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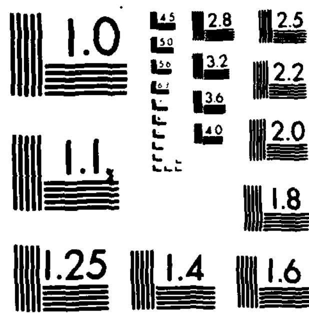

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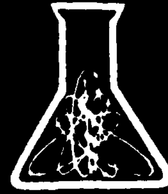

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This report contains a summary of the research projects of the Armed Forces Radiobiology Research Institute for the period 1 October 1979 through 30 September 1980.

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BEHAVIORAL SCIENCES DEPARTMENT

The Behavioral Sciences Department conducts investigations assessing the effects of ionizing radiation, chemicals, and drugs on combat performance. This program encompasses a spectrum of multidisciplinary approaches in experimental animal models, ranging from operant conditioning techniques to methods used in physiological psychology, neurochemistry, and neurophysiology. The Department addresses the specific behavioral consequences of exposure to ionizing radiation, and explores the biological mechanisms responsible for radiation-induced behavioral decrements. Collaborative efforts are pursued with other AFRRI departments, the Naval Research and Development Command, and the National Institutes of Health.

The Department has two Divisions: the Experimental Psychology Division and the Physiological Psychology Division. The Experimental Psychology Division uses behavioral and electrophysiological models to determine the conditions under which ionizing radiation and militarily relevant chemicals can degrade combat performance. Behavioral tasks that model specific aspects of cognitive and physical combat performance are used to determine the radiation levels that affect combat effectiveness. The information from these studies is compiled into a Database, which provides rapid retrieval, overall summary analyses, and development of extrapolated models applicable to battlefield conditions. Research in behavioral toxicology quantitates the changes in behavioral and neurophysiological capabilities due to exposure to chemicals and radiations that may be present in the military environment. This work uses a battery of tests designed to provide information on toxic dose levels and/or mechanisms by which these environmental hazards affect behavior.

The Physiological Psychology Division explores the mechanisms by which ionizing radiation and chemical toxins disrupt behavior. Behavioral, physiological, and neurochemical approaches are predominant. This information can be used to develop methods of preventing performance decrement.

The results obtained from the research of this Department are disseminated to the military services and appropriate government agencies by means of informal reports, committee assignments, working groups, and correspondence. Information is also transmitted through publication in the open scientific literature and through presentation at scientific meetings.

******

REDUCTION IN CYCLIC NUCLEOTIDE LEVELS IN THE BRAIN AFTER A HIGH DOSE OF IONIZING RADIATION

Principal Investigator: W. A. Hunt
Technical Assistance: T. K. Dalton

High doses of radiation can alter neural function in two ways. First, shortly after exposure, depression of the central nervous system is observed. This is characterized by reduced locomotor activity, disorientation, lethargy, and inability to avoid shock. Known as early transient incapacitation (ETI), this phenomenon lasts about 30 minutes; after that, the animals appear normal. At later periods, progressive and permanent incapacitation develops; it is accompanied by convulsions and unresponsiveness until time of death. This latter phenomenon is referred to as the central nervous system (CNS) syndrome.

Alterations in brain function are often accompanied by changes in cyclic nucleotide levels in various parts of the brain. For example, drugs that have an excitatory influence on the brain (such as amphetamine, apomorphine, pentylenetetrazol, and harmaline) elevate cyclic nucleotides, most notably guanosine-3',5'-monophosphate (cGMP). On the other hand, depressant drugs (such as diazepam, ethanol, morphine, and haloperidol) reduce cGMP levels. Stimuli other than drugs can also affect cyclic nucleotides. Stress can lead to elevated cGMP levels.

The purpose of this study was to determine the effect of ionizing radiation on the cyclic nucleotides cGMP and cAMP in various regions of the brain, and to see how any changes might relate to the behavioral decrement observed.

Cyclic nucleotide levels were measured in several areas of the brain by radioimmunoassay after doses of high-energy electrons (1). Both cGMP and cAMP were reduced maximally 10 minutes after exposure in the cerebellum, brain stem, caudate nucleus, and cerebral cortex. cGMP levels were depressed for at least 24 hours, while cAMP levels returned to control levels over a 4-hour period. Dose-response studies indicated that cGMP is more sensitive than cAMP to radiation. Reductions in cGMP could be seen at 5 krad and above, while 10 krad were needed with cAMP. Radiation doses of 10 krad did not alter the enzymes involved in the synthesis and degradation of cyclic nucleotides (2).

The data suggest that the response of the two cyclic nucleotides may represent different manifestations of radiation. The transient alterations in cAMP may be related to early transient incapacitation because of its correlation with dose and time course, while reductions in cGMP may involve development of the CNS syndrome.

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RADIATION-INDUCED DAMAGE TO BIOLOGICAL MEMBRANES

Principal Investigators: M. J. McCreery and W. A. Hunt
Technical Assistance: T. K. Dalton

Although many aspects of the effect of ionizing radiation on biological systems have been studied, the basic mechanisms of its action remain largely unidentified. Undoubtedly, the biological consequences of irradiation do not result from a common event, but rather from a multitude of divergent effects. Since the function of many cells depends on the integrity of the membranes surrounding these cells, we must know the effect of radiation on biological membranes to understand the overall consequences of irradiating living systems. Membranes provide a selective barrier between the inside of a cell and its surroundings, and are generally believed to play a significant role in the fundamental processes of life.

To determine how radiation interacts with biological membranes, molecular probes were used to monitor the environment in which membranes reside in order to find possible changes in structure. Unsealed erythrocyte ghosts were irradiated with either X rays, gamma rays, high-energy electrons, or neutrons. Using the probes diphenylhexatriene and anilinonaphthalene sulfonate, fluorescence intensity was reduced to a dose range of 10-100 krads. No consistent changes in fluorescence polarization and lifetimes were observed below 100 krads.

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DIFFERENTIAL SENSITIVITY OF VARIOUS AREAS OF THE BRAIN AFTER IRRADIATION USING EEG TECHNIQUES

Principal Investigator: H. Teitelbaum
Technical Assistance: J. H. Lee

High doses of ionizing radiation can induce a behavioral decrement of short duration, known as early transient incapacitation. Early transient incapacitation is characterized by reduced locomotor activity, disorientation, lethargy, and inability to avoid shock. Since discrete behaviors are affected by radiation, certain areas of the brain that might be involved with the expression of those behaviors could be more sensitive to disruption by radiation.

A study was undertaken to determine the sensitivity of different areas of the brain to radiation, using electroencephalograph (EEG) techniques. Electrodes were implanted in appropriate areas of the brain, and EEG recordings were made before and after irradiation with 5-krad and 10-krad doses of high-energy electrons.

Alterations in the EEG patterns were observed only at radiation doses of 10 krad. No effect was obtained at 5 krad. EEG amplitude and frequency were modified in

the medial thalamus and caudate nucleus but not in the hippocampus or parietal cortex. Power spectrum analysis indicated that EEG activity was shifted to lower frequencies. These changes paralleled the development and time course of early transient incapacitation.

The data support the concept that radiation can have a quantitatively different effect on different areas of the brain, especially those involved in motor function.

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BEHAVIORAL TOXICITY OF SOHIO SHALE DIESEL FUEL MARINE AND JET PROPULSION FUEL NUMBER 5

Principal Investigators: V. Bogo and R. W. Young
Technical Assistance: C. A. Boward and G. G. Kessell

The acute oral toxicity of Sohio shale diesel fuel marine (DFM) and jet propulsion fuel number 5 (JP5) was investigated in adult, male, Sprague-Dawley rats. For each fuel, six groups of six rats each were given either fuel or water at doses of 24, 30, 38, 48, or 60 ml/kg of fuel and 60 ml/kg of water (control). The measures used to evaluate fuel toxicity were daily weight, food and water consumption, home-cage activity, general condition, and survival time. All subjects were necropsied at the end of the studies. Blood was drawn for complete blood count and serum chemistry analyses of BUN, creatinine, SGOT (serum levels of glutamic-oxaloacetic transaminase), and SGPT (serum levels of glutamic-pyruvic transaminase). Selected tissues were taken for histological examination.

Sohio shale DFM. Compared to control levels, most measures of rat behavior were depressed at 1 day after administration of fuel. Food and water intake were depressed on days 1 to 6; this reversed on days 7 to 13 when intake was greater than control. The weight of the treated subjects decreased from days 1 through 6, at which time a normal gain in weight was observed. Overnight home-cage activity was depressed from days 2 to 8, and then returned to normal. In many cases, spontaneous movement was also affected. Walking was slow and labored. Some rats had arched backs, appeared stiff, and hopped when they moved. Matting and discoloration of the hair occurred, followed several days later by falling out of hair, redness of skin, and wrinkling of skin. Sensitivity to being handled was seen in several subjects.

Little evidence of pathology was found at necropsy. Blood counts and serum chemistries were essentially normal. Except for moderate congestion in the brain, liver, and lung observed in the three highest dose groups, most tissues were unremarkable. The LD 50/14 (lethal dose for 50% of animals after 14 days) for Sohio shale diesel fuel marine was 41 ml/kg.

Sohio shale jet propulsion fuel number 5. All treated subjects showed an initial change in behavior, but unlike shale diesel fuel marine, the shale jet propulsion

fuel number 5 did not always cause depression. Food consumption was lower during the first 4 days; then it increased until day 11, when it returned to normal. Water intake was depressed for 2 days after dosing, then increased through day 11, and then was normal throughout the remainder of the study. Weight loss occurred on days 1 to 3, but a normal weight increase began on day 4. On the first night after dosing, a marked increase occurred in activity, followed by depressed activity through day 4; then it returned to normal for the remainder of the study. Through the first week of assessment, body movement of some of the treated subjects was labored, unsteady, and characterized by an arched back and hopping, as though due to stiffness. Oiliness, matting, discoloration, and falling out of hair were observed. On the day after gavage, blood was found encrusted around the eyes, nose, and mouth of each fuel-treated rat.

The types of pathology found depended on the time of death. In rats that died within 24 hours, the subcutis, lungs, meninges, and epicardium were moderately to severely congested. In rats that died after 24 hours, the livers were swollen and mottled with accentuated lobular patterns. Further renal and hepatic damage after 24 hours was indicated by structural alterations. The most consistent renal change was the formation of eosinophilic hyaline droplets in the cytoplasm of the epithelial cells in the proximal distal tubules. Liver lesions consisted of moderate to severe cytoplasmic vacuolization of hepatocytes and hepatocellular degeneration around the portal triads. No significant differences were noted in the hematologic and serum chemistry assays. The LD 50/14 for Sohio shale jet propulsion fuel number 5 was 39 ml/kg.

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FREQUENCY CHARACTERISTICS OF SOMATOSENSORY EVOKED POTENTIALS IN THE RAT

Principal Investigators: R. M. Cartledge and R. W. Young

An underlying assumption in averaging somatosensory evoked potentials (SEPs) is that the background electroencephalograph (EEG) is random and independent (1). After exposure to ionizing radiation, however, the frequency spectrum of the EEG shifts to the left (2), becoming less random and less independent when compared with the SEPs. Since this shift in background activity could affect the latencies of the early peaks within the SEP, a study was conducted to identify the frequency spectrum of the SEP in the rat and to define the contributions to that spectrum of frequencies commonly found in the EEG.

Data indicate that the frequency spectrum of the early SEP components for adult rats is from 0.1 to 1000 Hz. The SEPs of six male Sprague-Dawley rats were filtered at high-frequency cutoffs of 10 kHz, 5 kHz, 3 kHz, 1 kHz, 600 Hz, 300 Hz, 200 Hz, and 100 Hz, all defined at the -3 dB points. Averaged SEPs for one subject filtered at three cutoff frequencies show that the shift in N1 and P1 latencies is more severe for 100 Hz than for either 1 or 10 kHz (Figure 1). As

seen in Figure 2, the mean N1 latency for the six subjects was 19.8 msec at 100 Hz, decreased to 13.4 msec at 1 kHz, and remained near that value through the 10-kHz cutoff point. A similar reduction in latency was observed for P1 as the filter setting was increased, with mean latencies of 32.7 msec at 100 Hz, 25.5 msec at 1 kHz, and 24.9 msec at 10 kHz.

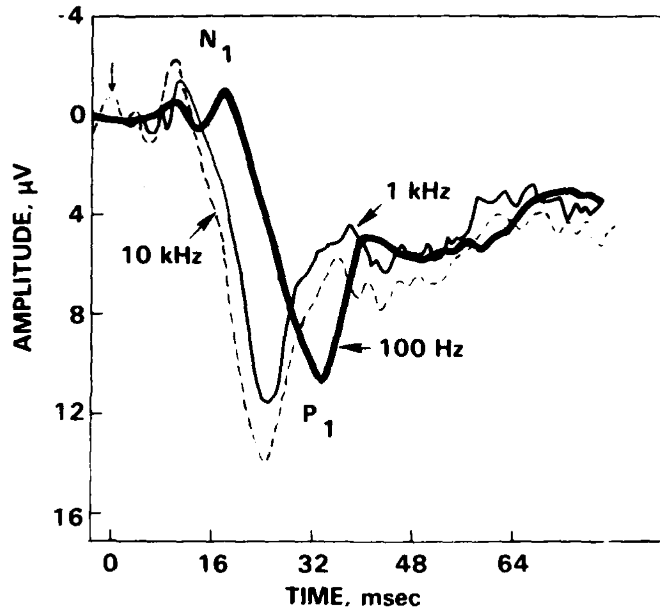

Figure 1. Effect of filtering SEP of adult rat at various high-frequency cutoff points. Filter settings were 10 kHz (dotted line), 1 kHz (thinner line), or 100 Hz (thicker line), all measured at -3 dB point. Stimulations were evoked at left plantar surface (arrow), at rate of 2/sec, 1024 responses/average, and recorded over right primary somatosensory area.

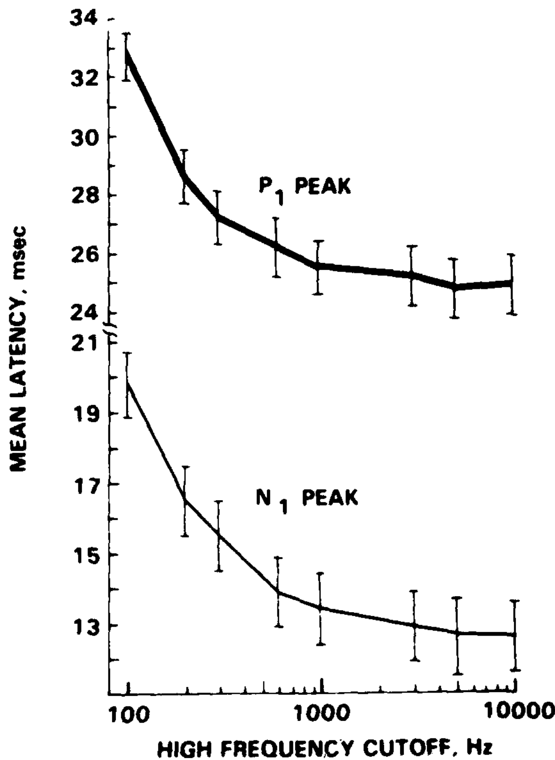

Figure 2. Changes in mean latencies of N1 (lower graph) and P1 (upper graph) SEP peaks for six rats as a function of filtering at various high-frequency cutoff settings. Standard error bars are shown. Horizontal axis is in logarithmic scale.

Although the SEPs include frequencies as high as 1000 Hz, most of the power is contained within the frequencies below 14 Hz. The mean total power for the 60-msec epochs, as determined with the fast fourier analysis, was 37.96 square microvolts, of which 82% was for frequencies between 0.1 and 14 Hz. Shifts in the percentage of slow-wave activity within the background EEG after irradiation must therefore be considered when interpreting changes in the latencies of SEPs.

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EXPERIMENTAL MODEL FOR PREIRRADIATION CUMULATIVE FATIGUE

Principal Investigators: C. G. Franz and R. W. Young
Collaborator: A. C. Bakarich
Technical Assistance: L. Clark

Military personnel are often required to perform physically demanding tasks for long periods of time, during which they have little opportunity to rest. Such activity may cause cumulative fatigue in which there is a loss of performance capacity and efficiency (1). Previous studies of monkeys (Macaca mulatta) have shown that ionizing radiation degrades the performance of a physical activity task when subjects are not fatigued (2). The purpose of this study is to develop a model of cumulative fatigue in monkeys in order to study the combined effects of fatigue and ionizing radiation on the performance of a physical activity task.

Cumulative fatigue has been produced in monkeys performing the physical activity task 6 hours per day, 7 days per week, for at least 16 days. This is a free-operant avoidance task in which subjects are required to turn a nonmotorized wheel at a speed greater than 1 mile per hour on a schedule of 10 min work/5 min rest (3,4). After 9 weeks of training and conditioning, performance is stabilized on a schedule of 2 hours per day, 5 days per week. This schedule is then increased to 6 hours per day, 7 days per week, in order to model cumulative fatigue. Each subject required about 6 days to adapt to this increased performance schedule, at which time its performance (measured by average speed throughout the workday) stabilized and remained asymptomatic for the next 10 days. From day 16 to the end of the study, each subject performed at its asymptomatic level during the early periods of work, but it could not sustain that performance throughout the

day. A monkey was considered to be fatigued if its average speed for the entire day was 20% below baseline for at least 2 consecutive days. A 20% decrease in performance is approximately the level at which the U.S. Army defines personnel as functionally impaired after exposure to nuclear weapons (5).

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BIOCHEMISTRY DEPARTMENT

Primary objectives of the Biochemistry Department have been (a) to obtain a better understanding of and to elucidate the biochemical mechanisms of injury, repair, and protection from the deleterious effects of radiation and other toxic agents on the mammalian organism, and (b) to develop reliable, sensitive, and easy-to-perform techniques for detecting and quantifying the extent of radiation-induced injury.

The Department consists of three Divisions: the Physiological Chemistry Division, the Molecular Biology Division, and the Immunological Chemistry Division.

Primary research goals of the Physiological Chemistry Division have been (a) to develop biochemical indicators of radiation-induced injury, (b) to systematically study the late effects of radiation and radiation-induced immunosuppression, and (c) to study the role of dietary constituents in protecting an organism from the effects of radiation. Investigations have continued on the radiation-induced changes in the levels of specific serum glycoproteins and protein-bound carbohydrates as indicators of radiation injury. Studies in collaboration with investigators at the National Institutes of Health included research on radiation-induced immunosuppression. The roles of various immunostimulants were evaluated as possible radioprotectors. Various dietary adjuvants such as vitamins C and E and other antioxidant drugs have also been tested for their radioprotective properties. The effect of radiation on the formation of bone and bone marrow has also been investigated.

The Molecular Biology Division has been concerned with (a) elucidation of the biochemical mechanisms of injury as a result of exposure of the organism to radiation and toxic chemicals, with emphasis on commercially available anticholinesterases. (b) Identification of radiation-induced damage to cellular membranes and identification of the sequence of biochemical events leading to this damage. The role of various radioprotective drugs has also been evaluated both *in vitro* and *in vivo* in alleviating the radiation-induced damage to these membranes. Emphasis has been given to the effects of radiation on lung tissue and lysosomes because of their importance in cellular function. (c) Elucidation of the mechanisms responsible for the release of histamine after exposure to ionizing radiation, and the possible role of calcium in mediating it. (Histamine is known to play a role in the manifestation of radiation injury.) The effects of radiation on DNA damage, repair, and protection are also under investigation.

Research aims of the Immunological Chemistry Division have included studies on the interaction of stromal tissue and hematopoietic cells and its importance in the regeneration of the hematopoietic system following irradiation. Techniques have been developed to specifically "tag" stem cells with a fluorescent dye; the stem cells can then be separated from other hematopoietic and progenitor cells using the fluorescence-activated cell sorter (FACS-II). Also under investigation have been the effects of ionizing radiation on endogenous interferon, and the role it may play in protecting hematopoietic stem cells against damage by ionizing radiation.

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RADIATION CHEMISTRY OF RADIOPROTECTANTS

Principal Investigators: C. R. Dobbs, C. E. Elhardt, and L. May
Technical Assistance: K. M. Hartley

When a compound is found to be a radioprotectant, whether it occurs naturally or is a drug, the following questions arise:

1. Does the molecular species itself protect the organism against ionizing radiation?

2. Is the compound first converted by the initial radiation to the radioprotectant species?

3. Does the organism itself convert the compound to a metabolite that acts as the radioprotectant?

One example of a radioprotectant compound is levamisole [(S)-(-)-2,3,5,6-tetrahydro-6-phenyl-imidazo-(2,1-b) thiazole], an immunomodulating drug and veterinary antihelminthic that is converted by tissues to a sulfhydryl derivative. The drug and its metabolite have mediating effects on lipid peroxidation in microsomal preparations. Because levamisole, as an inhibitor of lipid peroxidation, is a radioprotectant drug, it was of interest to study the response of the drug itself to ionizing radiation (1). Experiments were directed toward examining the effects of gamma radiation on aqueous solutions of levamisole. Chromatographic analysis (TLC) revealed two distinct groups of radiation products. Further separation and analysis of these groups by gas chromatography-mass spectrometry (GC-MS) demonstrated that each group of radiation products consists of several components, thus indicating that the gamma irradiation of nondeaerated solutions of levamisole gives rise to varying amounts of a multiproduct mixture, no constituent of which corresponds to the natural metabolite. Dose-effect curves for the levamisole irradiation indicate that the drug is markedly resistant to molecular alteration under experimental radiation conditions (Figure 1).

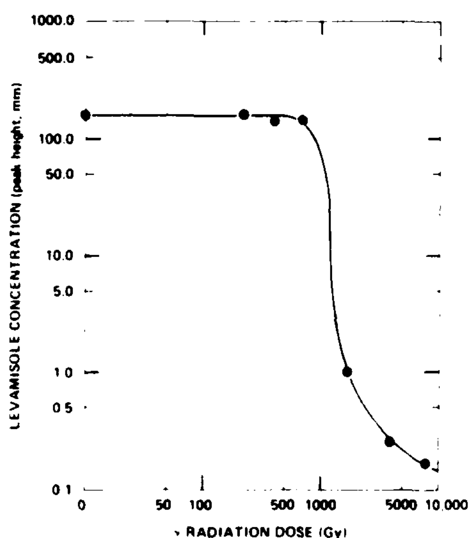

Figure 1. Effect of gamma radiation on levamisole in aqueous solution

Using similar techniques, studies on other experimental drugs with demonstrated radioprotective properties (WR 2721, N-2-mercaptopropionylglycine or thiola) are being carried out (2).

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ANTIOXIDANT AND RADIOPROTECTIVE PROPERTIES OF LEVAMISOLE

Principal Investigators: K. S. Kumar, C. R. Dobbs, and J. F. Weiss, *AFRRI*
Collaborator: M. A. Chirigos, *National Institutes of Health*
Technical Assistance: K. M. Hartley and W. W. Wolfe, *AFRRI*

During a survey of the radioprotective properties of drugs that affect the immune response, it was observed that the immunomodulator levamisole prolonged survival of irradiated mice of various strains. A series of studies were done to elucidate the mechanisms of the radioprotective properties of levamisole, including the role of sulfhydryl metabolite(s) of levamisole (1).

Levamisole was previously shown to protect rat liver microsomes from lipid peroxidation induced by adenosine diphosphate-iron complex (ADP-Fe) and one of the following: reduced nicotinamide adenine dinucleotide phosphate (NADPH), ascorbate, or X irradiation. The present experiments provide information about the mechanism of protection. Incubation of levamisole with a microsomal system containing ADP-Fe and NADPH resulted in protection of sulfhydryl groups, whereas reaction of levamisole with ascorbate (nonenzymatic system) indicated generation of a sulfhydryl metabolite. Production of a sulfhydryl metabolite of levamisole, dl-2-oxo-3-(2-mercaptoethyl)-5-phenyl-imidazolidine (OMPI), in either the enzymatic or nonenzymatic system was demonstrated by gas chromatography-mass spectrometry. While levamisole acts as an antioxidant at concentrations of 1.0 and 2.0 mM, OMPI had an enigmatic effect on microsomal lipid peroxidation induced enzymatically or nonenzymatically. OMPI exhibited a biphasic effect; at concentrations below 25 μM a prooxidant effect was observed, and at concentrations exceeding 50 μM an antioxidant effect was observed (Figure 1). The data suggest that the inhibition of microsomal lipid peroxidation by levamisole is due to the generation of sulfhydryl metabolite and that the active intermediate is probably OMPI.

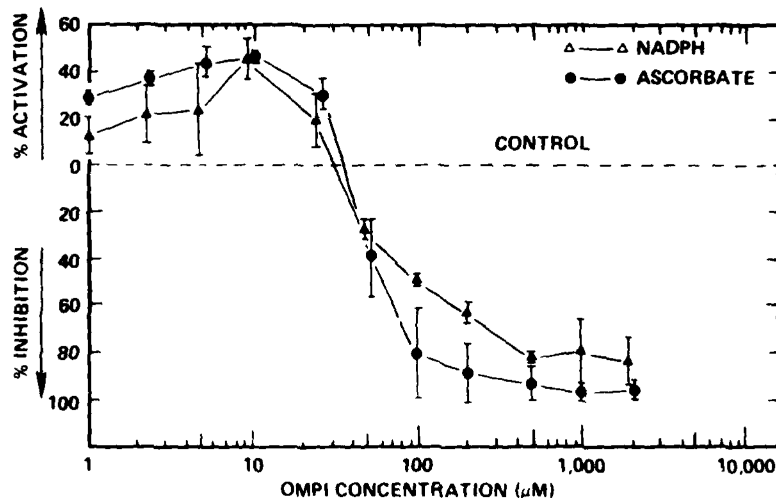

Figure 1. Effect of OMPI on microsomal lipid peroxidation. Microsomes (0.15 mg protein) were incubated with 0.15 mM NADPH or 1.0 mM ascorbate and 500 uM-5 uM ADP-Fe ($FeCl_3$ complexed with adenosine diphosphate) with varying concentrations of OMPI. Inhibition or promotion of lipid peroxidation (thiobarbituric acid-reactive material) due to addition of OMPI was determined by comparison to appropriate control reaction systems.

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RADIATION EFFECTS ON MEMBRANES

Principal Investigators: J. F. Weiss, C. R. Dobbs, K. S. Kumar, and G. N. Catravas
Technical Assistance: K. M. Hartley

Cellular membrane lipids can be damaged by the oxidizing effect of ionizing radiation, resulting in impairment of the integrity and function of the membranes, the membrane-associated enzymes, and therefore of the cell. Studies have been undertaken to determine radiation effects on cell membrane lipid components and enzymes (1) and on the release of lipid degradation products after radiation exposure (2).

Cholesterol and other lipids have been shown to modulate activity of membrane enzymes. Drugs that alter sterol biosynthesis may be used to assess the role of cholesterol in biological membranes. In the present experiment, 10 rats were fed AY-9944, an inhibitor of 7-dehydrocholesterol reductase, for 1 month at 500 mg/kg diet. While the sterol concentration of plasma was reduced, the total sterol content of isolated erythrocyte membrane ghosts was not depressed from that of controls. Erythrocyte membranes from drug-treated rats had a relatively greater 7-dehydrocholesterol:cholesterol ratio (about 2.5:1) than did the plasma from control animals (about 1.2:1). Membrane preparations were exposed to 4000 and 7000 rads of gamma radiation (cobalt-60). These doses of ionizing radiation did not appreciably alter the levels of membrane cholesterol or 7-dehydrocholesterol, and measurable amounts of oxygenated sterols were not found. The NA^+,K^+-ATPase activity of the membranes was depressed after AY-9944 treatment (18% less than controls, $p < 0.001$) as well as after radiation exposure (Table 1). The enzyme activities were: control > control + 4000 rads > AY-9944 > control + 7000 rads > AY-9944 + 4000 rads > AY-9944 + 7000 rads. Replacement of erythrocyte membrane cholesterol by 7-dehydrocholesterol may influence the Na^+,K^+-ATPase activity by altering its environment. Furthermore, the membranes from drug-treated animals appear to be more susceptible to radiation damage, as reflected in depressed enzyme activity.

Table 1. Effect of AY-9944 Treatment and *In Vitro* Irradiation on Na^+, K^+-ATPase Activity in Erythrocyte Membranes

	Na^+, K^+-ATPase ACTIVITY (μmP_i/mg protein/hr)
CONTROL	1.76 ± 0.07
CONTROL + 4000 R	1.68 ± 0.10
CONTROL + 7000 R	1.36 ± 0.05
AY-9944	1.44 ± 0.06
AY-9944 + 4000 R	1.29 ± 0.07
AY-9944 + 7000 R	1.07 ± 0.03

* $p < 0.05$, *** $p < 0.001$

MEAN VALUES ± S.E.M. (N = 10) were calculated as the total activity in the presence of Mg^{2+} + Na^+ + K^+ minus the activity in the presence of Mg^{2+} (ouabain insensitive).

Radiobiological damage may result from radiation-induced peroxidation of cellular lipids. We determined whether volatile hydrocarbons (postulated end products of fatty acid degradation) could be generated by lipid peroxidation of rat liver microsomes induced by enzymatic reaction or by ionizing radiation. Volatile compounds generated after incubation of microsomal preparations were sampled directly from the incubation flask and analyzed by gas chromatography on a Porapak Q column using flame ionization detection. Several hydrocarbons, including ethane and pentane, were produced after incubation of microsomes with adenosine diphosphate-iron complex (ADP-Fe) and reduced nicotinamide adenine dinucleotide phosphate (enzymatic promotion) or after irradiation (50-2000 Gy cobalt-60) in the presence of the promoter ADP-Fe. Production of pentane and

other hydrocarbons was related to radiation dose, incubation time, and concentration of ADP-Fe. Since the reactions leading to hydrocarbon production were inhibited by lipid antioxidants (butylated hydroxytoluene and levamisole), the principal source of hydrocarbons was probably the lipid moiety of the microsomal membrane. These in vitro experiments provide a rationale for our studies of hydrocarbons in the expired air of irradiated animals.

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IDENTIFICATION OF BETA-ADRENERGIC RECEPTORS ON PERITONEAL RAT MAST CELL GRANULE MEMBRANES

Principal Investigator: M. A. Donlon, *AFRRI*
Collaborators: G. N. Catravas and W. A. Hunt, *AFRRI*
M. A. Kaliner, *National Institute of Allergy and Infectious Diseases, NIH*

Mast cells release histamine in response to ionizing radiation. In an attempt to understand phenomena associated with this release at the cellular level, isolated rat peritoneal mast cells were studied. Histamine and other vasoactive substances are stored within membrane-bound granules in the mast cell. When these substances are activated by external receptors on the mast cell, an influx of calcium occurs, followed by fusion of the perigranular membrane with the plasma membrane as well as exocytosis of the granule constituents.

The components of the granule membrane have not been investigated. We have demonstrated the presence of beta-adrenergic receptors on the granule membrane of the mast cell using ^3H-dihydroalprenolol (^3H-DHA) binding with standard radioligand techniques.

Granules were isolated from mast cells on sucrose gradients. Different concentrations of ^3H-DHA were incubated with the granular preparation for 30 minutes at 4°C. This was done in the presence or absence of 10 μM D,L-propranolol, and the preparations were filtered through Gilson filter/type B glass filters. Results are shown in Figure 1. ^3H-DHA was bound specifically to intact granules and was saturable with a single population of binding sites.

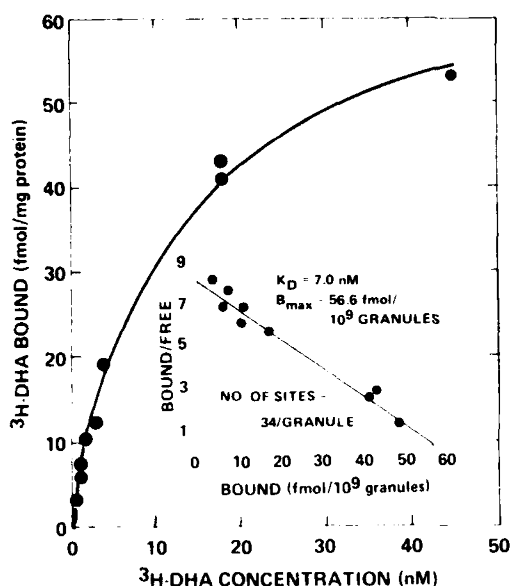

Figure 1. Saturation curve for ^{3}H-DHA-binding sites as indicator of presence of beta-adrenergic receptors on granule membranes in mast cells. Insert: Scatchard analysis of binding curve.

A dissociation constant of 7.0 nM and a maximum number of binding sites of 56.7 fmoles/10^9 granules were calculated from a Scatchard plot analysis. Each granule had 34 binding sites.

This is the first demonstration of receptor molecules occurring as an integral part of the granular membrane in mast cells.

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CHARACTERIZATION OF BETA-ADRENERGIC RECEPTORS ON RAT PERITONEAL MAST CELLS

Principal Investigator: M. A. Donlon, *AFRRI*
Collaborators: G. N. Catravas and W. A. Hunt, *AFRRI*
M. A. Kaliner, *National Institute of Allergy and Infectious Diseases, NIH*

The physiological effects observed after radiation injury originate at the cellular and biochemical levels. An understanding of the basic biochemical changes involved in the expression of radiation effects is essential in determining effective therapeutic approaches. The mechanism of radiation-induced histamine release from mast cells is unknown. The modulation of secretion in a variety of cells is initiated at the cell surface by binding to external receptors.

We have characterized the beta-adrenergic receptors on the surface of purified rat peritoneal mast cells. The purity of mast cell preparations was greater than 95%. The number of β-receptors on purified rat peritoneal mast cells was determined by the binding of H^3-dihydroalprenolol (DHA) after incubation (4°C) with cellular preparations. After 30 minutes, 3 ml of ice-cold buffer was added to the cell suspensions and the mixture was filtered through Gilson filter/type B glass filters under vacuum. The filters were washed and their radioactivity determined by liquid scintillation spectrometry. Specific binding was the difference between 3H-DHA binding and that found in the presence of 10 μM D,L-propranolol. The results are shown in Figure 1. A Scatchard analysis revealed two populations of binding sites. Each mast cell contains 120 x 10^3 high-affinity binding sites and 720 x 10^3 low-affinity binding sites, with a K_D (dissociation constant) of 10.6 ± 2.6 and 129 ± 4.7 nM and a B_{max} (maximum binding) of 186 ± 38 and 1200 ± 415 f mol/10^6 cells, respectively.

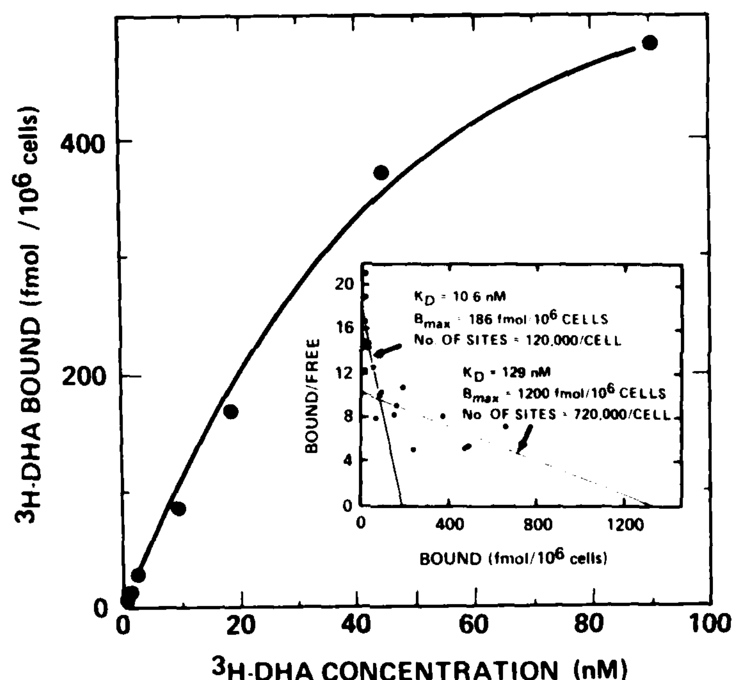

Figure 1. Binding of ^{3}H-DHA to isolated mast cells of rat as function of DHA concentration. Insert: Scatchard analysis of saturation curve.

This is the first description of beta-adrenergic receptors on the mast cell, and it suggests that β-adrenergic agonists may provide an effective mechanism of inhibiting histamine release in vivo.

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AMILORIDE-INDUCED HISTAMINE RELEASE FROM RAT PERITONEAL MAST CELLS

Principal Investigator: M. A. Donlon, *AFRRI*
Collaborators: G. N. Catravas, *AFRRI*
E. Chock and M. A. Kaliner, *National Institute of Allergy and Infectious Diseases, NIH*
Technical Assistance: L. Barr, *National Institute of Allergy and Infectious Diseases, NIH*

Histamine is released from mast cells as a result of midlethal and lethal doses of radiation in humans and in a variety of experimental animals. This research program studies mast cells directly using isolated and purified (>97% purity) rat peritoneal mast cells (RPMC). Our efforts have concentrated on understanding calcium transport in RPMC's and defining its role in the histamine release process, because increases in intracellular calcium levels are sufficient stimulus to cause histamine release.

An important mechanism for the transfer of calcium across cell membranes is Na^{+}-Ca^{2+} exchange. This is a carrier-mediated transport system that couples the movement of Ca^{2+} in one direction to the movement of Na^{+} in the other. We have studied the effect of Amiloride (N-Amidino-3,5-diamino-6-chloropyrazine carboxamide), which reversibly blocks Na channels on the external aspect of the plasma membrane of the RPMC. Since Amiloride competes with sodium in the medium, we examined the effect of replacing sodium in the medium on the dose-response curve and histamine release. Potassium was used to replace sodium in the buffer because potassium does not influence histamine release from RPMC. Purified RPMC's were incubated in either Na^{+}-buffer or in Na^{+}-free buffer for 5 minutes at 37°C.

The reaction was terminated by adding cold buffer (5°C) to the medium and centrifuging the mixture. Then the amount of histamine in the supernatant and pellet fractions was analyzed.

The results are seen in Figure 1. A concentration-dependent histamine release was seen from mast cells stimulated by Amiloride. The Amiloride-induced histamine response is much greater in cells incubated in Na-free medium than in those incubated in medium containing Na.

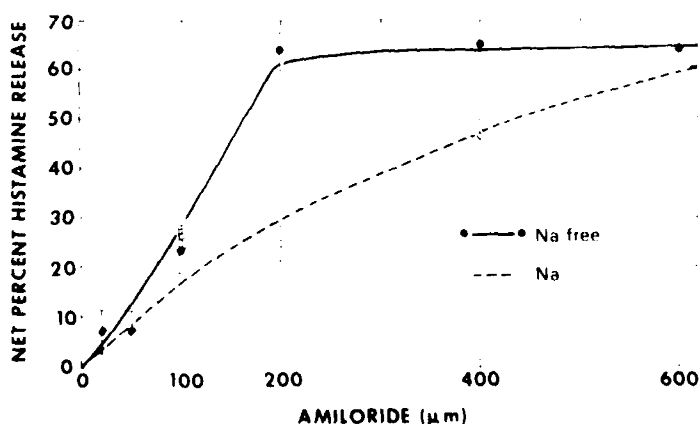

Figure 1. Effect of Amiloride on histamine release from rat peritoneal mast cells in presence and absence of buffer containing sodium. Error bars indicate standard error of mean in triplicate samples.

CHARACTERIZATION OF TWO CELLULAR CALCIUM COMPARTMENTS IN RAT PERITONEAL MAST CELLS

Principal Investigator: M. A. Donlon, *AFRRI*

Collaborators: G. N. Catravas, *AFRRI*

M. A. Kaliner, *National Institute of Allergy and Infectious Diseases, NIH*

Technical Assistance: E. Chock, L. Barr, and C. Bland, *NIAID, NIH*

Substances released from mast cells during radiation exposure are responsible for a variety of physiological effects. One of these substances, histamine, has been implicated in the hypotensive effects observed after radiation injury. An understanding of the factors that control mast cell secretion is essential for determining how radiation interacts with the mast cell in vivo. Calcium has been proposed as a coupling agent between (a) triggering events at the mast cell surface and (b) the secretory response.

One of the difficulties in the study of calcium changes associated with cells is the avid binding of calcium to membrane components such as proteins, phospholipids, and glycoproteins. This binding complicates the interpretation of calcium ion incorporation of data derived from only radioisotope measurements. It is difficult to determine to what extent calcium association reflects the transport properties of the plasma membrane or the affinity of calcium ion for extracellular phases. We have been able to identify the calcium changes in these two cellular calcium compartments on the basis of their accessibility to the impermeant calcium ion chelator EGTA [ethyleneglycol-bis-(8-amino ethyl ether)-N,N'-tetraacetic acid].

This research characterizes the changes in the two cellular calcium compartments in the isolated, purified rat peritoneal mast cell stimulated with a variety of secretogogues. The relationship between histamine release and internal versus total calcium pools is presented in Figure 1.

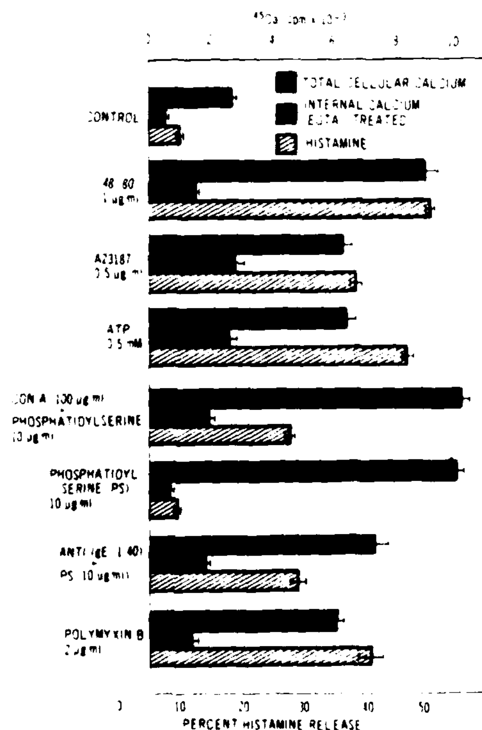

Figure 1. Effects of various secretagogues on mast cell calcium compartments and release of histamine. Both external and internal cellular calcium compartments showed increases associated with histamine release. Phosphatidylserine, an acidic phospholipid that binds calcium, caused increased calcium-45 in the external compartment without affecting histamine release. Error bars indicate standard error of mean of triplicate samples.

Rat peritoneal mast cells were incubated at 37°C in the presence of calcium-45 with a number of secretagogues [compound 48/80, A23187, Concanavalin A (Con A), anti-I_gE, and Polymyxin B].

Phosphatidyl serine (an acidic phospholipid known to augment histamine release induced by Con A and anti-I_gE) was also included in this study. The reaction was terminated by centrifugation of a portion of the cell suspension through silicone oil. The remaining portion was treated with 0.8 mM EGTA and handled in an identical manner. The supernatant was analyzed for histamine release and the calcium-45 associated with the cell pellet was determined. In each instance, significant net histamine release resulted in an increase in both total and internal cell-associated calcium. Phosphatidyl serine alone showed significant increases in the external calcium pool without altering the internal calcium pool or affecting histamine release. These data suggest that phosphatidyl serine might act by increasing the calcium pool within the cell membrane. Studies involving radiation-induced alterations in these calcium compartments during stimulation and secretion are now in progress.

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EFFECTS OF INDOMETHACIN ON LYSOSOMES, CYCLIC NUCLEOTIDES, AND PROSTAGLANDINS

Principal Investigators: G. N. Catravas and P. J. Trocha

Ionizing radiation has been shown to alter prostaglandin and cyclic nucleotide levels, to increase lysosomal enzyme activities, and to destabilize lysosomal membranes almost simultaneously (1,2). In order to determine if these changes in the lysosomal and cyclic nucleotide systems are indeed interrelated with prostaglandins, this study used the inhibitor of prostaglandin synthesis, indomethacin.

Sprague-Dawley rats (weighing 125-175 g) were exposed bilaterally to 1000 rads of gamma radiation at a dose rate of 500 rads/min. At designated intervals after irradiation and intraperitoneal injection of indomethacin (10 mg/kg), the rats were sacrificed. Spleen and liver tissues were rapidly excised and frozen with liquid nitrogen, except for portions (0.05-0.1 g) of each fresh tissue sample. The fresh spleen and liver aliquots were gently homogenized. A portion of each homogenate was centrifuged at 12,000 x g in order to obtain a supernatant free of lysosomes. The uncentrifuged tissue homogenates and the 12,000 x g supernatants were assayed for β-glucuronidase activity. Standard techniques were used to isolate and assay prostaglandin $F_{2\alpha}$ and the cyclic nucleotides cyclic adenosine-3',5'-monohosphate (cAMP) and cyclic guanosine monophosphate (cGMP) from the frozen tissue (3,4).

Indomethacin has been found to inhibit the initial increase in liver and spleen prostaglandin levels observed in rats at 3-6 hours after exposure to cobalt-60 radiation. However, an increase in spleen but not in liver prostaglandin levels at

1-10 days postirradiation was observed in irradiated rats treated with indomethacin at 70-90 min before sacrifice compared to irradiated controls not administered the drug. A significant stabilization of lysosomal membranes and reduction of lysosomal β-glucuronidase activity was observed in spleen but not in liver tissues removed from irradiated rats given indomethacin at various postirradiation intervals, compared to irradiated animals not given the drug. Indomethacin had no effect on liver cGMP levels at 1 hour to 10 days after radiation exposure, but more normal cGMP levels were found in spleen tissues taken from the treated animals at 3-10 days postirradiation. Analysis of nonirradiated animals given indomethacin showed that it had no effect on altering prostaglandin concentrations, cyclic nucleotide levels, or lysosomal membrane stability in spleen and liver, compared to rat tissues from nonirradiated controls given placebos.

The results of this investigation indicate an interaction between prostaglandin F_2 and the lysosomal cGMP systems. However, these interrelationships are different for the initial effects after radiation exposure (1-6 hours postirradiation) and the latent effects following radiation exposure (1-10 days postirradiation).

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CALCIUM ACCUMULATION AND RETENTION BY SYNAPTOSOMES IRRADIATED WITH 14.5-MeV ELECTRONS

Principal Investigators: M. J. Ely and G. N. Catravas

Exposure to ionizing radiation has been shown to alter membrane permeability in a variety of cell types. In mammalian neurons, this radiation-induced increase in passive permeability to sodium and potassium ions has been suggested as the cause of (a) partial membrane depolarization leading to increased neuronal excitability and (b) impaired restoration of polarization after an action potential (1). Considering the roles of calcium ions (Ca^{2+}) in the control of neuronal membrane potential, permeability, and stability (2), it seemed possible that supralethal doses of ionizing radiation might affect calcium disposition in nervous tissue, thereby increasing neuronal membrane excitability. Therefore, performance decrements (such as early transient incapacitation observed in laboratory animals after exposure to such large radiation doses) might be expressed as a result of this increased neuronal excitability.

In an attempt to assess the effect of ionizing radiation on neuronal membrane permeability to calcium ions, we measured accumulation and retention of calcium ions by synaptosomes (nerve-end particles prepared from homogenates of whole rat brain) after exposure to 14.5-MeV electrons from the AFRRI linear accelerator.

Irradiation of synaptosomes with doses of 50, 100, and 500 Gy did not result in significant changes in calcium accumulation when measured at 3, 7, and 17 minutes after exposure. Accumulation of potassium-stimulated calcium was likewise unaltered by doses of up to 500 Gy, as was calcium retention. Since calcium ions act to stabilize neuronal membranes by decreasing membrane permeability, we investigated the effect on calcium retention of altering the external calcium concentration of the incubation medium between 0.4 mM and 2.4 mM. Over this range of external calcium concentrations, we observed no significant changes in synaptosomal calcium retention at 3 and 5 minutes after a dose of 100 Gy.

These findings suggest that radiation-induced changes in neuronal calcium disposition, leading to increased neuronal excitability, do not play a major role in the mechanisms underlying performance decrements that occur transiently and within minutes of exposure to supralethal doses of ionizing radiation.

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HIGHLY REACTIVE IMPURITIES IN TRITON X-100 AND BRIJ 35: PARTIAL CHARACTERIZATION AND REMOVAL

Principal Investigators: Y. Ashani and G. N. Catravas

The use of non-ionic detergents, especially Triton X-100, resulted in several reports in which the observations may have been influenced by the properties of the detergent. Protein-detergent complex or selective removal of cofactors has accounted for various phenomena such as activation, inhibition, or modification associated with protein purifications (1,2).

We performed a systematic analysis of Triton X-100 (from three different suppliers) and Brij 35. It was found that they contain variable amounts of powerful oxidizing impurities representing a range of 0.04% to 0.22% H_2O_2 equivalents. These detergents also contain a considerable quantity of carbonyl compounds (0.5% to 2%) originating from carboxylic acids and ketones of aldehydes. Aqueous solutions of Triton X-100 and Brij 35 reacted readily with SH groups of protein and nonprotein molecules as well as with Fe^{++} ion. Both detergents were purified from the oxidizing impurities by treating aqueous solutions of detergent with either $NaHSO_3$ or $SnCl_2$ followed by an extraction procedure.

Our findings may clarify but also complicate the interpretation of previous studies in which these detergents were used for biologic purposes (especially for enzyme and protein purifications), or when they were used in procedures based on the formation or consumption of reducing reagents.

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COMBINED EFFECTS OF ANTICHOLINESTERASE DRUGS AND LOW-LEVEL MICROWAVE RADIATION

Principal Investigators: Y. Ashani, F. H. Henry, and G. N. Catravas

It has been previously reported that low-level microwave radiation may lead to physiologic changes in biologic systems (1,2) and possible alterations in the blood-brain barrier (3). Sublethal doses of anticholinesterase agents are known to cause hypothermia in rats. Therefore, we used this physiologic response as an indicator to study the possible interaction of two commercially available anticholinesterase drugs: phospholine iodide (which does not appreciably penetrate the blood-brain barrier) and paraoxon (which passes easily through the cellular membranes because of its solubility to lipids). Groups of rats were administered sublethal doses of phospholine iodide either 10 minutes before or after a 10-minute exposure to pulsed microwave irradiation with an average power density of 10 mW/cm^2.

As Figure 1 indicates, the hypothermia-inducing effects of phospholine iodide (body-core temperature measurements) showed a clear dose-response relationship over a dose range of 0 to 55 µg/kg. A statistically significant increase in hypothermia was seen at phospholine doses of 40 to 50 µg/kg if the animals were first administered the anticholinesterase drug and 10 minutes later exposed to pulsed microwaves (Figure 1a). However, when the animals were irradiated 10 minutes before administration of anticholinesterase, no statistically significant difference in body temperature was observed between these animals and those that had received phospholine without irradiation (Figure 1b). Statistically significant differences in body temperature were also observed between irradiated and nonirradiated rats given paraoxose (Figure 2).

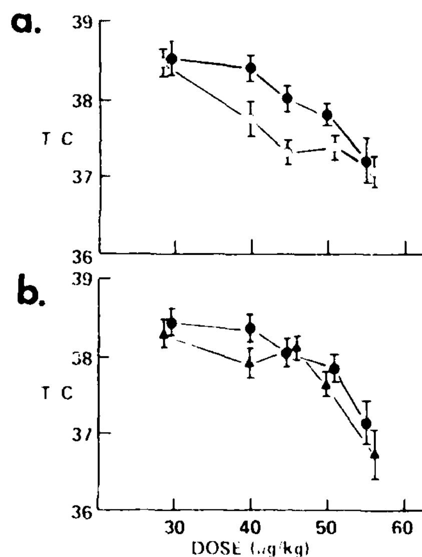

Figure 1. Temperature decrease as a function of phospholine iodide dose. (●), group that received phospholine without irradiation. ( ), group that received 10-min irradiation after phospholine administration. (▲), group that received 10-min irradiation before phospholine injection. Bars denote standard error from mean.

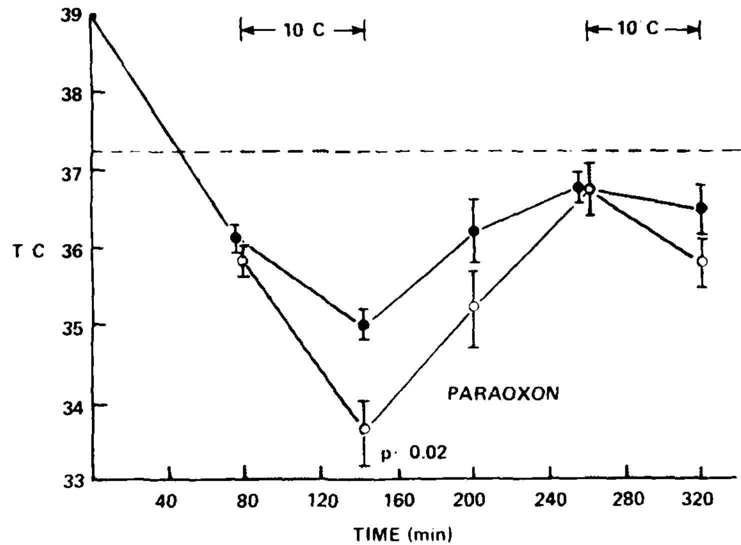

Figure 2. Hypothermia induced in rats by injection of paraoxon (200 ug/kg). (●), non-irradiated group; (○), group that received 10 mW/cm^2 irradiation for 10 min after injection of drug. Bars represent SE from mean.

Our data indicate that a reversible opening of the blood-brain barrier probably occurs when rats are exposed to microwave radiation. However, the possibility should not be excluded that other factors may have influenced the observed results.

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EXPERIMENTAL HEMATOLOGY DEPARTMENT

Exposure to ionizing radiation doses of 100-200 rads will damage or destroy bone marrow cells, resulting in the reduction of or the cessation in production of granulocytes, macrophages, and platelets, which are the first and major defense against infectious bacteria and their toxins. With increased radiation doses above 200 rads, these infections result in fatalities. Infections and fatalities can be decreased by procedures that protect bone marrow cells from these effects, or that enhance their endogenous production postirradiation, or that temporarily supply functional granulocytes until the recovery of radiation-damaged bone marrow. In addition, successful treatment is promoted by means to prevent the invasion of intestinal gram-negative bacteria into other tissues and organs of irradiated persons or at least to reduce the concentration of those bacteria. Successful treatment would permit (a) the exposure of persons to higher radiation doses if demanded by extreme military situations and (b) the use of enhanced nuclear weapons, since treatment would raise the radiation dose that causes 5% fatalities. Animal studies are also conducted to develop bases for (a) therapy for damage from exposures to higher radiation doses that do not completely destroy bone marrow stem cells and (b) replacement of bone marrow. In addition, one important project deals with the complex effects of multiple injuries, and another project deals with the possible late effects in survivors of low doses of radiation.

The Departmental program is divided into six project groups, each researching a specific area.

PROJECT GROUP 1: Stem Cell Physiology and Enhancement of White Cell Production Postirradiation

These projects are intended to elucidate (a) the mechanisms of normal stem cell renewal and differentiation, (b) the interaction of humoral substances released by functional white blood cells (lymphocytes and/or macrophages) in loci of inflammation and infection, and (c) the primitive precursor cells for increased production of adult functional cells. Of particular interest is the task of learning how to manipulate the radiation-injured precursor system by molecular engineering in order to enhance the production of white blood cells to fight against invading bacteria and their toxins. Significant progress has been made in the determination of humoral and cellular interactions, the proliferation capabilities of normal and postirradiation stem and precursor cells, and the relative biological effectiveness of neutron irradiation of these cells.

PROJECT GROUP 2: Studies of Origin and Prevention of Infection Postirradiation

These projects deal with experimental designs to discover the possible routes of bacterial invasion postirradiation, means of preventing this occurrence, and means of increasing the defense against the bacteria and their toxins in a radiation-injured organism. Studies were completed on the effects of *Corynebacterium parvum*, and new studies were initiated to determine the relative effectiveness of bacterial lipopolysaccharide (endotoxin) and yeast cell wall preparations (glucan) on the stimulation of the hematopoietic system and the nonspecific resistance to infection. Mutant mice that are resistant to endotoxin and extremely sensitive to gram-negative bacteria were introduced to help elucidate mechanisms involved in infectious disease.

PROJECT GROUP 3: Combined Injury

Military analysts have estimated that, in a future atomic war, more than 70% of the casualties will suffer certain injuries in addition to those caused by ionizing radiation. The greater percentage of those injuries will be wounds or burns. German and Russian studies with mice or dogs indicate that the presence of open wounds after irradiation will increase the number of fatalities whereas the immediate suturing of open wounds will not. Unfortunately, because of septic conditions, surgeons usually postpone the suturing of wounds in military field conditions. Since the hematopoietic system is involved in the healing of wounds, it is important to study that system's functional status in the irradiated animals. Studies to date point out that the incidence of survival is affected by the size of wounds and by the timing of trauma in relation to the exposure to radiation. It has been shown that wound-trauma before irradiation provokes an earlier and greater increase in clonogenic cells than in irradiated mice.

PROJECT GROUP 4: Physiological Assessment of Fresh and Cryopreserved Granulocytes and Macrophages Used for Postirradiation Transfusion

Bone marrow exposed to radiation doses of 350-500 rads still has the capability of recovering if the animal or human does not die from infection. The best treatment is the infusion of compatible granulocytes. Methodology for the isolation of granulocytes by counterflow centrifugation-elutriation (CCE) was continued and improved, and the separation of whole bone marrow cells was initiated. We demonstrated that the principles of CCE can be extended to an enlarged separation chamber for the isolation of therapeutic numbers of highly purified granulocytes and for animal-model transfusion studies with nucleated bone marrow cells. We also showed that CCE-isolated canine granulocytes displayed differential in vitro and in vivo efficacy in physiological function as a result of the dual leukapheresis, CCE-isolation, and storage/freezing procedures.

PROJECT GROUP 5: Transplantation of Bone Marrow Cells Into Lethally Irradiated Animals

Once radiation completely destroys the bone marrow, no endogenous recovery is possible. In such a case, the transplantation of bone marrow between genetically identical persons is the only means of treatment and recovery. However, genetically identical cells usually are not available (with the exception of those from identical twins), and the transplantation of incompatible bone marrow results in death. A study was conducted to assess the capability of compatible bone marrow to rescue mice from an LD_{50} dose (lethal dose for 50% of subjects) of neutron or gamma radiation. We determined that a significantly greater concentration of bone marrow stem cells was needed to rescue mice after neutron irradiation than after gamma irradiation.

PROJECT GROUP 6: Late Effects of Ionizing Radiation

In recent years, the question has been raised as to whether military personnel exposed to very low doses of radiation may show an increase of degenerative diseases years later. To obtain greater insight into this phenomenon, the studies in this project group were initiated.

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ENHANCED REPAIR OF RADIATION DAMAGE AND INCREASED RADIORESISTANCE OF HEMOPOIETIC STEM CELLS DURING ACTIVE CELL PROLIFERATION

Principal Investigator: M. P. Hagan
Technical Assistance: R. T. Brandenburg and J. L. Atkinson

Using continuous BrdUrd infusion in vivo and near-UV light irradiation in vitro (1), we have measured the time-integrated S-phase fraction of CFU_S derived from murine bone marrow (2). These measurements indicate a stochastic commitment to cycle of essentially the entire population of CFU_S, consistent with the existence of a noncycling cell-state G_0. The data indicate that for B6D2F1 female mice, the CFU_S are committed to DNA synthesis approximately once every 52 hours. Additionally, measurements of other CFU_S parameters showed no significant effects of the BrdUrd infusion. Hydroxyurea challenge of BrdUrd-infused mice indicates that the capacity of the CFU_S to respond to a proliferative stimulus during the infusion period is also unaffected by the BrdUrd (Figure 1).

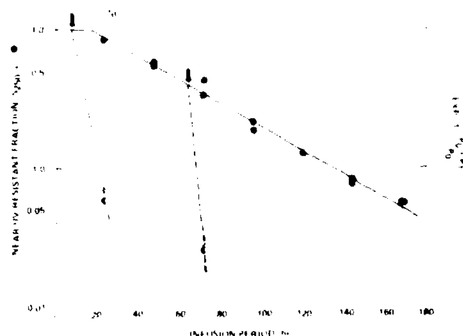

Figure 1. Fraction of CFU_S surviving a fluence of 250 J m^{-2} ($S_{250,t}$) is shown as a function of length of BrdUrd infusion period. Near-UV-resistant fraction, P_0, is also shown expressed in terms of P_0 and $P_0 + P_1$. Partially closed circles show survival values (±SE) of CFU_S from animals injected with *hydroxyurea* at time indicated by arrow. Symbol t_d represents dead time for assay.

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HEMATOPOIETIC RECOVERY IN PREGNANT MOUSE AFTER LOW-DOSE IRRADIATION

Principal Investigators: S. J. Baum, S. R. Weinberg, E. G. McCarthy, and T. J. MacVittie

Radiation injury to marrow and spleen tissue cellularity and hemopoietic stem cells following low-dose total-body irradiation (TBI) were studied in nonirradiated-virgin female mice, irradiated-virgin female mice, nonirradiated pregnant mice, and irradiated pregnant mice. Experimental mice at 10.5 days of pregnancy and virgin females received a total-body irradiation exposure of 0, 50, 100, 150, 200, or 300 rads of cobalt-60, gamma rays, at a rate of 40 rads per minute, and were sacrificed for study 4 days after irradiation. Plasma clot cultures were used to assess erythroid burst-forming cell (BFU-E) and erythroid colony-forming cell (CFU-E) activity. Double-layer agar cultures were established to evaluate granulocyte-macrophage colony-forming cell (GM-CFC) and monocyte-macrophage colony-forming cell (M-CFC) activity.

Fifty rads diminished marrow cellularity recovery of irradiated pregnant mice to 40% of nonirradiated pregnant mice, while an opposite enhancing effect was seen with irradiated virgin females. Spleen cellularity of both nonirradiated and irradiated pregnant mice was always 60% greater than nonirradiated and irradiated virgin females. Nonirradiated virgin-female marrow CFU-E and BFU-E were were greater compared to nonirradiated pregnant mice, reflecting the anemia caused by pregnancy. However, recovery of CFU-E per femur was depressed in both irradiated virgin females and irradiated pregnant mice following 100 rads and higher doses. Nonirradiated pregnant-mouse spleens were erythropoietically more active compared to nonirradiated virgin-female spleens. Although irradiated pregnant-mouse spleen CFU-E recovered rapidly after 50 rads, increasing total-body irradiation dose decreased levels of irradiated virgin females to below levels of nonirradiated females. After 50 rads, irradiated virgin females and irradiated pregnant mice were unable to recover marrow BFU-E to nonirradiated virgin-female and nonirradiated pregnant-mouse levels. BFU-E per spleen in irradiated pregnant mice recovered faster following 100 rads, whereas irradiated virgin-female values were below nonirradiated virgin-female values. Nonirradiated pregnant mice had higher marrow and spleen GM-CFC compared to nonirradiated virgin females. There was a reduced ability for marrow and spleen GM-CFC recovery of irradiated virgin females commencing with 50 rads.

The effect of pregnancy on M-CFC was different from that of GM-CFC and CFU-E. Total-body irradiation of 100 rads reduced marrow M-CFC recovery of irradiated pregnant mice but had no effect on spleen M-CFC of those mice. The anemia of pregnancy triggered (a) spleen hyperplasia, (b) increased spleen CFU-E and BFU-E, (c) increased marrow and spleen GM-CFC, and (d) decreased spleen M-CFC. Four days after 50 rads, femoral cellularity, erythropoiesis, and granulopoiesis of irradiated pregnant mice were still below those of nonirradiated pregnant mice. However, spleens of nonirradiated pregnant mice and irradiated pregnant mice appear to provide the increased compensatory hemopoiesis, with greater CFU-E and BFU-E recovery activity compared to irradiated virgin-female mice. Both erythroid burst-forming cell activity and monocyte-macrophage colony-forming cell activity of irradiated pregnant-mouse spleen were more radioresistant than BFU-E and M-CFC of the marrow of irradiated pregnant mice.

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EFFECT OF *IN UTERO* LOW-DOSE RADIATION ON FETAL LIVER HEMATOPOIESIS

Principal Investigators: S. R. Weinberg, E. G. McCarthy, T. J. MacVittie, and S. J. Baum

The influence of in utero low-dose ionizing radiation exposure on murine hematopoietic embryogenesis was investigated. In vitro assays such as plasma clot cultures and double-layer soft agar cultures served as sensitive biodosimeters to determine erythropoietic and granulopoietic injuries. Day-10.5 pregnant mice were irradiated with 0, 50, 100, 150, 200, or 300 rads of cobalt-60, gamma rays, at a rate of 40 rads per min, and day-14.5 fetal livers were studied for erythroid colony-forming cell, erythroid burst-forming cell, granulocyte-macrophage colony-forming cell, and macrophage-monocyte colony-forming cell activity. A decrease in day-14.5 fetal liver cellularity following 150 rads appeared to reflect a reduced ability of organ recovery. Two hundred rads diminished the recovery of erythroid colony-forming cells, 100 rads reduced the recovery of erythroid burst-forming cells and macrophage-monocyte colony-forming cells, and 50 rads decreased the potential for granulocyte-macrophage colony-forming recovery.

The difference in response between erythropoietin-dependent and erythropoietin-independent erythroid colony-forming cells strongly suggests the existence of two populations of fetal liver erythroid progenitor cells with different radiosensitivities. Our results showed that (a) fetal liver granulopoiesis is more sensitive to radiation injury than is erythropoiesis, and (b) fetal liver has greater potential for erythropoiesis recovery.

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LYMPHOMYELOPOIETIC EFFECTS OF SUBMANDIBULAR GLAND-CONDITIONED MEDIUM

Principal Investigators: D. F. Gruber and G. D. Ledney

Some military analysts have estimated that more than 70% of the casualties of a nuclear detonation would suffer combined injuries. In such a situation, it would be important to analyze the hematopoietic status of those injured persons and to take preventive steps when feasible.

Extract of submandibular glands is known to enhance wound healing (1,2) along with a number of other regulatory functions, including nerve growth factor, epidermal growth factor, granulocytosis influencing factor (3,4), and immunosuppressive actions (5). Since the hematopoietic system is intimately involved in wound healing, in this study we sought to clarify how the submandibular gland extracts and/or conditioned medium used in the treatment of the combined-injury recipient might affect the wound-healing process.

Myelopoietic Responses

Samples of pooled, cell-free medium collected from cultures of male mouse submandibular gland were examined for colony-stimulating capability (Table 1). Culture medium for submandibular gland (not shown), which contains fetal calf serum, RPMI-1640, and antibiotics as well as RPMI-1640 alone, did not stimulate clonal growth. Similar volumes (0.1 ml) of pregnant mouse uterine extract (PMUE) and submandibular gland-conditioned medium (SMG-CM) both demonstrated colony-stimulating activity of almost equal magnitude (14.65 versus 14.25). Microscopic evaluation of the SMG-CM- and PMUE-stimulated colonies demonstrated morphologic differences. SMG-CM appeared to promulgate only diffuse type colonies. In contrast, PMUE promoted mostly compact colonies, in a ratio of 6:1 with diffuse colonies. Another characteristic of the SMG-CM-stimulated cultures was their propensity to exhibit a decrease in total colony numbers between days 10 and 22 whereas PMUE-stimulated cultures increased twofold or more (Table 1).

Table 1. Clonal Growth of Bone Marrow Cells Grown in Submandibular Gland-Conditioned Medium (SMG-CM)[a,b]

Replicate[c]	SMG-CM		PMUE	
	Day 10 CFU-C	Day 22 M-CFC	Day 10 CFU-C	Day 22 M-CFC
1	14.2 ± 0.5	0[d]	14.5 ± 1.4	28.3 ± 0.9
2	14.3 ± 2.2	0[d]	14.8 ± 0.9	34.5 ± 1.9

[a]Volume of stimulating material(s), 0.1 ml.
[b]Counts are expressed as mean number of colonies/2.5×10^4 normal bone marrow cells (± S.E.M.).
[c]Minimum of 6 plates per group per replicate.
[d]Decrease noted in the number of day-22 colonies compared to day 10.

Lymphopoietic Responses

Splenic proliferative responses in the presence of SMG-CM consistently resulted in decreased [^{3}H]TdR uptake and decreased stimulation ratios (Table 2) in all mitogenic experimental groups assayed. The phytohemagglutinin-respondent populations were suppressed from normal response levels by 40%. The stimulation ratio indicates that addition of SMG-CM decreased the phytohemagglutinin-induced [^{3}H]TdR uptake by a factor of 2.53. Enhancement-suppression indices showed significant suppression.

Table 2. Counts Per Minute (cpm)[a], Stimulation Ratios (SR)[b], and Enhancement-Suppression (E/S)[c] Ratios of Mitogen-Stimulated Spleen Cells Incubated in SMG-CM

Treatments	No SMG-CM		50 µl SMG-CM			
Mitogen	cpm ± S.E.M.	SR	cpm ± S.E.M.	SR	E/S ratio[d]	Mean E/S ± S.E.M.
PHA (0.5 µg)	198108 ± 8212		120879 ± 6182		0.61	
	190582 ± 1962	81.71 ± 5.5	110509 ± 2665	32.42 ± 2.6	0.58	0.61 ± 0.01
	167822 ± 9649		105033 ± 10664		0.63	
CON-A (0.5 µg)	168043 ± 4481		122923 ± 8636		0.73	
	270130 ± 5955	106.31 ± 14.8	175223 ± 6072	45.47 ± 3.8	0.65	0.66 ± 0.04
	292391 ± 2230		179273 ± 3653		0.61	
LPS (0.5 µg)	59106 ± 1563		22093 ± 1423		0.37	
	48089 ± 1334	22.56 ± 2.5	18003 ± 414	5.44 ± 0.7	0.37	0.36 ± 0.01
	45981 ± 1500		16139 ± 1287		0.35	
Unstimulated	2149 ± 148		3208 ± 437		1.5	
	2405 ± 229		3758 ± 448		1.6	1.55 ± 0.05
	2277 ± 128		3483 ± 275		1.5	

[a]Based on [^{3}H]TdR uptake after 48 h of incubation.

[b]$SR = \frac{\text{Mean cpm of mitogen-stimulated cells}}{\text{Mean cpm of unstimulated cells}}$

[c]$E/S = \frac{\text{Mean cpm with SMG-CM}}{\text{Mean cpm with no SMG-CM}}$

[d]E/S ratios: >1.00 = enhancement; <1.00 = suppression

The Concanavalin-A-responsive populations were similarly suppressed but to a slightly lesser degree: 34% of normal values. The stimulation ratio again showed that addition of SMG-CM resulted in a 2.34-fold decrease in [^{3}H]TdR uptake. The Concanavalin-A enhancement-suppression indices compared to the phytohemagglutinin-stimulated population showed lesser but significant suppression.

The effects on the lipopolysaccharide-respondent population were suppressed by the greatest degree (64%) by the co-administration of SMG-CM. The stimulation ratio was concurrently depressed by a factor of 4.17--almost twofold greater than that seen in the phytohemagglutinin or Concanavalin-A groups.

These data support the hypothesis that the submandibular gland is a source of hematopoietic and immunoregulatory factors. Conditioned medium from the submandibular gland is found to be a source of colony-stimulating factor that induces myelopoietic response. The same conditioned medium suppresses the lymphopoietic response(s).

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ALTERATIONS INDUCED IN MACROPHAGE AND GRANULOCYTE-MACROPHAGE COLONY-FORMING CELLS BY A SINGLE INJECTION OF MICE WITH *CORYNEBACTERIUM PARVUM*

Principal Investigator: T. J. MacVittie
Technical Assistance: E. G. McCarthy, R. T. Brandenburg, J. L. Atkinson, and M. A. Brew

Corynebacterium parvum has been shown to be a nonspecific stimulator of the host reticuloendothelial and immune systems. Considerable evidence further implicated the monocyte-macrophage as target cells for mediation of the C. parvum antitumor effects. Recent detection of a colony-forming cell specific for monocyte-macrophage (M-CFC) production in the hematopoietic, lymphoid, and nonhematopoietic organs of the mouse prompted us to examine the alterations in this cell population(s) relative to those induced within the granulocyte-macrophage populations (CFU-c) by a single injection of C. parvum.

Marked changes were observed within both the CFU-c (Figure 1) and M-CFC (Figure 2) populations in all the organs assayed. A differential effect was noted within the marrow where CFU-c content gradually increased to values 200% of control while M-CFC content decreased to values 25% of control within 2 weeks following injection of C. parvum. Both CFU-c and M-CFC were mobilized into the circulation within 3 days following C. parvum. Absolute values of circulating M-CFC were approximately fiftyfold that of CFU-c per ml of blood, which indicated the marked potential of M-CFC for seeding the extramedullary mononuclear phagocyte system (MPS). The CFU-c content in the spleen increased significantly within 4 days while M-CFC rose significantly not only in the spleen but also in thymus and lymph node tissue. Absolute increases in M-CFC measured in these organs showed an approximate fivefold advantage over the content of CFU-c (Table 1). The M-CFC appeared to reside within and were capable of migrating throughout the MPS. These results implied that the proliferation and amplification of this large and widely disseminated macrophage precursor population would provide for a maximal increase in potential macrophage effector cells in animals bearing tumors.

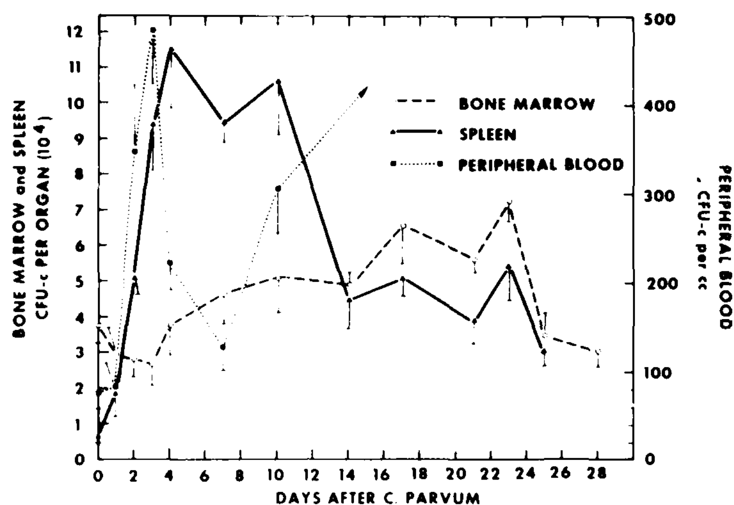

Figure 1. Alterations in total number of CFU-c per femur, spleen, and per ml of peripheral blood following a single injection of *C. parvum* (CN 6134). Values are means ± SEM of four replicate experiments.

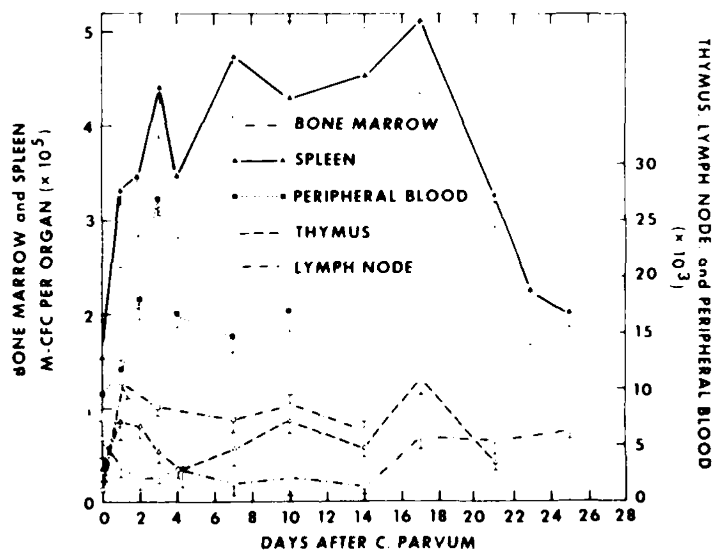

Figure 2. Alterations in total number of M-CFC per femur, spleen, thymus, cervical lymph nodes, and per ml of peripheral blood following a single injection of *C. parvum* (CN 6134). Values are means ± SEM of four replicate experiments.

Table 1. Average Relative and Absolute Values[a] of CFU-c and M-CFC Per Organ Over the Peak Response of Mice Injected With Saline or *Corynebacterium parvum (C.p.)*

	Average Relative Number per 1×10^5 Nucleated Cells per Organ					
	BM	SPL	PBMC	T	LNc	
CFU-c Saline	184[b]	4	3	—	—	
CFU-c *C.p.*	420	50	10	—	—	
M-CFC Saline	326	102	276	2	3	
M-CFC *C.p.*	300	120	500	10	25	
	Absolute Number per Organ[b]					
	BM	SPL	PBMC	T	LNc	Extramedullary[c]
CFU-c Saline	38	6.5	0.07	—	—	6.5
CFU-c *C.p.*	65	110.0	0.50	—	—	110.0
M-CFC Saline	62	150	9	2	1	162
M-CFC *C.p.*	70	475	27	6	8	516

[a] *C. parvum* values are the means of the values averaged over the time period of peak response to *C. parvum*.

[b] Organ values are $\times 10^3$.

[c] Extramedullary values equal SPL + PBMC + T + LN.

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HEMATOLOGIC RESPONSES INDUCED BY ENDOTOXIN IN NORMAL AND ENDOTOXIN-TOLERANT DOGS

Principal Investigators: T. J. MacVittie and R. I. Walker
Technical Assistance: R. Brandenburg, J. Atkinson, and E. McCarthy

Tolerance to the release of colony-stimulating activity (CSA) following injection of endotoxin was induced in beagle dogs by repeated, daily intravenous injections of endotoxin for 5 consecutive days. Several hematologic parameters were studied during the induction of tolerance and after a single challenge dose of endotoxin at 10 days and 60 days posttolerance. We studied circulating levels of CSA (Figure 1), granulopoietic activity of progenitor cells derived from marrow and peripheral blood as assayed by in vitro culture and in vivo diffusion chamber (DC) techniques, marrow M:E, and peripheral blood leukc te and platelet levels.

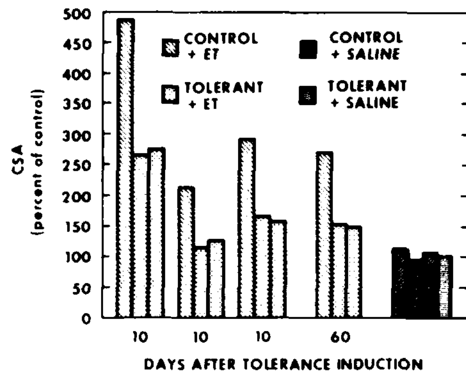

Figure 1. Plasma CSA expressed as percentage of mean control values (± SEM). CSA was assayed 6 hours after endotoxin (ET) or saline injection into dogs made ET tolerant and challenged 10 days and 60 days later. Six tolerant dogs were challenged 10 days after tolerance induction, and two dogs were challenged 60 days after tolerance induction.

The induction of CSA tolerance in the canine system was characterized by a marked increase in marrow granulopoiesis in association with a significantly diminished CSA response (Figure 1), both during induction of tolerance and through at least 10 days (but not 60 days) after induction of tolerance. Tolerant dogs responded to challenge doses of endotoxin with marked increases in marrow granulopoiesis (Figure 2), release of DC progenitors into the peripheral circulation, and more rapid return of peripheral leukocytes and platelets to normal levels. Although marrow granulopoiesis in tolerant dogs had returned to within normal values by 60 days after tolerance, their granulopoietic response to a challenge dose was significantly more rapid than that of normal dogs or tolerant dogs challenged at 10 days despite the reduced CSA (Figure 2). Increased

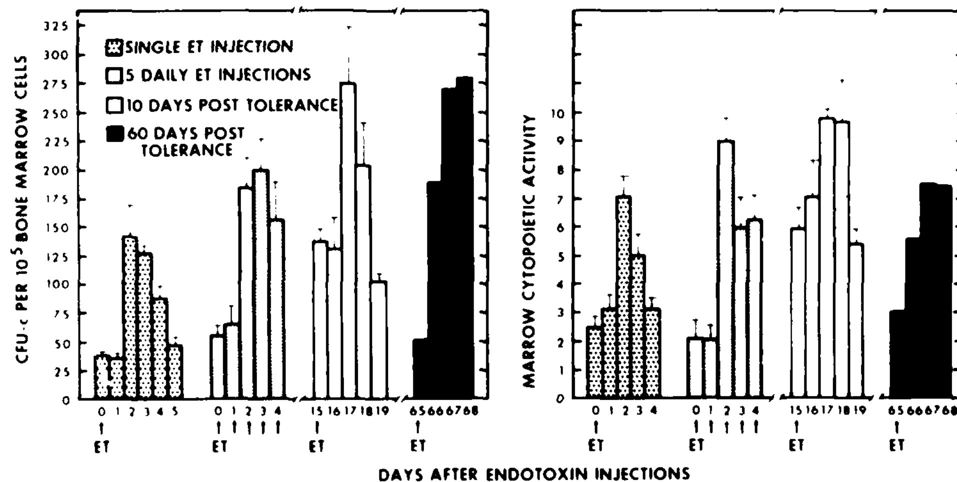

Figure 2a and b. Alterations in (a) concentration of bone marrow-derived $CFU_C/10_5$ nucleated cells and (b) cytopoietic activity of marrow-derived cells grown in diffusion chambers after endotoxin (ET) injections at times shown. Mean values are from six replicate (±SEM) experiments (one dog each) for the single ET injections and the 10-day posttolerance ET challenge. Three dogs were used for the five daily ET injections, and two dogs were used for the 60-day posttolerance ET challenge.

capability to mobilize leukocytes can increase resistance to the biological effects of endotoxin. One function of leukocytes is to reduce platelet aggregation in the microcirculation, thereby limiting the subsequent release of potentially deleterious mediators. Further knowledge of the hematologic parameters responsive to endotoxin tolerance will aid in elucidating the mechanism of increased resistance to endotoxin challenge.

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ACTIVE OXYGEN-METABOLIZING ENZYMES AND ENDOTOXIC SHOCK IN MICE

Principal Investigator: B. Gray

Mice challenged with Salmonella typhosa lipopolysaccharide W 0901 (endotoxin, Difco Laboratories) at sufficiently high doses undergo physiological stress and death (1). It has been hypothesized that irradiation leads to leakage of bacteria and endotoxin from the mammalian gut, causing stress. Female B6CBF1 mice at least 10 weeks old and weighing about 25 grams each were housed in a colony where they were allowed a standard laboratory diet and chlorinated water ad libitum. These mice were given 0.25 ml tail vein injections of solutions of superoxide dismutase (SOD) (Sigma, bovine blood, type I, 2900 units/mg) prepared by dissolving the enzyme in pyrogen-free sterile saline (Cutter Laboratories). The same mice were then injected intraperitoneally with 0.25 ml of an endotoxin solution made with pyrogen-free sterile saline. Table 1 shows that a higher percentage of mice given prophylactic injections of up to 8 mg SOD per mouse survived than did controls injected with sterile saline. Also, no mortality occurred in control mice injected with up to 8 mg SOD and sterile saline without endotoxin.

Table 1 Thirty-Day Survival of Mice

Endotoxin dose IP	Sterile saline IV	SOD 1 mg IV
500 μg	6/33 (18%)	17/33 (51%)
600 μg	3/21 (14%)	4/14 (29%)
700 μg	3/25 (12%)	7/25 (28%)

Additional mice were challenged with 250 μg of endotoxin; their lungs were removed, rinsed twice, and homogenized in 0.05 M sodium phosphate buffer (pH 7.8) at a 1:10 (w/v) ratio. Five thousand g max supernatants were assayed for SOD activity using the epinephrine autoxidation method. In addition, catalase and glutathione peroxidase were assayed in extracts. Over 95% of the SOD activity was in the original supernatant generated by centrifugation. Also, heat-stable factors removable by dialysis against 0.05 M sodium phosphate buffer (pH 7.8)

reduced apparent SOD activity of supernatants by about 20%. Figure 1 shows that endotoxin-treated mice had lung SOD levels that dropped to 60% of controls at 1 hour after injection, followed by recovery after 24 hours. Catalase and glutathione peroxidase levels did not change nearly as much. SOD injected into mice may alleviate the depression in endogenous SOD observed when challenged by endotoxin.

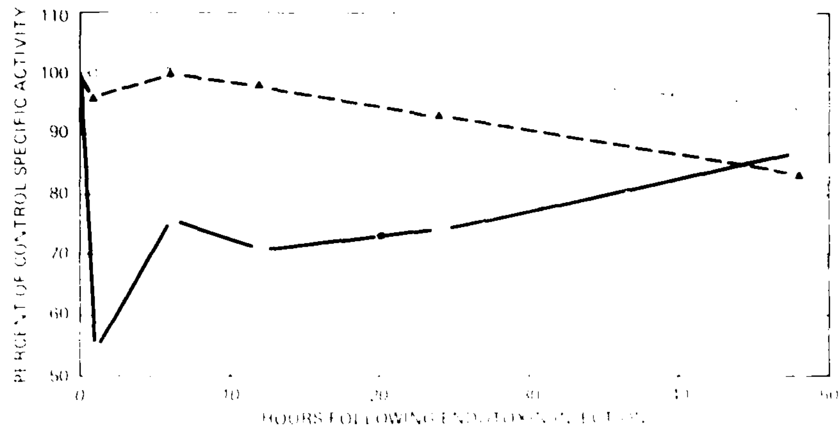

Figure 1. Enzyme-specific activity expressed as percent of control specific activity for mouse lung supernatant fluids. Five mice were used to prepare supernatants at each time point. Value for control specific activity for each enzyme was average of specific activities determined on each group of controls.

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WOUND TRAUMA-INDUCED SURVIVAL AND MYELOPOIETIC RECOVERY IN MICE EXPOSED TO LETHAL WHOLE-BODY RADIATION

Principal Investigators G. D. Ledney, D. A. Stewart, E. D. Exum, and P. A. Sheehy

Wound trauma, if given before lethal doses of radiation, may physiologically disturb the hematopoietic system, resulting in increased survival after exposure. We tested this idea by exposing mice to varying doses of whole-body cobalt-60 radiation within the hematopoietic death range. A 4%-body-surface skin wound was placed on the anterior dorsum 24 hours before irradiation. The LD 50/30 for B6CBF1 mice wounded before irradiation was 971 rads and 820 rads for control-irradiated mice (Figure 1). Thus, the dose reduction factor (DRF) for wounding before irradiation was 1.2. As seen in Figure 1, the LD 50/30 for mice wounded

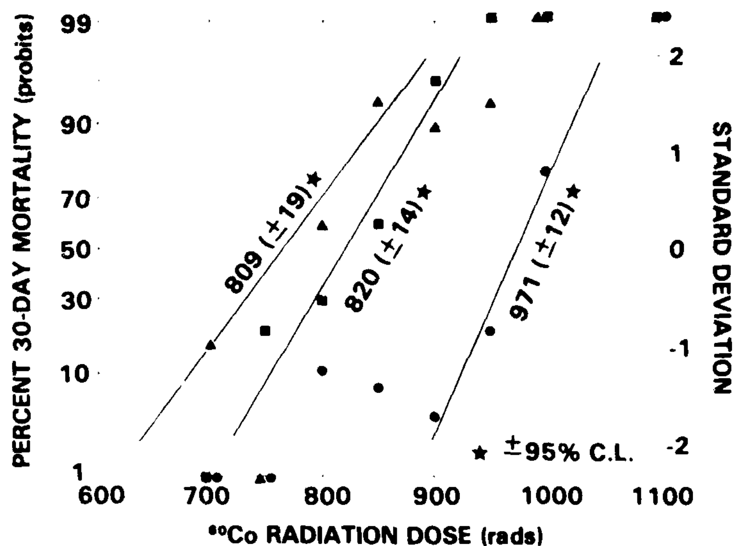

Figure 1. Mortality in skin-wounded irradiated mice. ▲, wounded 24 hours after irradiation; ■, irradiation controls; ●, wounded 24 hours before irradiation.

24 hours after irradiation was 809 rads, a value not statistically different from the control-irradiated mice.

The concentration and organ content of colony-forming units spleen (CFU-s), colony-forming units culture (CFU-c), and monocyte-macrophage colony-forming cells (M-CFC) were examined on days 3, 7, 10, and 14 after wound trauma given before 700 rads (Figures 2-4). During the times studied, the marrow clonogenic cell values for both irradiated animals and the combined injured animals never returned to control-nontreated quantities. However, wound trauma before irradiation provoked an earlier and greater increase in clonogenic cells than that seen in irradiated mice. Supranormal levels of splenic CFU-s and CFU-c were found in animals wounded before irradiation. These changes commenced sooner and reached greater levels than those detected in irradiated mice. M-CFC values, while greater for the combined injured animal than for the irradiated mouse, remained depressed throughout the time period studied. These data are important for individuals subjected to wound or surgical trauma before accidental or therapeutic radiation exposure.

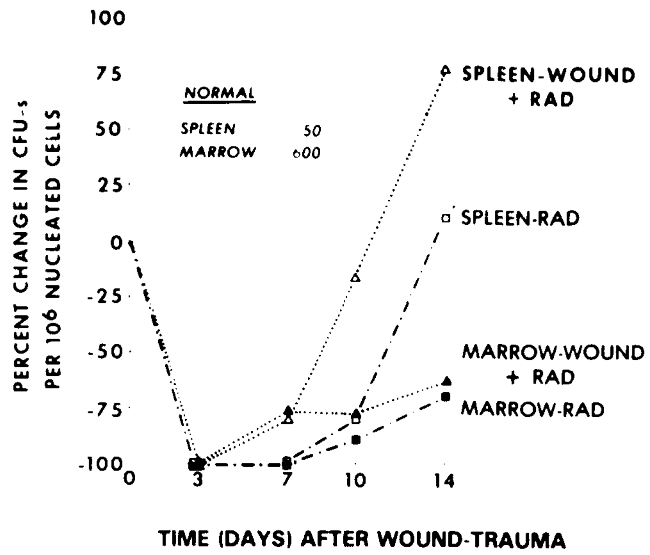

Figure 2. CFU-s in irradiated mice and in mice wounded before irradiation

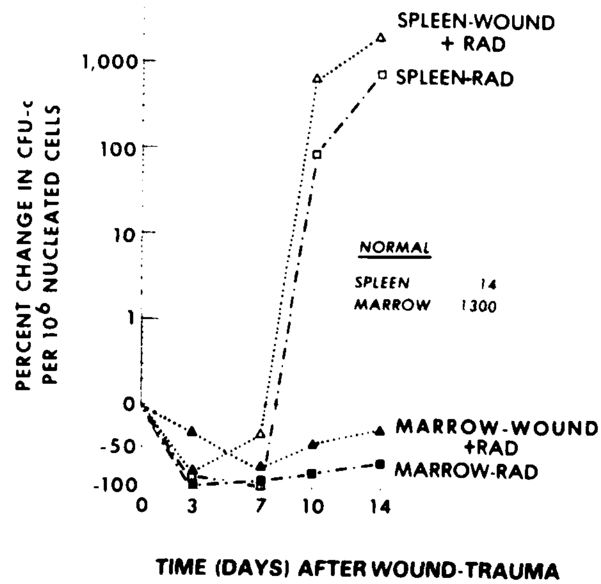

Figure 3. CFU-c in irradiated mice and in mice wounded before irradiation

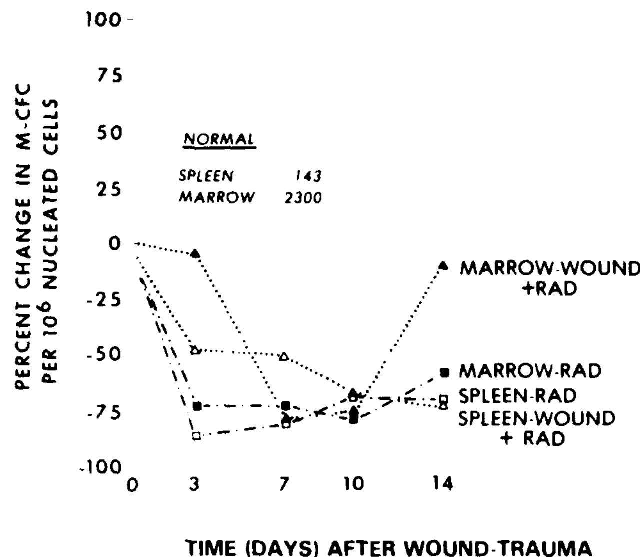

Figure 4. M-CFC in irradiated mice and in mice wounded before irradiation

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IN VIVO ANALYSIS OF PRESERVED GRANULOCYTES

Principal Investigators: J. F. Jemionek and W. H. Baker
Technical Assistance: D. A. Walden

The use of granulocyte transfusions as a beneficial adjunct to antibiotic therapy to combat sepsis in neutropenic patients as a result of chemical or radiation insult is well-documented in the literature. Standard procedures for granulocyte transfusions have used various leukapheresis procedures that yield 5 x 10^9 to 1 x 10^{10} granulocytes in addition to numerous red cells, platelets, and various numbers of monocytes and lymphocytes. The ability to store and recover cryopreserved human granulocytes would permit a more efficient use of these cells for transfusion purposes and would permit the development of blood banking protocols for granulocyte transfusion. The ability to cryopreserve a particular cell type has been enhanced if the cell type in question is the only cell population undergoing cryopreservation rather than cryopreserving a mixed population of cell types.

Using a principle called counterflow centrifugation-elutriation (CCE) (1-4), we have developed in our laboratory a rotor system and chambers that are capable of isolating 3-5 x 10^9 human granulocytes or 4-6 x 10^9 canine (animal model) granulocytes essentially free of erythrocytes, platelets, and other mononuclear leukocytes. Our data for granulocyte-rich leukapheresis concentrates isolated from canine animal models indicate that room temperature (20°C) appears to be

preferred to 6°C for short-term storage of granulocytes. The data also indicate that although the granulocytes isolated by CCE may retain in vitro functions of chemotaxis, phagocytosis, and bactericidal activity (5-7), the in vivo function of migration into skin chambers for isolated granulocytes is seriously impaired after storage for 18-24 hours at both 6°C and 20°C. This loss of in vivo function of stored granulocytes occurs in isolated granulocytes obtained by CCE or dextran sedimentation, and is not observed in the granulocyte-rich leukapheresis concentrates held at 20°C.

The results of these studies are fourfold: First, granulocytes freshly isolated by CCE display no apparent loss of either in vivo or in vitro function. Second, granulocytes isolated by CCE or by dextran sedimentation and stored at 6°C or 20°C are severely impaired in terms of their in vivo chemotactic function but display no loss of in vitro efficacy. Third, 20°C storage of granulocyte-rich leukapheresis concentrates for 18-24 hours is superior to 6°C storage. Fourth, in vitro analysis may be limited in its ability to indicate in vivo function as a measure of success in granulocyte preservation studies.

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ISOLATION OF PROGENITOR STEM CELLS FROM BONE MARROW

Principal Investigators: J. F. Jemionek and T. J. MacVittie
Technical Assistance: D. A. Walden

The use of autologous bone-marrow transplants as a method of hemopoietic reconstitution after high-dose chemotherapy or radiation therapy has been reported in the literature. The identification of granulocyte-macrophage colony-forming cells (GM-CFC) in culture is used as an indicator of progenitor cell isolation. The current physical methodologies of density gradients, unit gravity sedimentation, or cell sorters are used to isolate various cell populations (e.g., blasts, promyelocytes) from marrow. These procedures are generally limited in the numbers of cells they can isolate.

Bone marrow cells from murine, canine, primate, and human donors were fractionated by counterflow centrifugation-elutriation (CCE) using a continuous albumin gradient (1). Fractionation of 9×10^8 nucleated bone marrow cells (without removal of the erythrocytes) can be accomplished in less than 1.5 hours with 92% nucleated cell recovery, depending on the donor species (Figure 1).

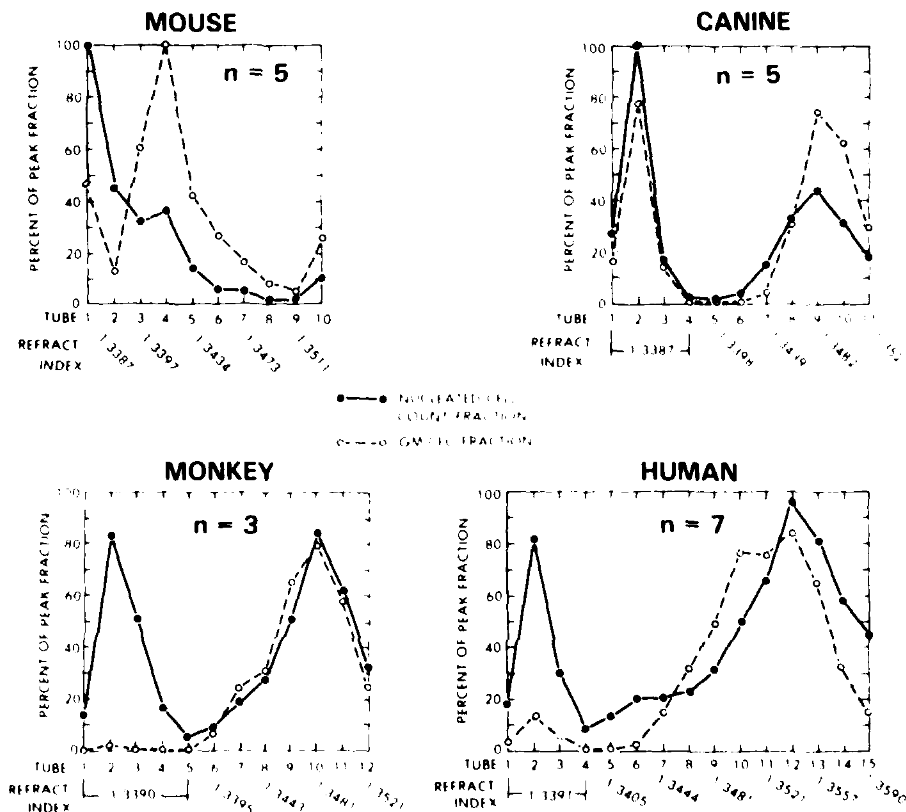

Figure 1. Total nucleated count and GM-CFC per fraction profile of density gradient elutriation of bone marrow aspirates

Murine bone marrow cell recovery after fractionation averaged 52%, whereas cell recoveries from canine, primate, and human donors averaged 85%, 92%, and 89%, respectively. The fractions were evaluated for total nucleated cell counts and GM-CFC. Each species studied presented a unique profile for nucleated cell recovery and associated GM-CFC activity. These profile variations may suggest significant differences in the modes of GM-CFC expression in the individual species that may depend on cell-to-cell interactions. Although CCE is unable to isolate a single population responsible for GM-CFC activity, CCE does permit a rapid, reproducible fractionation of large numbers of cells with minimal manipulation of sample. This makes the isolated sample ideal for further purification of progenitor cell populations.

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IN VITRO STUDIES OF LONG-TERM EFFECTS OF RADIATION DAMAGE TO HEMATOPOIETIC STEM CELL GROWTH AND DIFFERENTIATION

Principal Investigator: S. R. Weinberg
Associate Investigator: T. J. MacVittie

The methodology was established to prepare long-term liquid cultures (1) of *in vivo* irradiated mouse bone marrow and nonirradiated mouse bone marrow. These cultures were maintained for 10 to 13 weeks. Cell numbers and cell types per flask were monitored weekly as indicators of cell growth and cell differentiation. Preliminary studies with *in utero* low-dose irradiation (50-300 rads of total-body irradiation) have shown dramatic effects on the embryogenesis of the mouse hematopoietic system.

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NEUROBIOLOGY DEPARTMENT

The Neurobiology Department was dissolved and reorganized as the Physiology Department on 4 February 1980. During the reorganization, a large turnover occurred, with the loss of the Department Chairman, two Division Chiefs, and a majority of the scientific staff. A new Department Chairman was appointed in September 1980.

The objective of the newly reorganized Department is to design and direct an integrated research program toward evaluating the physiological and biophysical effects of radiation insult, either alone or in combination with other insults, on the whole animal. The Department was reorganized into three new scientific Divisions, with research goals as follows:

General Physiology: To conduct research on the physiological response of the various organ and tissue systems of the mammal (including the endocrine, gastrointestinal, and cardiovascular systems) to radiation and radiation-related injury.

Radiation Biophysics: To conduct research to determine the functional and biophysical changes that occur in membrane and cellular systems in response to radiation.

Neurophysiology: To conduct research on the physiological responses of the nervous system to ionizing radiation, including the combined effects of thermal insults, chemical insults, and ionizing radiation.

To comply with these objectives, an active recruitment program was started to find scientists with expertise in the study of the gastrointestinal, cardiovascular, and endocrine systems as well as in membrane physiology.

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CENTRAL NERVOUS SYSTEM MECHANISM OF THE MUSCLE STRETCH REFLEX

Principal Investigator: J. Wolpaw
Collaborators: D. Braitman and V. Kieffer

Development of a sensitive and quantitative system for assessing the acute and chronic effects of ionizing radiation on primate motor activity (including human) has advanced considerably in our laboratory during the past year. The electromyogram (EMG) recorded from muscle is a simple technique for determining the functional integrity of the spinal cord. Using the method of Cooke and his collaborators (1,2), we have identified various components of the muscle stretch reflex represented in EMG activity. The M1 component of the EMG reflects the monosynaptic activity of the spinal cord, and occurs within 10 milliseconds of stretching the arm. M2 activity occurs later, and reflects long loop reflexes via motor cortical pathways. We have trained rhesus monkeys on various paradigms in order to establish baseline data to determine the effects of ionizing radiation on motor performance and spinal cord activity.

Rhesus monkeys were required to maintain elbow angle at 90° ($\pm 1.5^{\circ}$) against constant extension force. Elbow angle and biceps EMGs from chronic intramuscular stainless-steel-wire electrodes were monitored by computer. If correct angle was maintained for a randomly selected period of 1-2 seconds, and if the average absolute value of biceps EMG (sampled at 10 kHz) for the final 0.5 seconds of the period fell within a preset range, a stimulus consisting of a 20-millisecond pulse of additional extension force occurred at the end of the period. The stimulus transiently extended the elbow (3°-4°), eliciting a stretch reflex. M1 was defined as the average absolute value of the EMG occurring at 12-25.5 milliseconds after stimulus onset, minus prestimulus EMG amplitude. The computer also followed stimulus-induced elbow angle change. Under the control condition, liquid reward was given 70 milliseconds after stimulus onset. Under the M1+ or M1- condition, reward was given only if M1 amplitude was greater than (M1+), or less than (M1-), a preset value.

Data were obtained from four animals over continuous recording periods of up to 3 months. The computer tabulated the number of trials, average prestimulus EMG amplitude, average M1 and M2 amplitudes, and average course of stimulus-induced elbow angle change, at hourly and daily intervals. Animals usually completed 3,000-10,000 trials per day. For each animal, EMG electrodes, prestimulus EMG amplitude, constant extension force, stimulus amplitude, and stimulus-induced change in elbow angle remained unchanged over the recording period.

Marked acute (hourly) and chronic (daily and weekly) changes occurred in average M1 amplitude independent of change in average prestimulus EMG amplitude. These preliminary studies suggested the following: (a) When animals working in constant light complete equal numbers of trials around the clock, a clear circadian variation in M1 amplitude often occurs. M1 may be up to 40% greater at midnight than at noon. (b) Under the impetus of the M1+ or M1- condition, we found that these amplitudes can be modified with appropriate training (Figure 1). (c) Without change in reward conditions, acute and chronic changes in M1 and M2 amplitudes can occur with considerable independence from each other.

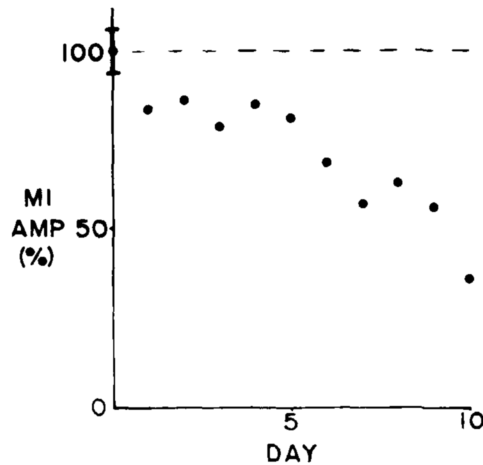

Figure 1. Daily average M1 amplitude for a single monkey for 10 days after onset of M1↓ training. M1 amplitude is given as percent of average daily M1 amplitude for the immediately preceding 7-day control period (indicated by initial point and dotted line). Brackets around initial point indicate ± 1 SD of daily M1 amplitudes for control period.

Our experiment is currently being applied to a study of radiation effects, in which a focal area of the monkey's cervical spinal cord will be irradiated using the AFRRI Linear Accelerator. This will allow us to determine the elements of synaptic function impairment, the location of the impairment, and some of the underlying mechanisms of radiation damage to the central nervous system (3).

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EXCITATORY NEUROTRANSMITTER CANDIDATES IN *IN VITRO* BRAIN SLICES OF PREPYRIFORM CORTEX

Principal Investigators: D. J. Braitman, C. R. Auker, and D. O. Carpenter, *AFRRI*
Collaborator: N. Hori, *Kyushu University, Fukuoka, Japan*
Technical Assistance: S. L. Hargett, *AFRRI*

Aspartate (Asp) and glutamate (Glu) are principal candidates for the excitatory neurotransmitter released by the lateral olfactory tract (LOT) in the prepyriform cortex in the central nervous system of the rat (1). Both are present in this region and are released when the LOT is stimulated. It has been demonstrated that ionizing radiation at levels as low as 250 roentgen causes a marked increase in the excitability of the prepyriform cortex in intact rats (2). However, it has not been shown that radiation acts directly on cells in the central nervous system or through central humoral agents.

In order to investigate this question, we have undertaken a study of the neurotransmitters in the prepyriform cortex of rat. Initial experiments have involved an attempt to identify the excitatory transmitter of the LOT in isolated slices of prepyriform cortex. This method provides, for the first time, a means of determining the direct effects of radiation on central nervous system tissue independent of humoral agents from the body. This distinction is critical for the development of protective agents and radiation antagonists to prevent incapacitation and prodromal syndromes after exposure to ionizing radiation.

In an earlier study (3), we found that bath perfusion of 2-amino-4-phosphonobutyric acid (APB), a presumed specific Glu antagonist, markedly decreased the amplitude of LOT-stimulated field potentials in rat prepyriform cortex slices. We used a similar preparation in an attempt to establish the identity of action of the LOT transmitter and either Glu or Asp. In the current study we recorded single-unit activity from the pyramidal cell layer of prepyriform cortex. We attempted to prove identity of action by blocking, with APB, both the LOT-stimulated activity and the activity evoked by ionophoretic application of various amino acids, including Asp and Glu.

Tangential slices (200-400 μ thick) of rat prepyriform cortex, including the LOT, were cut by hand and preincubated at 35°C in modified oxygenated Kreb's-Ringer solution. Individual slices were placed in a total-immersion chamber, and an electrode was driven into the pyramidal cell layer until an extracellular single-unit response evoked by LOT stimulation was recorded. A seven-barrel, glass ionophoretic electrode was then driven into the zone of termination of the LOT fibers onto the apical dendrites of the pyramidal cells (100-200 μ superficial to the recording tip). The barrels were filled with 1 M l-Glu, 1 M l-Asp, 1 M N-methyl-dl-aspartate (NMDA), 1 M dl-homocysteate (HC), 1 M l-cysteate (Cys), 10^{-2} M kainic acid (KA), or 0.5 M NaCl, each at pH 7-8.

Single units driven by LOT stimulation were also excited by ionophoretic application of all six amino acids. Bath perfusion of 10^{-3} M APB blocked the responses evoked by LOT stimulation but was ineffective in blocking the responses evoked by l-Glu, l-Asp, or l-Cys (Figure 1). However, responses to the three other excitatory amino acids (NMDA, HC, and KA, which do not naturally exist in

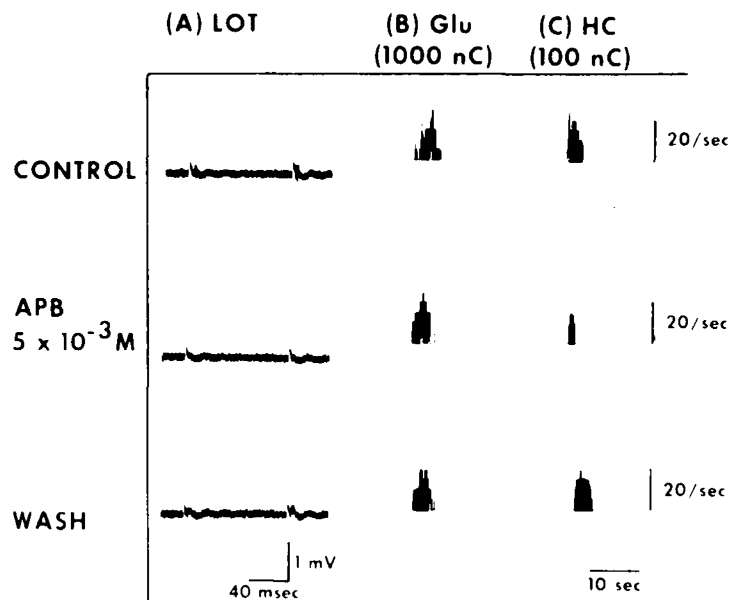

Figure 1. Comparison of effects of 2-amino-4-phosphonobutyric acid (APB) on responses of a rat prepyriform cortex to (A) lateral olfactory tract (LOT) stimulation; (B) ionophoretic application of glutamate (Glu), and (C) ionophoretic application of homocysteate (HC). (A) Single-unit responses. (B,C) Top trace of each pair is a rate meter output indicating rate of firing of single unit. Square wave on bottom trace of each pair indicates duration of ionophoretic pulse. (Amplitude of this square wave indicates only which substance is being ionophoresed.) Quality of change ionophoresed is given in nanocoulombs (nC) at top of each column. Data were recorded 4-5 min into APB perfusion period and 6-23 min into wash period. All data are from same single unit.

the brain) were markedly reduced by bath perfusion of 10^{-3} M APB (Figure 1). We tentatively concluded that (a) neither Asp nor Glu is the endogenous LOT transmitter, and (b) Glu, Asp, and Cys act at receptor sites different from those at which NMDA, HC, and KA act.

In future experiments we will examine the effects of ionizing radiation on the excitatory and inhibitory transmitter systems in prepyriform cortex.

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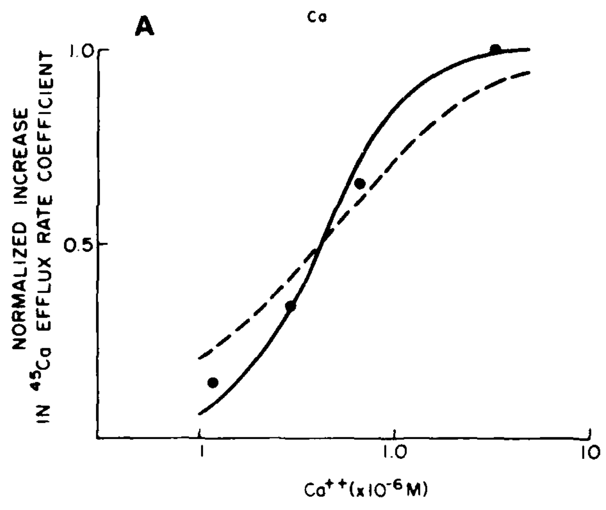
CALCIUM-45 UPTAKE AND CALCIUM-45 RELEASE BY ISOLATED *MYXICOLA* AXOPLASM

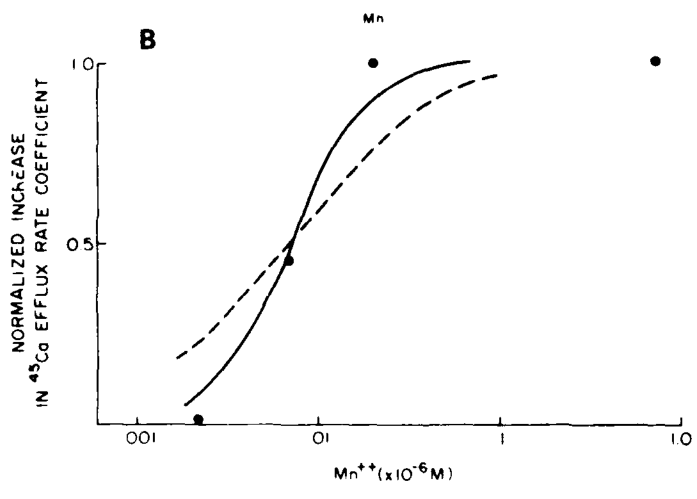
Principal Investigators: D. R. Livengood and L. M. Masukawa, *AFRRI*
R. F. Abercrombie and R. A. Sjodin, *University of Maryland School of Medicine*

Of the total calcium (Ca_T = 0.1 x 10^{-3}M) in nerve cells, only a small portion is in the ionized form (Ca_i^{2+} = 10^{-7} M). The un-ionized calcium is bound to cytoplasmic macromolecules (including proteins) and intracellular membranes, and is also sequestered within mitochondria and other organelles. Because a large relative fraction of bound Ca_T may be accessible for release, small changes in intracellular buffering may have large effects on Ca_i^{2+} and thereby on calcium-dependent processes within the cytoplasm. Studies on internal ionized calcium regulation in nerve cells suggest that although mitochondria can sequester large quantities of calcium when the ambient Ca^{2+} concentration is in the micromolar range, they do not function as the major physiological calcium buffer in the submicromolar physiological range of 0.02-0.1 μM (1,2).

Baker and Schlaepfer (3) have reported a mitochondrial Ca-binding component in Loligo and Myxicola axoplasm that requires ATP or succinate and a nonmitochondrial binding component that does not require metabolic energy. Little is known about the molecular makeup, the physiological response to calcium, or the mechanism for calcium binding of the cytosol buffers that are active in the physiological range of Ca_i^{2+}. The purpose of the present work is to examine the calcium-binding and -releasing characteristics of the nonmitochondrial components of Myxicola axoplasm.

Calcium-45 uptake and calcium-45 efflux from isolated samples of Myxicola axoplasm were studied. Calcium uptake consisted of two components: (a) an ATP-dependent uptake blocked by mitochondrial inhibitors or the calcium ionophore A23187, and (b) an energy-independent uptake unaffected by inhibitors or the ionophore. Energy-independent uptake occurred at a rate of 0.5-1.0 μM/kg-min at Ca^{2+} = 0.54 μM. The rate of this uptake varied almost linearly with the bath Ca^{2+} concentration. Calcium-45 efflux from samples preloaded with calcium-45 in the presence or absence of ATP increased when free calcium was present in the "efflux fluid." Cd or Mn also increased calcium-45 efflux (Figure 1), but the ions Co, Ni, Mg, Sr, and Ba were without effect on efflux rate coefficients. The dose-response curves representing the enhancement of the calcium-45 efflux rate coefficient by Ca, Cd, or Mn indicated that these ions acted at a very high affinity site and that some form of cooperativity was involved in their ability to release calcium-45. Similarities in the apparent ion affinities of the natural axoplasmic binding site, as judged from the dose-response curves, and in the ion affinities of EGTA (Table 1) suggest that the same type of chelating interaction occurs in both instances. Adding together the saturating concentrations of two of the divalent cations, Ca, Mn, or Cd, did not increase the calcium-45 efflux rate coefficient beyond the value shown with a saturating amount of a single divalent cation. This suggests that all three divalent cations acted at a common site.





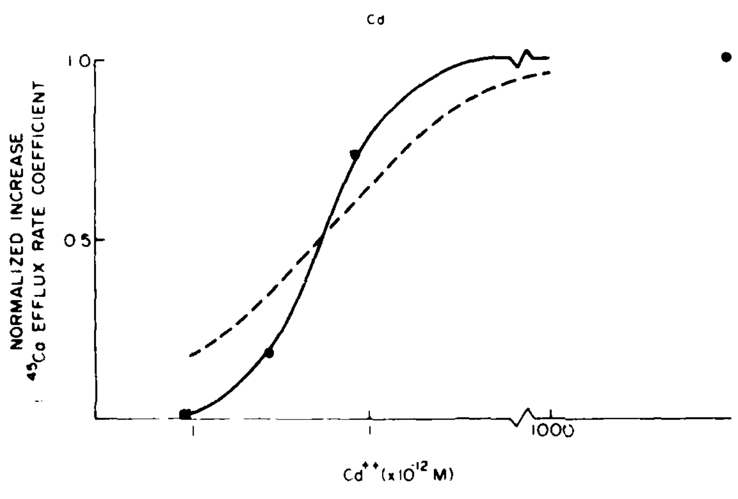


Figure 1 A-C. Concentration-response curves representing relative increase in calcium-45 release in presence of Ca^{2+}, Mn^{2+}, and Cd^{2+}. In these experiments, EGTA concentration was held constant at 100 μM as concentration of divalent was varied. Response was a steep, saturating function of free ion concentration. Solid curve is plotted according to $V = V_{max}/[1 + (K_d/M^{2+})^2]$, and the dotted curve is a plot of $V = V_{max}/[1 + (K_d/M^{2+})]$.

Table 1. Effect of Divalents on Release of Calcium-45 From *Myxicola* Axoplasm

Divalent Cation	Ligand Structure	Crystal Radius (A^{o})	Affinity for Axoplasm Site (pK_d^{site})	Affinity for EGTA* (pK_d^{EGTA})
Mg		0.66	< 2	1.1
Ni	Rutile (6 ligands)	0.72	< 5	9.5
Co		0.74	< 5	8.2
Mn		0.81	8.02	8.2
Cd		0.98	12.45	12.6
Ca	Fluorite (8 ligands)	1.00	6.42	6.9
Sr		1.14	< 5	4.4
Ba		1.32	< 5	4.2

*Calculated for pH 7.3, ionic strength 0.35 M

The dependence of the rate of calcium-45 displacement on Ca^{2+} apparently represents a specific interaction of Ca^{2+} with a major calcium-binding pool. With the present data, it cannot be determined whether this displacement reaction represents an isotopic exchange of unlabeled calcium (or calcium-like ions) for labeled calcium-45 at the axoplasmic binding site or a more complex interaction of ionized Ca^{2+} with other sites that modulate calcium-45 release. The rate of displacement is assumed to indicate the kinetics of this interaction. With this assumption in mind, the following conclusions may be drawn: (a) The calcium-interaction site has a definite size limitation for divalent cations with which it will interact. (b) The interaction between the site and divalent ions may be similar to the interaction between EGTA and these ions since the activation of calcium-45 release always occurred when EGTA was approximately half-saturated with Ca, Cd, or Mn. (c) Cooperativity may be involved in the calcium (Cd or Mn) interaction with this site.

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MACROPHAGE ELECTROPHYSIOLOGY: I. INWARD RECTIFICATION IN MOUSE MACROPHAGES

Principal Investigator: E. K. Gallin
Collaborator: D. R. Livengood

The electrical properties of cultured mouse thioglycollate-induced peritoneal macrophages were investigated using intracellular recording techniques. Thirty-five percent of the cells studied had membrane potentials ranging from -65 mV to -95 mV and exhibited S-shaped steady-state, current-voltage (I-V) relationships containing a transitional region (Figure 1, dotted line) (1). Analysis of currents in the transitional region from the rate of rise and fall of the voltage responses to current pulses indicated the presence of a negative resistance region in this area (Figure 1, dashed line). Tetrodotoxin (3×10^{-5}M), cobalt chloride (3 mM), 4-aminopyridine (4 mM), and tetraethylammonium chloride (8 mM) did not eliminate the transitional region of the I-V curves, as shown in Figure 1. On the other hand, the addition of barium chloride (4 mM) and rubidium chloride (3 mM) reduced or abolished the transitional region. Increasing the external concentration of potassium shifted the I-V relationship horizontally along the current axis but did not eliminate the transitional region (2).

These data indicate that macrophages exhibit a negative resistance region in their steady-state, current-voltage relationships. The data also support the view that the negative resistance region is produced by a voltage-dependent potassium conductance.

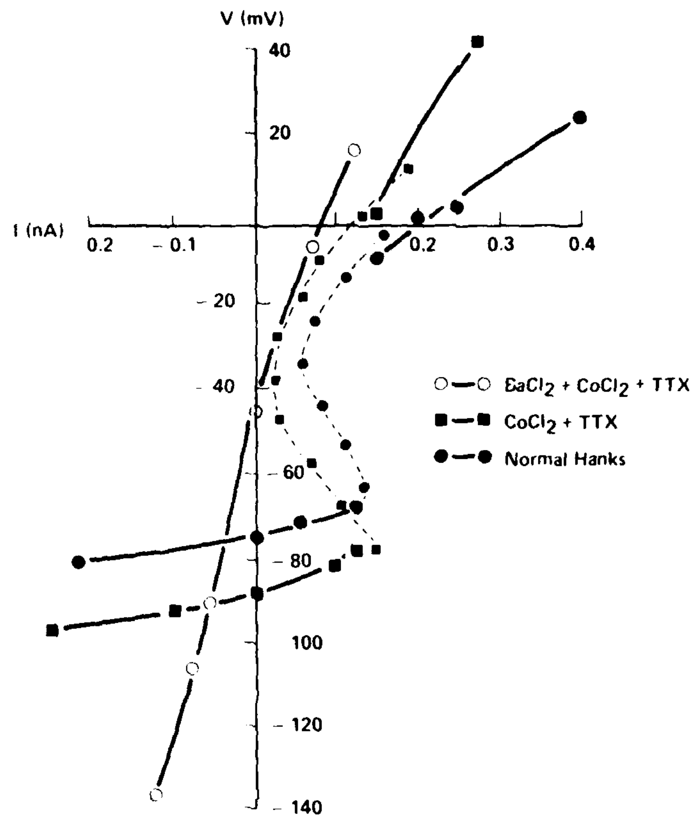

Figure 1. Effect of adding tetrodotoxin, cobalt chloride, and barium on I-V characteristics of a macrophage exhibiting an S-shaped I-V curve. Membrane potential is plotted on the ordinate,and current on the abscissa. I-V relationships before and after addition of tetrodotoxin (●), cobalt (■), and barium Cl ( ) to bath. Dotted portion of curves represents unstable region; dashed portion represents calculated currents.

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CALCIUM SPIKES IN CULTURED HUMAN RETICULAR CELLS FROM PERITONEAL EXUDATES

Principal Investigator: E. K. Gallin

Intracellular re‹ ›rdings of cultured human peritoneal exudate cells revealed that cells within the culture exhibit an active depolarizing response to injected currents that can reach positive potentials and resemble slow spikes (Figure 1).

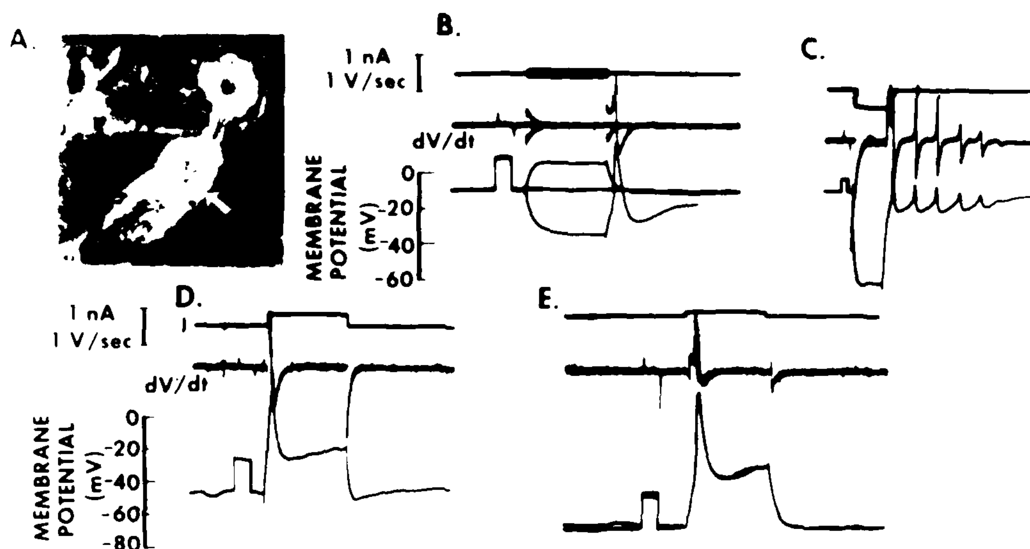

Figure 1. Photograph of cell (indicated by arrow) from which recordings were obtained (B-E) (X 330). Photograph was taken after tracing D. Diffraction pattern produced by microelectrode (in bath) is evident in left of picture. A-D: Voltage responses to injected current pulses (top tracing) are shown in bottom tracing. Middle tracing is differentiated voltage response that has been cut off in C-E. A 20-mV, 100-msec calibration pulse is superimposed on voltage responses in this and subsequent figures. Recordings B-D were made in normal Hanks. C and D are responses at resting membrane potential of -15 mV, and D at a membrane potential of -48 mV. E: Response of the cell to an outward current pulse at membrane potential of -60 mV after replacing bathing solution with choline chloride Hanks. Resting membrane potential in choline chloride Hanks was -15 mV.

The cells exhibiting spikes were similar to the reticular cells described by Stuart and Davidson (1) in that they were esterase (+) and acid phosphatase (+), and that they internalized colloidal carbon but not opsonized red blood cells.

The active depolarizing response was unaffected by either decreasing the external sodium concentration (Figure 1e) or by adding tetrodotoxin (3×10^{-5} M), whereas increasing the external calcium concentration increased both the spike amplitude and rate of rise, and the addition of cobalt (3 mM) blocked the response. Addition of barium increased the duration and amplitude of the spikes but reduced the afterhyperpolarization (2). These data indicate that cultured human reticular cells from the peritoneal cavity exhibit a calcium spike.

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EXPERIMENTAL DELAYED RADIATION NECROSIS OF THE BRAIN: EFFECT OF EARLY DEXAMETHASONE TREATMENT

Principal Investigators: A. N. Martins, R. F. Severance, and T. F. Doyle, *AFRRI*

Collaborator: J. M. Henry, *Armed Forces Institute of Pathology, Washington, DC*

These experiments were designed to detect a hitherto unrecognized interaction between high doses of the glucocorticoid dexamethasone and brain irradiation. Eighteen juvenile, male, rhesus monkeys received 1800 rads to the whole brain in 8.5 minutes. For 1.5 days before and 10.5 days after irradiation, 9 animals received 2.9 mg/kg/day of dexamethasone intramuscularly in addition to the radiation. The remaining nine animals received saline and served as the control group. After irradiation the animals were returned to their cages. They were observed and examined daily until they eventually died or until terminal inanition required that they be sacrificed. During the period of observation, at monthly intervals they were sedated and weighed, and blood was drawn for analysis. At 75, 106, and 136 days after irradiation, the fundi were examined for evidence of papilledema. Detailed notes were made daily to record each animal's appearance, response to stimuli, strength, coordination, appetite, posture, and seizure activity (if any). The experimental and control groups were compared for latency of onset of clinical signs; survival time; and number, distribution, and location of lesions of radionecrosis.

Large, biologically active doses of dexamethasone did not alter the susceptibility of the primate brain to delayed radionecrosis. These results imply that the common practice of using glucocorticoids together with radiation therapy neither protects the normal brain from the deleterious effects of ionizing radiation nor increases the risk of developing delayed radiation necrosis.

The most prevalent lesion in our histopathology samples and in those of Kemper et al. (1) was the sharply demarcated microscopic focus of necrotic tissue that had a predilection for white matter and spared the cerebral and cerebellar cortex. We observed both macroscopic and microscopic hemorrhages in the brains of all nine saline-treated animals and in eight of the dexamethasone-treated animals.

The pathogenesis of delayed radionecrosis of the brain remains the subject of continuing controversy and debate (2). One widely held view is that most of the lesions develop as the consequence of injury to blood vessels (3-5). According to this hypothesis, ionizing radiation damages stem cells that supply mature endothelial cells to replace those lost through natural attrition. This sets the stage for progressive failure of the microcirculation of the brain, leading to a break in the blood-brain barrier, chronic vasogenic edema and demylination, petechial hemorrhages, and thrombosis. The morphology of the lesions observed in this study supports this hypothesis.

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EFFECTS OF LITHIUM ON EXCITATORY RESPONSES TO SEROTONIN AND DOPAMINE IN *APLYSIA*

Principal Investigators: A. N. Williamson, T. C. Pellmar, and D. O. Carpenter

Aplysia californica neurons can exhibit ionic conductance increases similar to those of several neurotransmitters. This has led to the suggestion that there are common ionophores for each class of ionic response (1). If the ionophores mediating the fast sodium conductance increases in response to serotonin and dopamine are identical, then these ionophores should respond similarly to sodium substitutes. Since a different ionophore probably underlies the slow sodium conductance increase to serotonin [the A' response of Gerschenfeld and Paupardin-Tritsch (2)], sodium substitutes might act differently on this response.

We tested the effects of substituting for 50% and 100% of the sodium in artificial seawater with lithium (Li-seawater) on the excitatory responses to iontophoretically applied serotonin and dopamine. Preliminary results suggest that the slow excitatory response to serotonin is reduced in amplitude and prolonged by 50% Li-seawater. There is a further reduction of amplitude in 100% Li-seawater, but the response was not completely abolished. The reduction in amplitude was partially reversible. The fast conductance increases due to serotonin and dopamine were also reduced in amplitude at both lithium concentrations (Figure 1). Those responses were not prolonged by lithium. Normal seawater partially reversed the reduction in amplitude of the responses to both neurotransmitters.

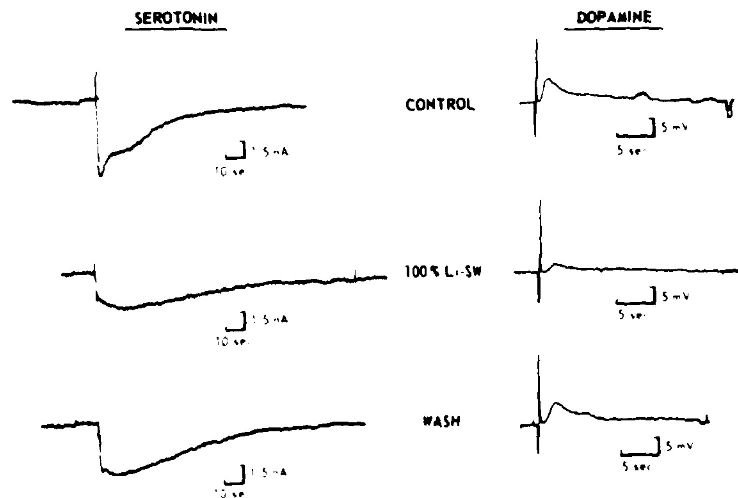

Figure 1. Responses to ionophoretic application of dopamine and serotonin in 100% artificial seawater with lithium (Li-SW)

The observation that lithium affects the fast excitatory responses to dopamine and serotonin in a similar manner supports the hypothesis that they are medidated by the same ionophore. Since lithium acts differently on the slow excitatory response to serotonin, a different ionophore may be involved.

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INVOLVEMENT OF CYCLIC AMP IN VOLTAGE-DEPENDENT CALCIUM CURRENT ELICITED BY SEROTONIN

Principal Investigator: T. C. Pellmar

Intracellular pressure injection of cyclic AMP *induces a slow voltage-dependent,* inward current at depolarized potentials in LB and RB neurons of Aplysia californica (Figure 1). Two other methods of increasing cellular cyclic AMP (phosphodiesterase inhibitors and sodium fluoride activation of adenylate cyclase) also induce an inward current at depolarized potentials. The time course, voltage dependence, and ionic sensitivity of the response to cyclic AMP are nearly identical to those of the voltage-dependent calcium current induced by serotonin in the same neurons. The response to cyclic AMP is unaffected by changes in extracellular concentration of chloride or potassium. The current is slowly but minimally reduced by a sodium-free solution. The calcium channel blocker cadmium blocks the current elicited by injection of cyclic AMP. The data suggest that cyclic AMP can induce a voltage-dependent calcium current.

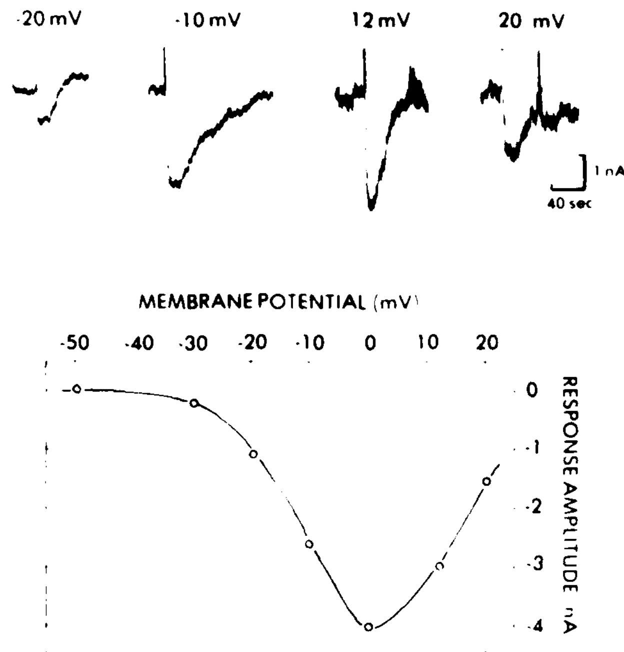

Figure 1. Voltage-dependent inward current evoked by intracellular pressure injection of *cyclic AMP*. Traces are sample responses to *cyclic AMP* elicited while membrane voltage was clamped to potential indicated. Inward current is represented by downward deflection. Plot of response *amplitude versus membrane potential illustrates* voltage dependence of cyclic AMP-induced current. Response is absent at potentials more negative than -30 mV. Response has a maximum amplitude near 0 mV.

Using agents that alter cyclic AMP metabolism, we tested the possibility that cyclic AMP mediates the voltage-dependent response to serotonin. The phosphodiesterase inhibitors do not enhance the response to serotonin; rather, they block the current induced by both cyclic AMP and serotonin. DTNB (an adenylate cyclase blocker) and imidazole (a phosphodiesterase activator) do not reduce the current elicited by serotonin as would be expected if a cyclic nucleotide were mediating the response. If cyclic AMP were second messenger in the response to serotonin, serotonin should increase cyclic AMP levels in the same neurons. Preliminary results indicate that serotonin did not increase cyclic AMP levels in LB and RB neurons. These results are inconsistent with a cyclic AMP mechanism.

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EFFECTS OF PROPRANOLOL AND OTHER ADRENERGIC DRUGS ON RESPONSES OF THE COCHLEA, AUDITORY NERVE, AND BRAIN STEM TO ACOUSTIC STIMULI

Principal Investigator: M. L. Wiederhold

Intravenous administration of beta-adrenergic-blocking agents has been shown to decrease glucose metabolism in nuclei throughout the auditory central nervous system (1). In an attempt to determine where this effect might occur, we have recorded click-evoked electrical responses generated in the cochlea (cochlear microphonic, CM), auditory nerve (N_1), and brain stem (BSER recording). All potentials were recorded simultaneously from the external ear canal of barbiturate-anesthetized cats before, during, and after intravenous infusion of propranolol for 1 hour. Dose rates from 0.01 to 1.0 mg/kg/min were used. The amplitudes of both N_1 and BSER in response to clicks approximately 40 dB above threshold were reduced up to 70% in a dose-dependent manner by propranolol. A half-maximal effect was obtained at approximately 0.3 mg/kg/min. Maximal effects were generally seen about 10 minutes after infusion was stopped. Recovery began immediately thereafter but was never complete within 2 hours. No consistent dose-dependent effects on CM were seen. Since comparable reductions of N_1 and BSER responses were obtained at all dose rates, a major portion of the effects of propranolol in these experiments must be mediated within the cochlea. Dose-response relationships for the effects of propranolol on CM, N_1, and N_4, a brain-stem-evoked potential, are shown in Figure 1.

To test for possible antagonistic effects between alpha and beta receptors, clonidine (an alpha agonist) was also infused. This caused no change in CM or N_1 but did reduce the BSER amplitude slightly. To test for specificity of the propranolol effect, sotalol (a beta-adrenergic blocker locking the local anesthetic properties of propranolol) was used. Sotalol appears to have no effect on CM or N_1, whether given intravenously or directly into the fourth ventricle. This finding suggests that some property other than the beta-blocking effect of propranolol is responsible for its reduction of neural responses.

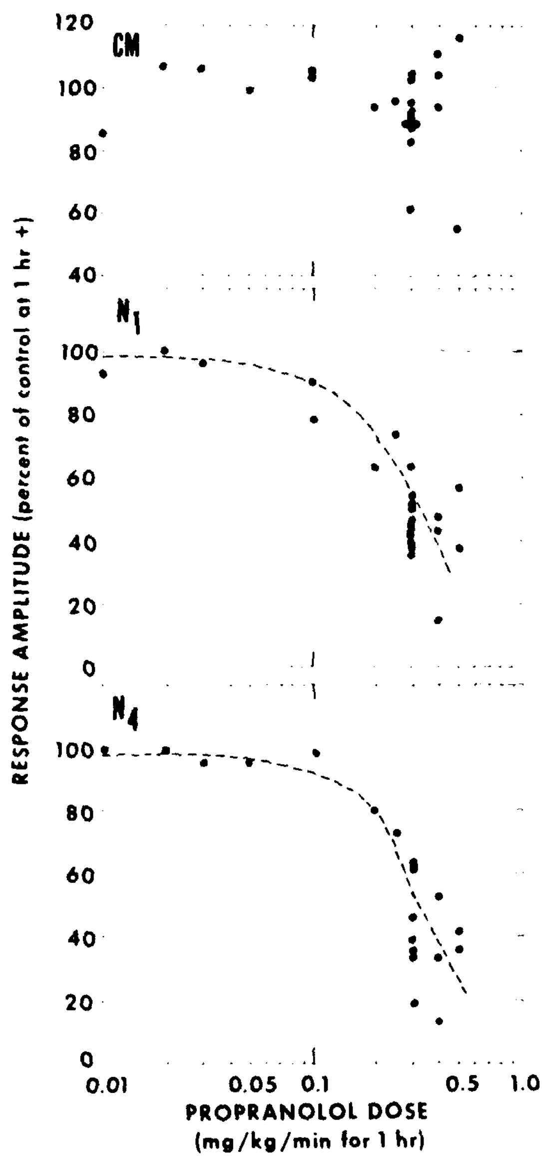

Figure 1. Dose-response curves for effects of systemic intravenous infusion of propranolol on cochlear microphonic (CM), auditory *nerve* (N_1), *and brain stem* (N_4) responses to condensation clicks approximately 40 dB above *threshold.*

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MUSCARINIC ACETYLCHOLINE RECEPTOR ACTIVATION SUPPRESSES A NOVEL VOLTAGE-DEPENDENT POTASSIUM CHANNEL

Principal Investigator: J. E. Freschi

Voltage-clamp technique was developed for studying individual ionic currents in cultured mammalian neurons. It was found that cultured superior cervical ganglion neurons from rat have distinct potassium channels that are linked to muscarinic acetylcholine receptors (Figure 1). At the ionic channel level, then, the kinetics of the action of chemical warfare agents and their antidotes can be studied.

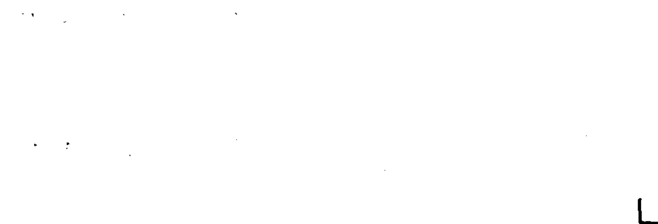

Figure 1. Muscarinic-sensitive potassium current seen in voltage-clamped cultured rat sympathetic neuron. Upper trace = voltage; lower trace = current. Holding potential, -40 mV. Hyperpolarizing voltage steps produce fast ohmic step followed by slow relaxation that is initially inward but becomes outward after steps beyond -80 mV. On repolarization, current relaxation is outward. Ohmic step is of greater amplitude at step onset than at offset, indicating that step moves from higher to lower conductance state. Calibration: 20 mV, 2 nA, 0.4 sec.

The kinetics of the potassium channels that underlie muscarinic acetylcholine action appear to be unaffected by ionizing radiation in doses of up to 5000 rad (cobalt-gamma).

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SCIENTIFIC SUPPORT DEPARTMENT

The Scientific Support Department is composed of three divisions: the Scientific Instrumentation Division, the Radiological Physics Division, and the Radiation Sources Division.

During fiscal year 1980, the Scientific Instrumentation Division concentrated on five major areas of study:

Gastrointestinal: For evaluation of the efficacy of an antiemetic drug administered to canines before whole-body cobalt-60 irradiation.

Receptor-binding radiopharmaceuticals: For evaluation of radiolabeled agents potentially valuable for quantitating muscarinic receptor and function.

Fluorine-18 production in AFRRI's TRIGA reactor: For eventual incorporation into biologically active substances.

Membrane damage postirradiation: For membrane analysis using electron paramagnetic resonance spectrometry.

Muscarinic receptors: For study of the effect of physostigmine and mecamylamine on the response to acetylcholine in *Aplysia*.

Although the Scientific Instrumentation Division (SID) has supervision of the electron microscopy and the gas chromatographic mass spectrometers unit (support instruments), SID's function continues to be primarily radiobiological research. SID utilizes radionuclides and sophisticated radiopharmaceuticals to perform not only *in vitro* animal studies but also noninvasive imaging studies of canines and primates. The former studies allow rapid and more extensive analysis of radiation insult to biological models, setting the basis for the latter studies, which allow more intricate, noninvasive evaluation of a higher order animal model. These radiotracer studies help to evaluate biological transport and metabolic events as well as other physiological functions in experimental animal models. In addition to radiotracer studies, SID is involved in electron paramagnetic resonance investigation of membrane damage postirradiation.

One of the life-threatening situations for combat pilots is emesis postexposure to a nuclear detonation. The use of an antiemetic drug before irradiation may possibly prolong their operational function. One of the projects in SID is the evaluation of the efficacy of an antiemetic drug administered to canines before whole-body cobalt-60 irradiation.

To evaluate parasympathetic autonomic function that has been insulted by irradiation and/or chemical agents, specific radiolabeled muscarinic agents are potentially useful for quantitation of damage. SID has undertaken an extensive collaborative research effort to develop a suitable muscarinic radiopharmaceutical for this purpose. In addition, studies on effects of physostigmine and mecamylamine on the response to acetylcholine in an *Aplysia* model was studied.

One of the most rapidly developing noninvasive probes in nuclear medicine for quantitation and qualitation of radiotracer data is positron emission computerized tomography (PECT). The advantage of PECT is the specificity of the enormous numbers of radiolabeled biochemicals that can be utilized for assessing biodistribution, metabolism, and excretion in radiobiological studies. One of the important radionuclides for PECT studies is fluorine-18. SID has been investigating the production of fluorine-18 in AFRRI's TRIGA reactor.

The Radiological Physics Division provides dosimetry support for all radiation sources at AFRRI. Although its function is primarily supportive in nature, it is a highly scientific function requiring extensive in-house dosimetry research. Its main areas of study are (a) measurement of tissue-to-air ratios (TARs); conversion of air doses to tissue doses using tissue-equivalent phantoms; (b) field mapping; measurement of dose distribution; (c) the study of new dosimetry systems for adaption to the AFRRI program.

Specific areas of dosimetry research included one-dimensional and three-dimensional transport calculations of AFRRI's TRIGA reactor spectra. These spectra were partially verified by activation foil measurements. In addition, LiF-100 TLD variability was studied over a wide range of doses.

The Radiation Sources Division maintains and performs quality control checks on AFRRI sources and accelerator (TRIGA reactor, Cobalt-60 source, Theratron, Phillips X-ray unit, and LINAC) on a scheduled basis. This insures that AFRRI and outside investigators can complete their experiments in a timely and accurate fashion.

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GASTRIC EMPTYING AND VOMITING POSTIRRADIATION AND INTERVENTION WITH AN ANTIEMETIC DRUG

Principal Investigators: A. Dubois and D. O. Castell, *Uniformed Services University of the Health Sciences*
J. P. Jacobus, M. P. Grissom, R. R. Eng, and M. F. Corral, *AFRRI*

Twenty dogs were studied on 2 separate days, blindly and in random order, after intravenous injection of either a placebo or 10 mg domperidone. On a third day, they received 800 roentgens of cobalt-60 whole-body irradiation after receiving either the placebo ($n = 10$) or domperidone ($n = 10$). Before each study, each dog was fed chicken liver tagged in vivo with technetium-99m-sulfur colloid as the solid marker and indium-111-DTPA (diethylenetriamine pentacetic acid) in water as the liquid marker. Radionuclide imaging was performed at 10-min intervals for 3 hours.

The slope of the exponential clearance of intragastric contents (%/hour) of technetium-99m (solids) and indium-111 (liquids) was determined for each study. Vomiting was observed within 6 hours after irradiation in 9 of 10 dogs given placebo but in only 1 of 10 dogs pretreated with domperidone ($p < 0.01$). In addition, as shown in Table 1, neither domperidone treatment nor irradiation after placebo administration modified the emptying of liquids or solids. In contrast, gastric emptying was suppressed by irradiation after domperidone ($p < 0.01$).

Table 1. Exponential Slopes Determined

	Placebo		Domperidone	
	Basal	Irradiation	Basal	Irradiation
K Liquids (In)	0.22 ± 0.05	0.20 ± 0.05	0.19 ± 0.03	0.04 ± 0.01
K Solids (Tc)	0.09 ± 0.03	0.10 ± 0.03	0.07 ± 0.01	0.02 ± 0.01

K = rate constants for clearance
In = indium-111
Tc = technetium-99m

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EVALUATION OF RECEPTOR-BINDING RADIOPHARMACEUTICAL-BETA ADRENOCEPTOR LIGANDS

Principal Investigators: M. Grissom, F. Vieras, and R. Eng, *AFRRI*
W. Eckelman and J. Phillips, *George Washington University, Washington, DC*

Receptor-binding radiopharmaceuticals can be potentially useful for assessing physiological function of the heart. A tissue distribution study was performed on a group of radioiodinated beta adrenoceptor agents [I-125 iodohydroxybenzyl-pindolol (IHYP), I-125 iodopindolol (IPIN), and I-125 iodocyanopindolol (ICYP)].

The IHYP gave low heart/blood ratios but was displaceable in the lungs. IPIN did not show high heart/blood ratios (0.41-0.65) in rats, but the ICYP did (3.8-5.3). In addition, at 2 hours after injection, ICYP was clearly displaced from the heart (78%) and lungs (88%) by pindolol, thus indicating specific receptor binding to these organs (Table 1).

Table 1. Distribution and Displacement of Beta Adrenoceptor Agents

	Time	Blood	Heart	Lung
^{125}IPIN	¼	0.49±0.04	0.33±0.03	2.09±0.45
	2	0.32±0.04	0.13±0.03	0.50±0.12
^{125}ICYP	¼	0.18±0.04	0.95±0.33	5.14±2.61
+ 100nmol PIN	¼	0.67±0.01	0.84±0.44	1.25±0.59
^{125}ICYP	2	0.17±0.03	0.64±0.13	5.68±0.65
+ 100nmol PIN	2	0.06±0.01	0.14±0.03	0.66±0.12

It is concluded that although ICYP has good heart/blood ratio, it probably will not be useful as a cardiac imaging agent because of high pulmonary localization. The same problem applies to IPIN. ICYP labeled with an appropriate gamma-emitting radionuclide may be potentially useful for receptor imaging of the lungs.

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PRODUCTION OF FLUORINE-18 IN THE AFRRI TRIGA REACTOR

Principal Investigators: R. Eng and M. Grissom

Fluorine-18 is one of the most important positron-emitting radionuclides used in radiopharmaceuticals for studies with positron emission computerized tomography (PECT). Fluorine-18 is produced mainly via a cyclotron using either proton or deuteron bombardment on specific target materials. For those research centers that do not have access to a cyclotron but do to a reactor, there is a method to produce fluorine-18.

Quartz tubes, 2 mm in diameter, were filled with enriched lithium-6 carbonate (100 mg/tube). These tubes were placed in an aluminum holder and lowered into the core of the TRIGA reactor. During the irradiation, fluorine-18 was produced via the $^{6}Li\ (n,\alpha)\ T$ - $^{16}O\ (T,n)\ ^{18}F$ nuclear reactions. Energetic tritium atoms are produced after neutron irradiation of the lithium-6. The energetic tritium atoms interact with adjacent oxygen atoms to produce fluorine-18. With an incore neutron flux of 9×10^{12} neutrons/cm^2/sec, a yield estimate of 4 mCi/g lithium-6 carbonate was obtained for a 15-minute irradiation. Although yields may not be dramatic, it is sufficient for developing radiochemicals, and possibly for producing enough radiochemicals for small-animal work.

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MOLECULAR DAMAGE TO ERYTHROCYTE GHOST MEMBRANES INDUCED BY IONIZING RADIATION

Principal Investigators: M. J. McCreery, W. A. Hunt, and T. K. Dalton

Radiation-induced damage to biological membranes may represent a common pathway of structural and biochemical aberrations leading to cellular dysfunction and death. We have used hydrophobic and site-specific molecular probes possessing a fluorescent or nitroxide moiety to monitor changes due to ionizing radiation in sealed and unsealed ghosts of freshly drawn human or rat erythrocytes. Fluorescent lifetimes, polarization, and intensity of DPH, ANS, or an anthroyl stearate intercalated into the membrane were measured. Alternatively, the order parameter of doxyl stearates was determined. From these data, correlation times were calculated yielding approximations of membrane fluidity and polarity. X rays, cobalt-60 gamma-rays, and 18-MeV electrons from a linear accelerator were compared for their ability to perturb these biophysical parameters.

Our results suggest that accelerated electrons are most effective in inducing these membrane perturbations. The data also suggest that sealed ghosts are more sensitive to radiation-induced changes than unsealed membrane fragments. It remains to be determined whether these changes are the result of molecular damage to phospholipids which the probes directly measure, or are secondary to constituent protein aberrations, or a combination of both.

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EFFECTS OF PHYSOSTIGMINE AND MECAMYLAMINE ON THE RESPONSE TO ACETYLCHOLINE IN *APLYSIA*

Principal Investigators: M. J. McCreery and D. O. Carpenter, *AFRRI*
M. G. Filbert and J. T. Moffitt, *U.S. Army Medical Research Institute for Chemical Defense, Aberdeen Proving Grounds, MD*

It is widely accepted that the effects of physostigmine (physo) can be attributed to the accumulation and prolonged action of acetylcholine (Ach) due to inhibition of acetylcholinesterase (AChE). However, evidence also suggests a direct action of physo on postsynaptic membranes (1,2). It has been shown that physo blocks binding of ^{125}I-α-Bungarotoxin (α-Btx) to ganglionic homogenates of Aplysia and also blocks inhibition by α-Btx of the electrophysiological response to Ach (3). Recently, mecamylamine (meca), a nicotinic blocker, was observed to inhibit α-Btx binding to Aplysia homogenates as well. The I_{50}'s for the inhibition of α-Btx binding by physo and meca were comparable to that of d-tubocurarine (4).

We have examined the effects of these cholinergic agents on the electrophysiological responses of Aplysia neurons to iontophoretic application of Ach and carbechol (Carb). Iontophoresis of physo onto the cell just before Ach or Carb almost always resulted in a reduced response, whereas its perfusion sometimes caused an increase in the amplitude and duration of the response to Ach as expected with inhibition of AchE. The sensitivity of the Ach and Carb response to inhibition by physo was dependent upon the conductance change elicited, $g_{Na^+} > g_{Cl^-} >> g_{K^+}$. Iontophoresis of meca resulted in diminution of the response, irrespective of whether the voltage change was due to an increase in conductance to Na^+, Cl^-, or K^+.

These results suggest that the classic anti-AChE physo can also interact directly with the ACh receptor. At low concentrations of physo, enzyme inhibition predominates, leading to potentiation of the ACh response. Higher concentrations of physo result in its binding to the ACh receptor, which reduces the ACh response amplitude and offsets the effects due to enzyme inhibition. Only a reduction in the response to the nonhydrolyzing substate Carb was observed with physo. These data are consistent with the binding curves for physo with AChE (I_{50} = 0.25 μm) and for its inhibition of α-Btx binding (I_{50} = 5.0 μm) (3). The ACh response is reduced by meca, which has no enzyme-inhibiting activity.

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BIOMATHEMATICS AND STATISTICS OFFICE

EARLY BIOLOGICAL EFFECTS FROM NUCLEAR RADIATION IN HIROSHIMA AND NAGASAKI

Principal AFRRI Investigator: S. G. Levin
Principal Contractor's Investigator: D. Summers, *The Dikewood Corporation, Albuquerque, NM*
Collaborators: T. Jones and G. Kerr, *Oak Ridge National Laboratories, Oak Ridge, TN*
D. Groce, *Science Applications, Incorporated, San Diego, CA*

Joint Commission

The original study by the Joint Commission for the Investigation of Effects of the Atomic Bomb in Japan was under the direction of COL Ashley Oughterson, U.S. Army Medical Corps, and resulted in six volumes (1) produced for the Atomic Energy Commission. Later a summary volume by Oughterson and Shields Warren (2), titled "The Medical Effects of the Atomic Bomb in Japan," became the basic source of information on almost all of the early radiation effects. The Atomic Bomb Casualty Commission has continued the work, but its major concern is late effects.

Because of the mass destruction, unavailability of accurate maps, lack of knowledge of the location of the epicenter, and lack of other dosimetric information, the Oughterson-Warren and later analyses used concentric rings of 500-meter radius to locate persons at the time of detonation of the bombs. The center ring includes individuals from ground zero down to approximately 260 rads free in air; the second ring ends at about 20 rads. Thus, almost all cases of military interest are included in these two rings, and meaningful doses cannot be associated with the observed effects. Furthermore, the specific symptoms associated with the rings were not limited to only radiation. For example, for vomiting, the observed cases in the third ring (1.6 to 20 rads) are between 7% and 20%, while observed cases of diarrhea are 30% to 40% in the same ring. Clearly the category "radiation injury" includes combined blast and/or burns. The percentages of persons with specific symptoms were obtained by dividing the number of persons in a particular category who showed the symptom by the number of persons examined. This method contains a built-in bias because the majority of those examined had been injured.

Dikewood Corporation

The present study was undertaken to overcome the shortcomings of the previous work. The original forms that had been used by the Army Institute of Pathology contained data on exact location of individuals at the time of exposure, their injuries, and their shielding. The Dikewood Corporation of Albuquerque, New Mexico, had coded all available data onto a consistent set of forms and then transcribed the results onto magnetic tape, under previous contracts to the Atomic Energy Commission, the Office of Civilian Defense, and the Defense Nuclear Agency (DNA). In its reports (3-5), the Dikewood Corporation did not examine specific symptoms and did not assign a dose to each individual. Instead, it grouped the effects into categories of radiation, blast, and burn injury. Furthermore, the Corporation used distance instead of dose.

Present Study

The previously developed data tapes were used; however, for this study, the specific requirements relative to the AFRRI mission were addressed. The actual computations were done by the Dikewood Corporation under DNA contract 001-79-C-0025. In order to eliminate the bias inherent in the original analyses, additional data that had been recorded on the forms were used. Groups were formed that consisted of persons at the same site when the bomb was detonated, with the number in the group confirmed by two or more persons. The sum of all these groups then became the population to be studied. Although this does not create a random sample, it does permit an unbiased estimate of the percentage of individuals with a given symptom. It is assumed that this sample is representative of the entire exposed group at the dose ranges of interest.

Since each record contains information on all symptoms, in order to delineate radiation-only injuries, the following were deleted from the cases with symptoms: those persons with worse than mild burns (> 10%-body-area first-degree burns) and those with more than moderately severe secondary blast injury (single laceration, abrasion, and/or simple fracture not of the long bone). This method was used because the examining physicians had no training in diagnosing or treating radiation injury. Only cases that had received 1-650 rads of combined neutron-plus-gamma (free in air) radiation were included, because little shielding information was available for cases with less than 1 rad, and the very high mortality in the over-650-rad group reduced that group's numbers and reliability.

Only individuals who had been in wood-frame dwellings or had been shielded by wooden buildings were included because good estimates of dose were possible for them. Furthermore, cases of persons who had been in the open invariably had severe burns and could not be included, and those in brick or concrete structures were deleted because doses to those persons cannot be accurately estimated.

The present study calculates doses in total rads (gamma plus neutron) attenuated for transmission through walls of wood-frame dwellings, using the tentative 1965 dose estimates (6) that were considered "state of the art" until very recently. Cases were sorted according to dose and grouped in 25-rad intervals. The percentages of persons who showed specific symptoms of vomiting, diarrhea,

purpura and petechiae, other hemorrhages, scalp epilation, and reproductive system anomalies have been calculated, and have been presented in two reports (7,8) to DNA.

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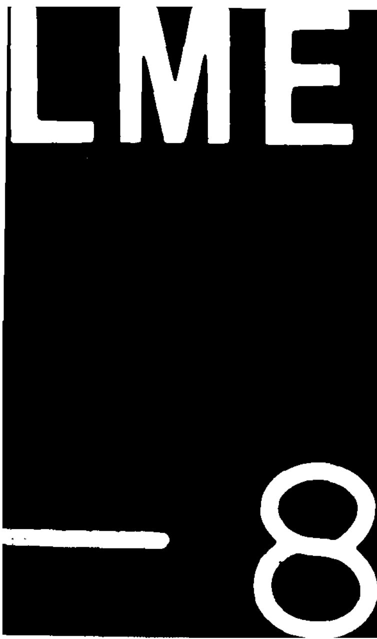
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