 7-12.

B. H. Erickson

ORAU Traveling Lecture Series, Stillman College, Tuscaloosa, AL, "Effects of Ionizing Radiation on the Mammalian Embryo," March 4-5.

Fifth International Congress of Radiation Research, Seattle, WA, to present paper entitled "Effect of Dose and Dose Rate on the Stem Spermatogonia of the Prepuberal Rat," July 14-18.

ORAU Traveling Lecture Series, The University of Tennessee Center for the Health Sciences, Memphis, TN, and Meharry Medical College, Nashville, TN, "Effects of Ionizing Radiation on the Mammalian Embryo" and "Mammalian Gametogenesis and its Modification by Radiation and Other Noxious Agents," December 10-11.

Symposium of the Society for Developmental Biology of Reproduction, Athens, GA, June 9-12.

S. L. Hansard

66th Annual Meeting of the American Society of Animal Science, College Park, MD, July 26-August 1.

25th Annual Convention of the Tennessee Feed Manufacturers' Association, Gatlinburg, TN, November 7-8.

American Society of Animal Science Committee on Minerals and Vitamins, Gainesville, FL, December 16-19.

P. G. Martin

Fifth International Congress of Radiation Research, Seattle, WA, to present paper entitled "Effects of Continuous $\gamma$ Irradiation on the Pig Brain," July 14-18.

ORAU Traveling Lecture Series, The University of Tennessee Center for the Health Sciences, Memphis, TN, "The Postnatal Development of the Brain for Prenatally Irradiated Rats," November 21.

17th Annual Meeting of the American Society of Hematology, Atlanta, GA, December 6-10.

A. F. McFee

12th Annual Somatic Cell Genetics Conference, Park City, Utah, January 6-10.

ORAU Traveling Lecture Series, University of Texas, Austin, TX, "Radiation Effects at the Chromosome Level," February 19-22.

ORAU Traveling Lecture Series, Tennessee Polytechnic Institute, Cookeville, TN, "Problems and Promises of Genetic Engineering," May 1-2.

Symposium of the Society for Developmental Biology of Reproduction, Athens, GA, June 9-12.

J. K. Miller

Southern Divisional Meeting of the American Dairy Science Association, Memphis, TN, to present papers entitled "Effects of Simulated Fallout Radiation on Iodine-131 Metabolism in Lactating Cows" and "Effect of Thiocyanate and Thyroid Status on Absorption and Gastric Secretion of Iodine in Cattle," February 3-7.

ORAU Traveling Lecture Series, Virginia Polytechnic Institute and State University, Blacksburg, VA, "Iodine Metabolism in Dairy Cattle," February 18-19.

66th Annual Meeting, American Society of Animal Science, College Park, MD, July 26-August 1.

T. R. Noonan

Fifth International Congress of Radiation Research, Seattle, WA, July 14-18.

L. B. Sasser

University of Missouri's 8th Annual Conference on Trace Substances in Environmental Health, Columbia, MO, June 10-14.

International Symposium on Trace Elements and Human Disease, Detroit, MI, July 11-12.

G. E. Spalding

Fifth International Congress of Radiation Research, Seattle, WA, July 14-18.

H. E. Walburg

Research Triangle Park, NC, for consultation with Dr. David Hoel, National Institute of Environmental Health Sciences, January 14-15.

Albuquerque and Los Alamos, NM, for conferences with Dr. Roger McClellan, Lovelace Foundation—Inhalation Toxicology Research Institute—and Dr. C. R Richmond, Los Alamos Scientific Laboratory, February 17-22.

International Congress of Radiation Research Program Committee Meeting, Argonne National Laboratory, Chicago, and American Institute of Biological Sciences Governing Board Meeting, Washington, DC, March 3-8.

Donner Radiation Laboratory, Berkeley, CA, March 17-24.

Radiobiology Laboratory, Davis, CA, and Nevada Test Site Operations Office visit, Las Vegas, NV, May 5-11.

Fifth International Congress of Radiation Research, Seattle, WA, July 14-18.

National Institute of Environmental Health Sciences Workshop, "Statistical Problems in Developing Procedures for Estimating Risks to Man from the Results of Animal Experiments," Wrightsville Beach, NC, September 29-October 2.

National Institutes of Health On-Site Visit, Savannah River Plant, Augusta, GA, November 4-6.

National Institute of Environmental Health Sciences, Research Triangle Park, NC, November 20-21.

## 1975

M. J. Constantin

National Academy of Sciences National Research Council Assembly of Mathematical and Physical Sciences, Washington, DC, Committee on Managed Terrestrial Ecosystems, January 6-11.

ERDA Division of Biomedical and Environmental Research, Washington, DC, for conferences with Dr. Hanson on the feasibility of using plant cell systems to study carcinogens, mutagens, and teratogens, April 10-11.

Third International Barley Genetics Symposium to present paper entitled "Mutations for Chlorophyll-Deficiency in Barley: Comparative Effects of Physical and Chemical Mutagens," Grunbach, Germany, July 4-12.

World Soybean Conference, Urbana, IL, to present paper "Creating Genetic Variability by Mutations," August 3-9.

Annual Meeting of the S-9 Committee on Plant Introductions for the South and Southeastern States, Griffin, GA, August 20-23.

67th Annual Meeting, American Society of Agronomy, Knoxville, TN, August 24-30.

The University of Tennessee Tobacco Experiment Station, Greeneville, TN, November 5.

G. R. Eisele

Workshop on the Biological Effects of the Toxicity of $^{239}Pu$ and $^{226}Ra$, Sun Valley, Idaho, October 5-10.

B. H. Erickson

Radiation Research Society Annual Meeting, Miami Beach, FL, to present paper "Effect of $\gamma$ Radiation on the Stem and Differentiating Spermatogonia of the Rat," May 10-15.

International Atomic Energy Agency Symposium, Chicago, IL, November 2-8.

ORAU Traveling Lecture Series, Texas Woman's University, Denton, TX; Paul Quinn College, Waco, TX; The University of Tennessee Center for the Health Sciences, Memphis, TN; "Effects of Ionizing Radiation on the Mammalian Embryo" and "Mammalian Gametogenesis and Its Modification by Radiation and Other Noxious Agents," December 1-3.

S. L. Hansard

American Society of Animal Science Directors' Meeting, Denver, CO, January 16-19.

Director and Chairman of the Animal Science Section, Southern Division of the American Society of Animal Science, New Orleans, LA, February 2-6.

American Society of Animal Science Feed Additive and FDA Relations Committee, Beltsville, MD, February 24-26.

American Society of Animal Science Annual Meeting to present paper entitled "Metabolism of Magnesium in Gravid Ovine," Fort Collins, CO, July 25-31.

Grass Tetany Workshop, Atlanta, GA, November 9-10.

American Society of Animal Science Task Force Vitamin and Hormone Meeting, Lexington, KY, December 18-19.

R. R. Henke

Plant Cell and Tissue Culture Program of the Gordon Research Conference, Andover, NH, June 8-13.

26th Annual American Institute of Biological Sciences, Corvallis, OR, August 17-22.

67th Annual Meeting of the American Society of Agronomy, Knoxville, TN, August 24-30.

The University of Tennessee Tobacco Experiment Station, Greeneville, TN, November 5.

B. J. Kelman

Federation of American Societies for Experimental Biology, Atlantic City, NJ, to present paper entitled "Calcium, Sodium, and Potassium Movements Across the Perfused Guinea Pig Placenta," April 11-20.

P. G. Martin

Radiation Research Society Annual Meeting, Miami Beach, FL, to present paper entitled "Absorption and Distribution of $^{59}$Fe as Affected by Pregnancy and Iron-Deficiency," May 10-15.

18th Annual Meeting of the American Society of Hematology, Dallas, TX, December 5-9.

A. F. McFee

ORAU Traveling Lecture Series, The University of Tennessee Center for the Health Sciences, Memphis, TN, "Leukocyte Cultures as a Genetic Tool," January 16.

17th Southeastern Developmental Biology Conference, Atlanta, GA, to present paper entitled "Hematological Effects of Low-Level Irradiation Throughout Prenatal Development in Swine," May 23-26.

Radiation Research Society Annual Meeting, Miami Beach, FL, to present paper entitled "Chromosome Aberrations in Leukocytes of Swine Chronically Exposed to Low-Level Gamma Radiation," May 10-15.

Invited lecturer, Oak Ridge Associated Universities Short Course on Environmental Pollutants, Oak Ridge, TN, August 25.

27th Annual Meeting of the American Society of Human Genetics and the Johns Hopkins Centennial Symposium on Human Genetics and Development, Baltimore, MD, October 7-10.

Invited lecturer, Genetics Seminar, Texas A&M University, College Station, TX, "Chromosome Studies in Irradiated Large Animals," October 29.

14th Annual Somatic Cell Genetics Conference, Houston, TX,

ORAU Traveling Lecture Series, Paul Quinn College, Waco, TX, and The University of Tennessee Center for the Health Sciences, Memphis, TN, "Radiation Effects at the Chromosome Level" and "Genetic Studies with Cultured Leukocytes," November 10-12.

J. K. Miller

Annual Meeting of the Southern Section, American Society of Animal Science, New Orleans, LA, to present paper entitled "Retention of Feed Residues in the Calf Gastrointestinal Tract," February 2-6.

70th Annual Meeting of the American Dairy Science Association, Kansas State University, Manhattan, KS, to present paper entitled "Effect of Protein Source and Supplemental Iodine on Thyroxine Secretion in Calves," June 22-25.

University of Tennessee Agricultural Experiment Station, Jackson, TN, July 15-16.

Grass Tetany Workshop, Atlanta, GA, November 9-10.

L. B. Sasser

Fifteenth Annual Hanford Life Sciences Symposium, Richland, WA, to present paper "The Influence of Selenium on the Distribution of Methyl Mercury and Mercury Chloride in the Pregnant Rat," September 29-October 1.

M. D. Schneider

First International Symposium on Equine Hematology, East Lansing, MI, May 22-June 3.

Tennessee Blood Club Program, Fall Creek Falls State Park, TN, to present paper entitled "Mechanism of Retention of Particulate Matter in Blood Microemboli During Bypass," October 3-4.

Conference on Environmental Impact of Water Chlorination, Holifield National Laboratory, Oak Ridge, TN, October 22-24.

50th Annual Meeting of the American Society of Parasitologists, New Orleans, LA, November 10-14.

G. E. Spalding

New York Management Center Seminar on Minicomputers, Washington, DC, March 9-14.

H. E. Walburg

Stony Brook, NY, for collaboration with A. C. Upton, January 5-7.

Research Triangle Park, NC, for discussions with Drs. Falk and Hoel of the National Institute for Environmental Health Sciences, April 9.

Battelle Pacific Northwest Laboratories, Seattle, WA, July 21-25.

J. F. Weiss

Conference on Environmental Impact of Water Chlorination, Holifield National Laboratory, Oak Ridge, TN, October 22-24.

## 1976

P. J. Bredderman

Annual Meeting of the American Society of Biological Chemists, San Francisco, CA, June 6-10.

R. J. Chertok

ORAU Traveling Lecture Series, Clinch Valley College, Wise, VA, "Active Transport of Sugars Across the Renal Proximal Tubule," March 5.

Federation of American Societies for Experimental Biology, Anaheim, CA, April 11-17.

M. J. Constantin

Bethesda and Beltsville, MD, DHEW Committee on Mutagenicity Testing, consultation with Dr. Paul Bottino, University of Maryland, and meetings with Drs. Jerrel Powell, Gideon Schaeffer, and J. G. Moseman, USDA Plant Industry Station.

Annual Meeting of the Tissue Culture Association, Philadelphia, PA, June 7-10.

Joint Meeting of the Southern Seed Certification officials, Southern Seed Control officials, and Foundation Seed personnel, Nashville, TN, July 6-8.

73rd Annual Meeting of the American Society for Horticultural Science, Baton Rouge, LA, August 12-13.

S-9 Committee Plant Introduction Meeting, Miami, FL, August 17-22.

Brookhaven National Laboratory, Upton, NY, Plant Research Program On-Site Review, September 7-9.

ORAU Traveling Lecture Series, Grambling College, Monroe, LA, "Mutagenesis in Cultured Cells and Tissue," September 29-October 1.

AUA-ANL Bicentennial Conference, "Accomplishments and Challenges for American Life Sciences," Argonne National Laboratory, Argonne, IL, October 10-14.

E. B. Darden, Jr.

USDA Agricultural Research Service, Baton Rouge, LA, and Boll Weevil Radiation Laboratory, Starkville, MS, March 21-23.

G. R. Eisele

Symposium on the Dynamics of Transuranics in Terrestrial and Aquatic Environments, Nevada Applied Ecology Group, Gatlinburg, TN, October 5-7.

Invited lecturer, "Gastrointestinal Uptake in Perinatal Animals," Section Seminar Series, Oak Ridge National Laboratory Medical Physics and Internal Dosimetry Section, December 14.

B. H. Erickson

ORAU Traveling Lecture Series, John A. Gupton College, Nashville, TN, "Effects of Ionizing Radiation on the Mammalian Embryo," March 4.

7th Annual Meeting of the Environmental Mutagen Society, Atlanta, GA, March 11-15.

ORAU Traveling Lecture Series, Transylvania College, Lexington, KY, "Mammalian Gametogenesis and Its Modification by Radiation and Other Noxious Agents," April 8.

Joint Meeting of the Health Physics Society and the Radiation Research Society, San Francisco, CA, to present paper entitled "Late Effects of $^{60}Co$ $\gamma$-radiation on the Bovine Oocyte as Reflected by Oocyte Survival," June 27-July 2.

Invited lecturer, "Effects of Continuous Gamma Radiation on the Primitive Mammalian Germ Cell," Staff Seminar, Medical and Health Sciences Division, Oak Ridge Associated Universities, August 31.

First Annual Course in Principles and Practices of Genetic Toxicology, University of Texas Medical Branch, Galveston, TX, September 19-24.

Invited lecturer, "Mammalian Gametogenesis and Its Modification by Ionizing Radiations," The University of Tennessee Department of Animal Science, November 1.

ORAU Traveling Lecture Series, The University of Tennessee Center for the Health Sciences, Memphis, TN, "Effects of Ionizing Radiation on the Mammalian Embryo," November 16.

Society of Toxicology and National Institute of Environmental Health workshop on Target Organ Toxicity: Gonads (Reproductive and Genetic Toxicology), Vanderbilt University, Nashville, TN, November 30-December 3.

D. E. Foard

Invited lecturer, "Low Molecular Weight Soybean Trypsin Inhibitors," The University of Tennessee Memorial Research Center, Knoxville, TN, September 22.

S. L. Hansard

American Society of Animal Science, Mobile, AL, February 1-4.

American Society of Animal Science Regulatory Agency Task Force, Beltsville, MD, February 9-11.

International Symposium on Feed Composition and Animal Nutrient Requirements, Logan, UT, July 10-17.

American Society of Animal Science Annual Meeting, College Station, TX, August 13-19.

American Society of Animal Science Task Force Meeting, Gainesville, FL, December 13-17.

R. R. Henke

Invited speaker, "The Plant Research Program at the Comparative Animal Research Laboratory," The University of Tennessee Department of Botany, Knoxville, TN, January 26.

Symposium on Molecular Biology and Plants, University of Minnesota, St. Paul, MN, March 24-27.

27th Meeting of the American Institute of Biological Sciences, Tulane University, New Orleans, LA, to present paper entitled "Organogenesis and Plantlet Formation from Organ- and Seedling-Derived Calli of Rice (*Oryza sativa* L.)," May 30-June 3.

NATO/FEBS/EMBO Institute—CNRS Colloquium on "Nucleic Acids and Protein Synthesis in Plants," Strasbourg, France, July 12-28.

Invited lecturer, "Selection of Biochemical Mutants in Higher Plants," Rochester Institute of Technology, Rochester, NY, December 14.

B. J. Kelman

Society of Toxicology, Atlanta, GA, March 14-17.

Federation of American Societies for Experimental Biology, Anaheim, CA, to present paper entitled "Movements of Methylmercury Across the Perfused Guinea Pig Placenta," April 11-17.

10th Annual Conference on Trace Substances in Environmental Health, Columbia, MO, June 7-10.

P. G. Martin

Society of Toxicology, Atlanta, GA, March 14-17.

10th Annual Conference on Trace Substances in Environmental Health, Columbia, MO, June 7-10.

A. F. McFee

7th Annual Meeting of the Environmental Mutagen Society, Atlanta, GA, March 11-15.

Southeastern Developmental Biology Conference, Clemson, SC, March 18-20.

Joint Meeting of the Health Physics Society and the Radiation Research Society, San Francisco, CA, June 27-July 2.

First Annual Course in Principles and Practices of Genetic Toxicology, University of Texas Medical Branch, Galveston, TX, September 19-24.

ORAU Traveling Lecture Series, Fort Hays, Kansas, State College, "Radiation Effects at the Chromosome Level," September 28-30.

ORAU Traveling Lecture Series, Rice University and the University of Texas, San Antonio, TX, "Promises and Problems of Genetic Engineering," November 1-3.

ORAU Traveling Lecture Series, The University of Tennessee Center for the Health Sciences, Memphis, TN, "Radiation Effects at the Chromosome Level," November 30-December 1.

J. K. Miller

Annual Meeting of the Southern Section, American Society of Animal Science, Mobile, AL, to present paper entitled "Titanium-44 Metabolism in Ruminants." Annual Meeting of the Southern Division, American Dairy Science Association, Mobile, AL, to present paper entitled "Effects of Ingested Soil on Ration Utilization by Dairy Cows," February 1-4.

F. R. Mraz

Joint Meeting of the Health Physics Society and the Radiation Research Society, San Francisco, CA, to present paper entitled "Absorption of $^{95}$Nb by Rats Varying in Age," June 27-July 2.

Invited lecturer, "Gastrointestinal Uptake of Niobium as a Function of Age," Section Seminar Series, Oak Ridge National Laboratory Medical Physics and Internal Dosimetry Section, Oak Ridge, TN, December 7.

F. J. Ryan

Invited speaker, Oak Ridge National Laboratory, Biology Division Journal Club, Oak Ridge, TN, "The Reaction of Pyridoxal 5′-Phosphate with Ribulose Bisphosphate Carboxylase," February 15.

L. B. Sasser

ORAU Traveling Lecture Series, Florida State University, Tallahassee, FL, "The Interaction of Environmental Contaminants with Nutrients," February 23-24.

Society of Toxicology, Atlanta, GA, March 14-17.

Environmental Protection Agency Symposium on Biochemical Effects of Environmental Pollutants, Cincinnati, OH, June 1-3.

10th Annual Conference on Trace Substances in Environmental Health, Columbia, MO, June 7-10.

M. D. Schneider

New York Academy of Sciences Conference on Behavior of Blood and Its Components at Interface, New York, NY, March 24-27.

Walter Reed Army Institute of Research Comparative Animal Pathology Course, Armed Forces Institute of Pathology, Washington, DC, May 10-12.

Boll Weevil Research Laboratory, Starkville, MS, July 26-30.

Invited lecturer, "Concept of the Sterile-Male Technique for the Control and Eradication of Insect-Pest Populations," Agricultural Biology Department, The University of Tennessee, Knoxville, TN, November 8.

G.E. Spalding

Symposium on the Dynamics of Transuranics in Terres-

trial and Aquatic Environments, Nevada Applied Ecology Group, Gatlinburg, TN, November 5-7.

H. E. Walburg

Retreat for Scientists Working on the Health Problems on Energy Technologies, Pinehurst, NC, January 6-9.

Plutonium Information Conference, Nevada Applied Ecology Group, Las Vegas, NV, February 11-14.

Consultation with Drs. David Hoel, H. Malling, and David Coffin, National Institute of Environmental Health Sciences, Research Triangle Park, NC, February 16-17.

Invited speaker, "Capabilities and Facilities of the Comparative Animal Research Laboratory," Inter-Service Committee on Technical Facilities, Southeastern USA, Museum of Atomic Energy, Oak Ridge, TN, February 23.

Conference on the Problems of Extrapolating the Results of Laboratory Animal Data for Man and Extrapolating the Results from High-Dose-Level Experiments to Low-Dose-Level Exposures, Pinehurst, NC, March 9-12.

USDA Agricultural Research Service, Baton Rouge, LA, and Boll Weevil Radiation Laboratory, Starkville, MS, March 21-23.

Invited speaker, "CARL Programmatic Goals," Oak Ridge National Laboratory Health Physics Division, Oak Ridge, TN, March 29.

Invited speaker, "CARL Program Goals," USDA Club, Knoxville, TN, April 14.

Invited speaker, "Overview of Research in Progress at the Comparative Animal Research Laboratory," The University of Tennessee Department of Animal Science, Knoxville, TN, May 3.

Environmental Protection Agency Symposium on Biochemical Effects of Environmental Pollutants, Cincinnati, OH, June 1-3.

Joint Meeting of the Health Physics Society and the Radiation Research Society, San Francisco, CA, June 27-July 2.

Symposium on the Dynamics of Transuranics in Terrestrial and Aquatic Environments, Nevada Applied Ecology Group, Gatlinburg, TN, October 5-7.

Invited speaker, "Tensions Between the Scientific Community and the Public Sector," East Tennessee Chapter, Society for Technical Communications, Knoxville, TN, November 11.

Invited lecturer, "Overview of CARL's Metabolism Program," Section Seminar Series, Oak Ridge National Laboratory Medical Physics and Internal Dosimetry Section, Oak Ridge, TN, November 30.

J. F. Weiss

Joint Meeting of the Health Physics Society and the Radiation Research Society, San Francisco, CA, to present paper entitled "Liquid Scintillation Alpha Counting and Spectrometry and Its Application to Bone and Tissue Samples," June 27-July 2.

Symposium on the Dynamics of Transuranics in Terrestrial and Aquatic Environments, Nevada Applied Ecology Group, Gatlinburg, TN, October 5-7.

## 1977

R. J. Chertok

Invited lecturer, "Glucose Binding to Isolated Renal Brush Borders," The University of Tennessee Department of Zoology, Knoxville, TN, January 21.

S. L. Hansard

Annual Meeting of the Southern Section of the American Society of Animal Science, Atlanta, GA, February 6-9.

American Society of Animal Science Regulatory Agency Committee Task Force Meeting, Beltsville, MD, February 17-19.

ORAU Traveling Lecture Series, "Fetal-Maternal Mineral Nutrition in Animals," Longwood College, Farmville, VA, February 24-25.

R. R. Henke

Southern Sectional Meeting of the American Society of Plant Physiologists, Atlanta, GA, February 6-8.

A. F. McFee

Environmental Mutagenesis Society, Colorado Springs, CO, February 13-17.

J. K. Miller

Annual Meeting of the Southern Section of the American Society of Animal Science, Atlanta, GA, to present paper entitled "Effects of Feeding Magnesium Oxide or Corn Meal to Cows on Spring Pasture," February 6-9.

Invited lecturer, "Mineral Nutrition Related to Animal Health," University of Georgia, Athens, GA, February 21.

M. D. Schneider

Colloquium and Workshop of the Knoxville-Oak Ridge Associated Researchers in Cancer on "Biomarkers as a Measure of Malignancy" and "Mechanisms Regulating Excretion of Modified Nucleosides," Knoxville Academy of Medicine, Knoxville, TN, and Oak Ridge National Laboratory, Oak Ridge, TN, January 5-6.

H. E. Walburg

First International Cadmium Conference, San Francisco, CA, and Nevada Operations Office, Las Vegas, NV, January 30-February 4.

National Research Council, Washington, DC, February 20-23.

## COLLABORATIVE PROJECTS

M. J. Constantin

Mutation Induction in Soybeans, N. S. Hall, L. N. Skold, and F. Allen, The University of Tennessee Agricultural Biology and Plant and Soil Science Departments, 1971-continuing.

Preliminary Effort to Induce Corn Mutants Resistant to H. maydis, L. M. Josephson, The University of Tennessee Plant and Soil Science Department, 1971-1973.

Begonia Improvement Through Mutagenesis, Mikkelsens, Inc., Ashtabula, OH, 1970-1974.

Effects of Seed Irradiation on Production of Crop Plants, G. Burton, USDA-Agricultural Research Service, Tifton, GA, 1970-1974.

Effects of Seed Irradiation on 6X Triticale, V. T. Sapra, Alabama A&M University, Normal, AL, 1974-continuing.

Mutation Induction in Peaches, Apples, and Pecans, J. Thompson and J. Payne, USDA-Agricultural Research Service, Byron, GA, 1974-continuing.

Mutation Induction in Ornamental Plants, J. E. Love, Louisiana State University, Baton Rouge, LA, 1970-continuing.

E. B. Darden, Jr.

Effects of Fission Neutrons and X-rays on the Mouse Embryo, W. Friedberg, Federal Aviation Administration, Civil Aeromedical Institute, Oklahoma City, OK, 1970-continuing.

Radiation Induced Opacification of the Lens in Mice: Effects of Radiation Quality and Rate of Delivery, J. J. Beauchamp, Oak Ridge National Laboratory, Oak Ridge, TN, 1968-continuing.

G. R. Eisele

Numeric Data Retrieval Systems, Nevada Applied Ecology Information Center, Ecological Science Information Center, Oak Ridge National Laboratory, Oak Ridge, TN, 1974-continuing.

Validation of the Uptake, Distribution, and Metabolism of Pu Under Ordinary Agricultural Conditions, R. Dahlman, Oak Ridge National Laboratory, Oak Ridge, TN, 1976-continuing.

B. H. Erickson

Effects of Prenatal Irradiation on Gametogenesis in the Bonnet Monkey, A. G. Andersen Radiobiology Laboratory, University of California at Davis, 1973-present.

D. E. Foard

Sequencing of Amino Acid in Soybean Trypsin Inhibitors, Davis Lin, The University of Tennessee Memorial Research Center, Knoxville, TN, 1976-continuing.

Development of Antiserum Against Protease Inhibitors, D. Hwang, W. Yang, Oak Ridge National Laboratory, Oak Ridge, TN, 1975-continuing.

Leaching of Lectins During Soybean Germination, W. Yang, Oak Ridge National Laboratory, Oak Ridge, TN, 1975-continuing.

J. F. Weiss

Validation of the Uptake, Distribution, and Metabolism of Pu Under Ordinary Agricultural Conditions, R. Dahlman, Oak Ridge National Laboratory, Oak Ridge, TN, 1976-continuing.

## INTERAGENCY AGREEMENTS

Defense Civil Preparedness Agency, "Effects of Fallout Radiation on Important Agricultural Crops and Food Producing Livestock," 1968-1975.

Environmental Protection Agency, "Influence of Diet on the Gastrointestinal Absorption of Cadmium," January 1975–January 1980.

United States Department of Agriculture Agricultural Research Service, "Mass Sterilization of Boll Weevils," March 1, 1976–September 30, 1977.

## MANAGEMENT SEMINARS

In the fall of 1976, seven of CARL's thirteen administrative staff members were accepted into membership in the American Management Association. To facilitate interaction among our management personnel, a weekly management seminar was instituted. These seminars provide an opportunity for development of standard management philosophies and practices, introduction to established management techniques, and general discussions related to current management problems.

## JOURNAL CLUB

Researchers at CARL have long felt the need for greater interaction among individuals in our three research areas: animal metabolism, animal effects, and plant research. In the fall of 1976, under the direction of Dr. Frederick Ryan, the organization of the Journal Club was completed. As the participants represent diverse scientific backgrounds, discussions necessarily cover a broad range of topics. Weekly meetings are held over lunch to keep the group informed of significant developments in the respective fields.

## RESEARCH PROGRESS SEMINAR

A weekly luncheon seminar was initiated in mid-1975 to foster communication among the varied research groups within the laboratory. Initially, each investigator discussed his current work and future research direction. With recent increased research cooperation among groups, the need for this type seminar has declined, and the group now meets sporadically when specific subject matter presents itself.

## EMPLOYEE QUALIFICATION IMPROVEMENT PROGRAM

CARL's Employee Qualification Improvement Program (EQIP) provides an opportunity for employees to improve and/or broaden their skills in a variety of ways. Through the fees waiver program of the University of Tennessee, employees may register for one credit course each quarter on the undergraduate or graduate level. A recently-initiated correspondence course program also provides opportunities for independent study in the areas of maintenance, secretarial skills, and management. Employees may also register for short-term seminars in a variety of subject areas. In these ways CARL is attempting to meet the educational needs of its employees.

## SEMINARS PRESENTED AT CARL

O. J. Schwarz, Assistant Professor, The University of Tennessee Department of Botany. "The Effects of Paraquat on Oleoresin Production in Species of Southern Pine."

G. J. Wagner, Department of Biology, Brookhaven National Laboratory, Upton, NY. "Current Research on Vacuole Isolation and Intracellular Localization of Pollutants in Plants."

Michael Shelby, Environmental Mutagen Information Center, Technical Information Center, Oak Ridge, TN. "Information Data Base on Plant Mutagenesis."

Jerome Dobson, Oak Ridge National Laboratory, Oak Ridge, TN. Fuel From Biomass.

J. M. Stewart, The University of Tennessee Department of Plant and Soil Science. "In Vitro Culture of Cotton Ovule."

D. K. Dougall, W. Alton Jones Cell Science Center, Lake Placid, NY. "Towards the Biochemistry of Adventive Embryogenesis in Carrot Tissue Cultures."

Alan Poole, Institute of Energy Assessment, Oak Ridge Associated Universities. Fuels from Biomass.

B. V. Conger, The University of Tennessee Department of Plant and Soil Science. Summary of Visits to European Laboratories.

M. L. Salin, National Institutes of Health Postdoctoral, Departments of Biochemistry and Biological Sciences, Duke University. "Organic Acids as Hill Agents in Mesophyll Cells Isolated from $C_4$ Plants."

G. D. Winget, Associated Professor, University of Cincinnati Biochemistry and Biological Sciences Department. "The Coupling of Light Energy to ATP Synthesis: A Model of the Chloroplast."

A. Kleinhofs, Department of Agronomy and Genetics Program, Washington State University. "Prospects of Genetic Engineering in Plants."

G. A. Rosenthal, University of Kentucky. "Biochemical Studies of Canavanine Toxicity in Tobacco Hornworm."

## VISITING STAFF AND STUDENTS

### Visiting Staff

S. P. Arora, Food and Agriculture Organization Fellow, on leave from Indian Council of Agricultural Research/ Ministry of Food and Agriculture, New Delhi, India, July 1975.

Hendratno, International Atomic Energy Agency Fellow, on leave from Pasar-Jumat Research Center, Jakarta, Indonesia, June 1975-September 1975.

P. Kale, Oak Ridge Associated Universities Faculty Research Participation Program, from Knoxville College, Knoxville, TN, June 1975-August 1975.

Abul Mansur, International Atomic Energy Agency Fellow, on leave from Institute of Nuclear Agriculture, Mymensingh, Bangladesh, September 1975-September 1976.

Ahmad Mokhtarzadeh, on leave from Pahlavi University, Shiraz, Iran, September 1976-September 1977.

Krisda Samphantharak, International Atomic Energy Agency Fellow, on leave from Kasetsart University, Bangkok, Thailand, October 1976-October 1977.

Jarnail Singh, Oak Ridge Associated Universities Faculty Research Participation Program, from Stillman College, Tuscaloosa, AL, June 1973-August 1973.

### Graduate Students

D. E. Lentz, Animal Science, The University of Tennessee, Knoxville, TN, 1975-1976.

F. C. Madsen, Animal Science, The University of Tennessee, Knoxville, TN, 1973-1975.

R. C. Tsou, Physiology, The University of Tennessee, Knoxville, TN, 1972-1974.

R. Wahnbaech, Botany (Plant Physiology), The University of Tennessee, Knoxville, TN, 1975-present.

### Oak Ridge Associated Universities Undergraduate Research Participation Program

Janet Frances Laprade, College of Our Lady of the Elms, Chicopee, MS, Summer 1974.

Jacquelyn W. George, A&T State University, Greensboro, NC, Summer 1975.

Dwana M. Bush, Emory University, Atlanta, GA, Summer 1975.

Jane L. Rohrer, Carson-Newman College, Jefferson City, TN, Summer 1975.

Margaret P. Mitrane, St. Peter's College, Jersey City, NJ, Summer 1975.

Charles Tysinger, Campbell College, Buies Creek, NC, Summer 1976.

Timothy R. Silvis, Emory University, Atlanta, GA, Summer 1976.

Samuel E. Steinmetz, Eastern Illinois University, Charleston, IL, Summer 1976.

# VISITORS

## 1975–1977

The University of Tennessee Veterinary School personnel E. D. Gage, A. M. Legendre, and R. E. Roberts toured facilities and discussed with H. E. Walburg the potential for cooperative research projects between the veterinary faculty and the CARL staff, May 1, 1975.

Carl Guston, Nuclear Regulatory Commission, Washington, DC, toured the facilities and conferred with H. E. Walburg, May 15, 1975.

Art Shearon, ERDA Controller's Office, Washington, DC, toured the Laboratory, May 21, 1975.

Wei-Li Chen and Sudernaique F. Deus, Holifield National Laboratory, Oak Ridge, TN, conferred with H. E. Walburg concerning arrangements for cooperative research programs, May 30, 1975.

Dr. and Mrs. V. T. Sapra, Alabama A&M University, Normal, AL, visited the Laboratory to discuss projects of mutual interest with M. J. Constantin, June 6, 1975.

Dr. Agnar Nilsson, Research Institute of National Defence, Sundbyberg, Sweden, visited the Laboratory for conferences with H. E. Walburg, July 3, 1975.

Dr. L. B. Davich, USDA Boll Weevil Research Laboratory, Starkville, MS, conferred with H. E. Walburg and E. B. Darden concerning a cooperative sterilization experiment of boll weevils as part of an eradication program, July 16, 1975.

Mr. Clarence Mitchell, Extension Office, Jessamine County, Kentucky, and a group of Kentucky agricultural workers and farmers toured the facilities, July 24, 1975.

Drs. D. M. Gossett, T. J. Whatley, and R. R. Johnson, The University of Tennessee Institute of Agriculture, discussed research programs with H. E. Walburg, August 15, 1975.

Dr. B. Shore, Lawrence Livermore Laboratory, Livermore, CA, visited H. E. Walburg, August 25, 1975.

Dr. J. Widholm, University of Illinois, Urbana, IL, discussed the plant mutagenesis program with R. R. Henke and M. J. Constantin, August 25, 1975.

Drs. H. R. Caffey, W. Patrick, and B. L. Miller, Louisiana State University, Baton Rouge, LA, consulted with M. J. Constantin, August 26, 1975.

M. J. Constantin held discussions and led a tour for Abbas Ali Zali, Syrus Abdmishani, B. Y. Samadi, all of Iran, and Ciba-Geigy Corporation representatives R. Brown, C. Laidle, A. Cocke, D. Taylor, W. Wilcox, and D. Reicosky, August 27, 1975.

Drs. J. B. Storer and R. J. Julian, Holifield National Laboratory, Oak Ridge, TN, visited H. E. Walburg, September 2, 1975.

Dr. L. S. Valberg, University of Western Ontario, Canada. visited H. E. Walburg, September 11-12, 1975.

Dr. P. W. Durbin, Lawrence Berkeley Laboratory, Berkeley, CA, visited H. E. Walburg, September 15-16, 1975.

Mr. and Mrs. F. Finley, Atomic Energy of Canada, Ltd., toured the facilities, September 18, 1975.

ERDA Review Team Members G. W. Casarett, University of Rochester Medical School, A. Conger, Temple University School of Medicine, J. R. Laughnan. University of Illinois, T. H. Roderick, Jackson Laboratory, M. F. Sullivan, Battelle-Pacific Northwest Laboratory, D. R. Van Campen, U. S. Department of Agriculture, R. A. Doherty, University of Rochester Medical School, and R. G. Cuddihy, Inhalation and Toxicology Research Institute, September 23-24, 1975.

Energy Research and Development Administration, Division of Biomedical and Environmental Research, Washington, DC, visitors C. H. Hobbs, N. Carter, G. Stapleton, D. Mahlum, D. Parker, and B. W. Wachholz, visited the Laboratory in connection with the Review, September 23-24, 1975.

Energy Research and Development Administration, Oak Ridge Operations Office, visitors R. E. Benson, J. A. Lenhard; Holifield National Laboratory representatives S. I. Auerbach, J. A. Auxier, and R. C. Dahlman visited the Laboratory in connection with the Review, September 23-24, 1975.

Dr. J. A. Ewing, Dean, Agricultural Experiment Station, The University of Tennessee, Knoxville, TN, visited the Laboratory in connection with the Review, September 23-24, 1975.

Dr. R. E. Roberts, The University of Tennessee Veterinary School, consulted with H. E. Walburg, October 7, 1975.

The University of Tennessee personnel Drs. J. A. Martin, T. J. Whatley, D. H. Luttrell, J. I. Sewell, R. J. Johnson, J. H. Gibbons, and Albert Bedinger met with CARL

personnel to discuss potential research projects involving solar energy in agriculture. Dr. S. E. Beall, Holifield National Laboratory, Oak Ridge, TN, also participated in the discussions, October 14, 1975.

Drs. N. K. Gossilino and T. L. Bolfueva, USSR, visited the Laboratory as part of the USA-USSR Bilateral Exchange Program, October 24, 1975.

Members of the Economic Development Department of the Oak Ridge Chamber of Commerce met with H. E. Walburg and other staff members, November 25, 1975.

Dr. L. M. Josephson, The University of Tennessee, and Dr. W. P. Grobbelaar and Mr. H. C. Kuhn, Department of Agriculture, Republic of South Africa, discussed plant research projects and toured the facilities with CARL plant research staff, November 26, 1975.

Dr. Ralf Mott, North Carolina State University, and Dr. Karen Hughes, Department of Botany, The University of Tennessee, met with Drs. B. V. Conger, M. J. Constantin, and R. R. Henke to discuss plant cell tissue culture techniques and tour the facilities, January 5, 1976.

Dr. Wallace Friedberg, Federal Aviation Administration, Oklahoma City, OK, discussed cooperative research with E. B. Darden, January 6, 1976.

Drs. Ted Davich and John Dawson, USDA-Agricultural Research Service, conferred with H. E. Walburg and E. B. Darden concerning cooperative research between the Boll Weevil Research Laboratory and CARL, January 15-16, 1976.

Drs. James M. Stewart and Ms. Cecilia Hsn, The University of Tennessee (through USDA-Agricultural Research Service), discussed research with M. J. Constantin, January 21, 1976.

Dr. C. M. Haaland, Oak Ridge National Laboratory, Oak Ridge, TN, discussed the effects of fallout from a nuclear war on crop production with M. J. Constantin, January 28, 1976.

A. G. Linton and J. L. Holdaway, Environmental Protection Agency, Atlanta, GA, consulted with D. M. Robie on waste management as part of an overall review of Oak Ridge Operations, January 28, 1976.

Drs. Jerry Payne and Jim Thompson, USDA Fruit and Nut Laboratory, Byron, GA, visited M. J. Constantin for radiation experiments, February 10-11, 1976.

Dr. J. A. Ewing, Dean, Agricultural Experiment Station, The University of Tennessee, Knoxville, TN, and Drs. T. J. Whatley and D. M. Gossett, The University of Tennessee Institute of Agriculture, conferred with H. E. Walburg, M. J. Constantin, and R. R. Henke, February 24, 1976.

Dr. Wallace Friedberg, Federal Aviation Administration, Oklahoma City, OK, conferred with E. B. Darden concerning collaborative research pertaining to air transport of radioisotopes, February 26, 1976.

Mrs. Leslie Braunstein, ERDA Public Affairs, Washington, DC, conferred with D. M. Robie and toured the facilities, March 23, 1976.

Dr. Mats Holmberg, National Defense Research Institute, Sundbyberg, Sweden, visited the Laboratory to confer with H. E. Walburg concerning CARL's facilities and programs, April 25-28, 1976.

Dr. Omer Idris, IAEA Fellow, Cornell University, visited J. K. Miller and S. L. Hansard, May 27-28, 1976.

L. V. Miranda and Everett Freeman, ERDA Headquarters, Washington, DC, conferred with D. M. Robie, September 29, 1976.

Mary G. White, Scientific Program Manager, Nevada Applied Ecology Group, ERDA Nevada Operations Office, toured CARL's facilities and conferred with H. E. Walburg, October 8, 1976.

Drs. Rene Sotomayor, Oak Ridge National Laboratory Biology Division, Oak Ridge, TN, and Mario Roman, Chilean Ministry of Agriculture, visited W. J. Kopp for a briefing of CARL's animal programs, October 22, 1976.

A group of researchers from the Agricultural Research Service Boll Weevil Research Laboratory, Mississippi State University, met with H. E. Walburg, M. D. Schneider, and E. B. Darden concerning their cooperative project in boll weevil radiation sterilization, November 4, 1976.

A group from Meharry Medical College School of Graduate Studies, Nashville, TN, visited H. E. Walburg for a program review, December 2, 1976.

Dr. Wallace Friedberg, Federal Aviation Administration, Oklahoma City, OK, consulted with E. B. Darden regarding ongoing collaborative research, January 11, 1977.

## COMPARATIVE ANIMAL RESEARCH LABORATORY PUBLICATIONS

### Reprints Available

562. EISELE, G. R., and J. L. WEST. 1973. Bacteriological evaluations of swine exposed to lethal levels of gamma radiation. *J. Animal Sci.* 37: 27-32.

568. MILLER, J. K., E. W. SWANSON, and W. A. LYKE. 1973. Iodine concentration in nonthyroid tissues of cows. *J. Dairy Sci.* 56: 1344-1346.

569. SASSER, L. B.. M. C. BELL, and F. H. CROSS. 1973. Hematologic response of sheep and cattle to whole-body gamma irradiation and gastrointestinal and skin beta irradiation. *Am. J. Vet. Res.* 34: 1555-1560.

570. EISELE, G. R., F. R. MRAZ, and M. R. JOHNSTON. 1974. Effects of whole-body gamma irradiation on various chemical properties of muscle. *J. Animal Sci.* 38: 20-23.

571. SASSER, L. B., L. WADE, JR., and M. C. BELL. 1974. Effect of radiation on metabolism of selected minerals in cattle. *J Animal Sci.* 38: 178-185.

572. BYRNE, W. F., and M. C. BELL. 1974. *Fallout facts for sheep producers.* Agricultural Extension Service, The University of Tennessee and the UT-AEC Agricultural Research Laboratory of the Tennessee Agricultural Experiment Station, RCD-9.

573. BELL, M. C., and W. F. BYRNE. 1974. *Fallout facts for swine producers.* Agricultural Extension Service, The University of Tennessee and the UT-AEC Agricultural Research Laboratory of the Tennessee Agricultural Experiment Station, RCD-8.

574. MILLER, J. K., E. W. SWANSON, W. A. LYKE, B. R. MOSS, and W. F. BYRNE. 1974. Effect of thyroid status on digestive tract fill and flow rate of undigested residues in cattle. *J. Dairy Sci.* 57: 193-197.

575. CONGER, B. V., and M. J. CONSTANTIN. 1974. The effectiveness of fission neutrons, 14.7-MeV monoenergetic neutrons and $^{60}$Co gamma radiation on seedling growth reduction and induction of chlorophyll-deficient mutations in barley. In *Biological effects of neutron irradiation,* pp. 417-432. Vienna: IAEA.

576. CONGER, B. V. 1973. The effects of ascorbic acid and sodium azide on seedling growth of irradiated and non-irradiated barley seeds. *Radiat. Bot.* 13: 375-379.

583. KILLION, D. D., and M. J. CONSTANTIN. 1974. Effects of separate and combined beta and gamma irradiation on the soybean plant. *Radiat. Bot.* 14: 91-99.

584. EISELE, G. R. 1974. Bacterial and biochemical studies on gamma-irradiated swine. *Am. J. Vet. Res.* 35: 1305-1308.

585. McFEE, A. F., M. W. BANNER, and M. N. SHERRILL. 1974. Chromosome aberrations in the leukocytes of partial-body and whole-body irradiated swine. *Radiat. Res.* 60: 165-172.

589. CONSTANTIN, M. J., D. D. KILLION, and A. C. BLAKE. 1974. *Fallout facts for corn producers.* Agricultural Extension Service, The University of Tennessee and the UT-AEC Comparative Animal Research Laboratory of the Tennessee Agricultural Experiment Station, RCD-12.

590. BROWN, D. G. 1975. Physiologic responses to exercise of irradiated Shetland ponies: A five-year study. *Am. J. Vet. Res.* 36: 645-652.

591. MILLER, J. K., B. R. MOSS, E. W. SWANSON, and W. A. LYKE. 1975. Effect of thyroid status and thiocyanate on absorption and excretion of iodine by cattle. *J. Dairy Sci.* 58: 526-531.

592. CONGER, B. V. 1975. Maximum r.b.e. of fission neutrons for induction of somatic mutations in maize. *Int. J. Radiat. Biol.* 27: 271-281.

593. HANSARD, S. L., F. C. MADSEN, G. M. MERRIMAN, and J. B. McLAREN. 1975. Tennessee grass-fed cattle need supplementary magnesium. *Tenn. Farm Home Sci.*, Progress Report 93, pp. 36-38.

594. MILLER, J. K., E. W. SWANSON, W. A. LYKE, and W. F. BYRNE. 1975. Altering iodine metabolism in the calf by feeding iodine-binding agents. *J. Dairy Sci.* 58: 931-937.

597. MADSEN, F. C., G. E. SPALDING, J. K. MILLER, S. L. HANSARD, and W. A. LYKE. 1975. Magnesium movement in hypothyroid sheep. *Proc. Soc. Exp. Biol. Med.* 149: 207-214.

598. CONGER, B. V. 1975. Radioprotective effects of ascorbic acid in barley seeds. *Radiat. Bot.* 15: 39-48.

600. KILLION, D. D., and M. J. CONSTANTIN. 1975. *Effects of fallout radiation on crop production.* Final

report on DCPA Work Order No. DAHC 20-70-C-0312, Work Unit No. 3223F. Washington: Defense Civil Preparedness Agency. ORO-747.

603. MRAZ, F. R., and G. R. EISELE. 1975. Increased $^{144}Ce$ uptake in fetal rats after the addition of carrier. *Radiat. Res.* 64: 399-400.

606. WEST, J. L. 1975. Bovine pheochromocytoma: Case report and review of literature. *Am. J. Vet. Res.* 36: 1371-1373.

607. SASSER, L. B., M. C. BELL, and G. E. JARBOE. 1975. Influence of acute tissue injury on in vitro incorporation of $^{65}Zn$ by sheep erythrocytes. *J. Animal Sci.* 41: 1679-1685.

608. CONGER, B. V. 1976. Effectiveness of fission neutrons versus gamma radiation for inducing somatic mutations in presoaked seeds of maize. *Mutat. Res.* 34: 223-232.

609. CONSTANTIN, M. J., W. D. KLOBE, and L. N. SKOLD. 1976. Effects of physical and chemical mutagens on survival, growth, and seed yield of soybeans. *Crop Sci.* 16: 49-52.

610. CONGER, B. V., L. W. SKINNER, and L. N. SKOLD. 1976. Variability for components of yield induced in soybeans by seed treatment with gamma radiation, fission neutrons, and ethylmethane sulfonate. *Crop Sci.* 16: 233-236.

611. MADSEN, F. C., D. E. LENTZ, J. K. MILLER, D. LOWREY-HARNDEN, and S. L. HANSARD. 1976. Dietary carbohydrate effects upon magnesium metabolism in sheep. *J. Animal Sci.* 42: 1316-1322.

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# Facilities Construction and Improvement

Facilities and equipment of CARL, representing an investment of approximately $4 million, include a central laboratory and office building; a unique variable-dose-rate irradiation facility; a multiple-source, low-dose-rate irradiation facility; a high-level radioisotope laboratory building; surgical facilities for large and small animals; an autopsy laboratory; large animal isolation facilities; greenhouses and field plots; and facilities and land for housing and pasturing swine, cattle, sheep, and burros.

During the period covered by this report, numerous construction projects were initiated and completed.

In January, 1974, work began on extensive modifications to the Men's Change Room. The existing change room provided no shower facilities for the main laboratory complex, and only limited shower facilities were available for personnel in outlying areas. Because the Laboratory environment is monitored according to strict microbial control and because laboratory personnel must be provided adequate facilities to protect their home environments from transfer of harmful agents, modification of existing facilities was undertaken at an estimated cost of $25,000.

Most existing experimental radiation data from animals other than the laboratory rodent are from the beagle dog. In addition, inter-species comparisons developed at CARL are to be related to extensive, diverse pools of data from other ERDA programs. Therefore, the beagle dog is an essential experimental animal for existing research programs involving radiation, technology, and internal dosimetry. Approval was received in May, 1974, to begin construction of a canine facility to house a minimal number of beagle dogs at a projected cost of $96,000.

An increased research activity in the area of plant mutation genetics and biochemistry necessitated the addition of approximately 250 square feet of laboratory space and remodeling of the Cell Culture Laboratory. In the fall of 1974, at a cost of $13,000, this renovation not only provided the needed additional space but also upgraded the Cell Culture Laboratory as a centrally located facility to accommodate new techniques utilized in plant cell tissue culture.

In order to develop a herd of specific-pathogen-free swine at CARL, the Laboratory maintains an isolation facility designed for housing these animals. Due to the increased population of this herd, it was necessary to initiate a project involving construction of a Swine Waste Disposal System to meet acceptable sanitary standards for animal waste effluents. Without the addition of this disposal system, a considerable amount of time and labor would be required to remove waste from the area each day. This project was approved in April, 1975, at an approximate cost of $11,000.

Plans for the construction of a Small Animal Actinide Facility, at a cost of $20,000, were approved in May, 1975. Research on the metabolism and effects of internally deposited radionuclides has historically been supported by the Energy Research and Development Administration. The actinides are one group of radionuclides which impose an obvious health hazard because of their long half-life, high-energy emission, and tendency to localize in critical tissues. In order to carry out satisfactory experimentation on inter-species relationships, careful studies of the location and retention times of realistic physical-chemical forms of radionuclides must be carried out in several species in the same laboratory with the same treatment material.

During the past four years, two major fencing projects were initiated, and one has been completed. The first, in May, 1975, involved installing 3½ miles of fencing at a cost of $3,000. The second, begun in April, 1976, at an estimated cost of $10,000, calls for installation of additional fencing needed to contain and protect research animals and provide appropriate security for the research facility.

Research in the areas of basic biochemical and structural aspects of intestinal absorption by epithelial cells

Facilities Construction and Improvement

(a) Recently acquired Data General Eclipse Mini-Computer which has permitted improved efficiency in biomodeling and metabolism studies. (b) Construction of an animal metabolism facility is nearing completion. (c) A recently installed electronic gate provides the needed security for CARL property. (d) New swine waste disposal system at Freels Bend.

began in the spring of 1975 with the construction of an $88,000 Biochemistry Laboratory and Service Area. Inherent in this project was modification of several existing laboratories to accommodate equipment and techniques required by the programmatic research in biochemistry. This project also provided centralization of the Laboratory's service area for washing and sterilizing large volumes of glassware.

Continued expansion of energy needs and the exploitation of all efficient energy-producing technologies have identified considerable gaps in our knowledge about the interaction of effluents from both nuclear and non-nuclear sources with the environment and man. In May, 1976, approval was granted for work to begin on the construction of a Large Animal Containment Facility. This facility will enable further experiments on the metabolism of selected radionuclides as well as utilization of radiotracer techniques for non-nuclear studies. Metabolic data from large, long-lived animals are essential for establishing the risks to man from environmental pollutants and for assessing transport to man through food chains. Very little data on large animals are now available. Utilizing diverse large animal species, information on the kinetics of metabolism and distribution of pollutants can be assured. Studies are needed on localization and retention times following various modes of introduction into the body; for example, ingestion. Realistic physical-chemical forms of important radionuclides and non-nuclear effluents will be studied in several species at CARL using the same treatment material. The projected cost of this facility is $200,000.

Problems of the current exhaust and air conditioning system, such as excessive down time, non-uniform temperature control, dust buildup in ducts, and potential loss of experimental data and specimens due to excessive temperature and/or dust contamination, will be corrected with proposed modifications to the existing system. Initiated in June of 1976, this project will cost approximately $80,000 and will also provide expanded radionuclide laboratory space by adding adequate filtration systems to existing hood and room exhausts.

Existing radioisotope containment facilities require a special waste water collection and testing system to provide maximum protection to personnel and to restrict environmental releases below acceptable levels. Begun in June, 1976, at a cost of $50,000, this project will also permit upgrading of the existing sanitary sewer system. This system will consist of double contained lines from "hot" sinks to storage tanks from which the hot waste will be transported for burial and a containment tank for testing of "cold" waste water from these facilities before release to the waste water system.

Considerable new equipment reflecting the new program directions and techniques has been purchased during the past four years. This includes chromatography equipment and radioisotope counting equipment, including alpha particle counting equipment. Of special note is the acquisition of a Data General Eclipse Mini-Computer which has permitted the expansion of our biomodeling program and much increased the efficiency of our metabolism studies.