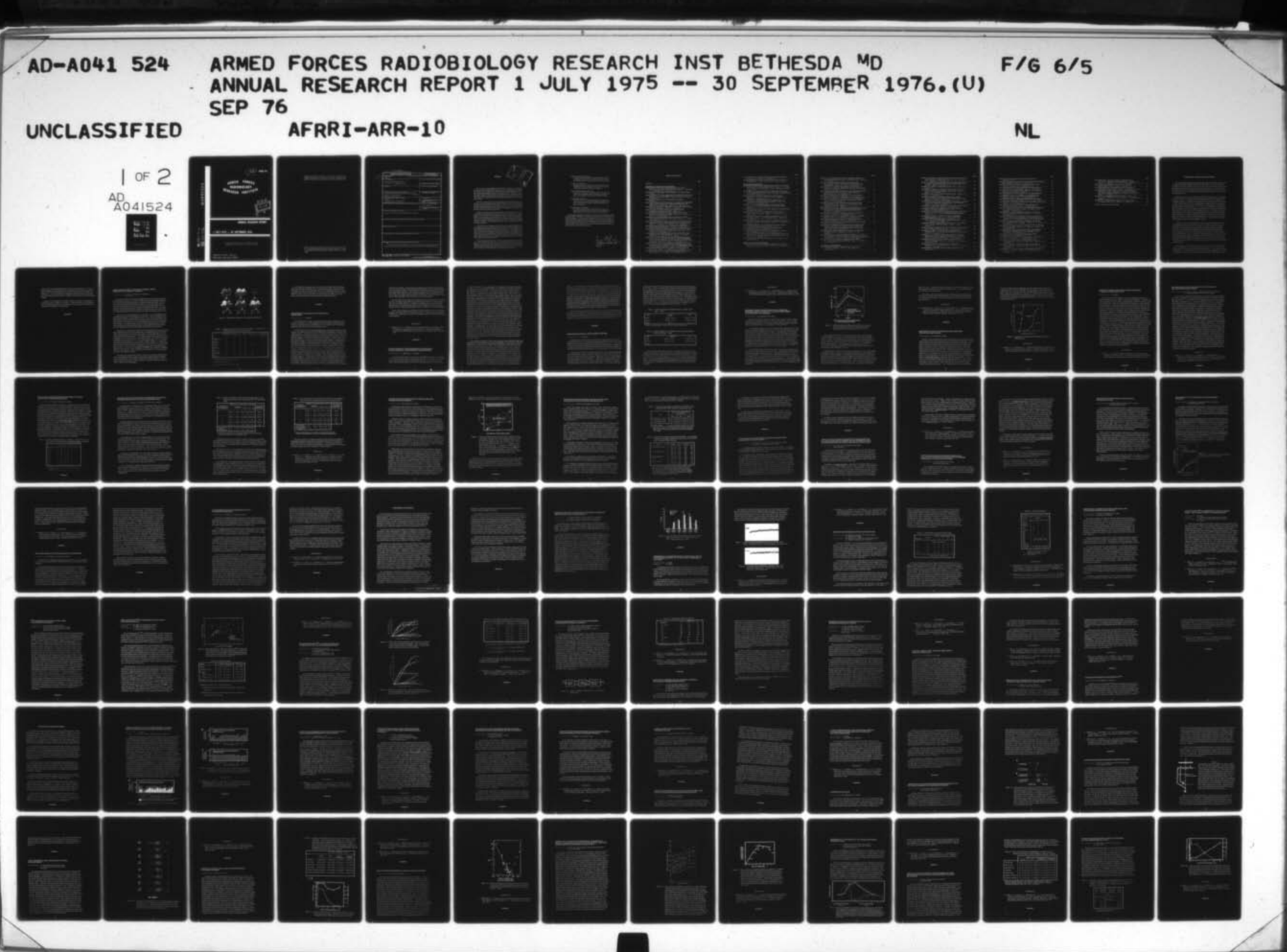

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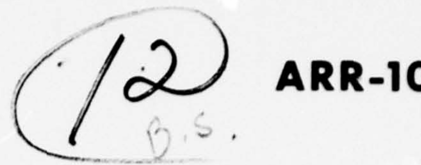

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This report contains a summary of the research projects of the Armed Forces Radiobiology Research Institute for the period 1 July 1975 to 30 September 1976.

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PREFACE

The Armed Forces Radiobiology Research Institute (AFRRI) is a command of the Defense Nuclear Agency (DNA), the joint services organization which plans and coordinates the Department of Defense's nuclear weapons programs, including nuclear weapons effects research. AFRRI's research is undertaken to meet DoD requirements in biomedical effects of nuclear weapons. AFRRI also acts in an advisory capacity to the Department of Defense on all medical problems associated with nuclear weapons systems and on the hazards associated with incidents and accidents.

The areas of current interest in the Biomedical Research Program have increased in scope and complexity with the identification of a number of operational problems related to tactical nuclear weapons. There are critical requirements for expansion of efforts in areas of research such as combined injury, collateral damage, casualty prediction, and the diagnosis and prognosis of radiation injury as well as treatment of nuclear casualties.

AFRRI has assessed its projects and programs, studied them in the context of identified major requirements of the three services and to probable requirements in the foreseeable future, evaluated the relative urgency of its responsibilities in the areas noted, and reallocated its resources to achieve optimum results for the accomplishment of its mission. An important move was the realignment of the institute's departments to facilitate a clearer approach to research problems by grouping related programs to permit closer evaluation and direction of program progress.

The present research programs of the institute encompass the following: nuclear radiation-induced incapacitation and performance decrement studies in animal models to allow better prediction of man's response; medical aspects of nuclear weapons effects for use in nuclear weapons effects medical planning; molecular and cellular radiobiology as well as nonmammalian and mammalian radiobiology in the study of mechanisms of radiation interaction with biological systems; radiation dosimetry in the evaluation and application of biomedical effects data; and facilitation of repair of radiation damaged tissues and protective agents in the study of recovery and treatment.

The scientific departments of the institute as now constituted and a brief summary of their projects are given below. A more detailed statement of each department's activities during this reporting period is given in the main body of this publication with summary reports on particular projects of that department. These reports represent a major portion of the research results for the year but do not cover all aspects of the ongoing long-range programs of the institute.

Behavioral Sciences Department:
research on the acute effects of radiation and other injuries on behavior, performance and psychoneurological integrity to establish casualty criteria for tactical nuclear operations

Biochemistry Department:
biomedical research in disciplines of biochemistry and radiobiology with special emphasis on the basic radiochemical mechanism of radiation injury and its diagnosis

Experimental Hematology Department:
research on the effects of ionizing radiation and combined injuries on mammalian species with special emphasis on doses in the midlethal range related to collateral damage

Neurobiology Department:
research to elucidate the normal function of peripheral and central nervous systems and their malfunction in disease and injury to study basic mechanisms underlying high dose casualties and mortality

Nuclear Sciences Department:
biomedical, biological and radiological physics research using radioactive materials to increase the understanding of disease processes and organ function in combat injuries.

AFRRI hosted meetings of the Board of Governors, the Biomedical Nuclear Weapons Effects Development Coordination Committee and the Joint Medical Research Committee. To increase its competence to carry out its mission, staff members attended courses and panel meetings on Special Nuclear Weapons Effects, armament problems and a reexamination of the data on radiation effects of nuclear weapons on combat personnel. In addition to the publication of papers in scientific journals and laboratory reports, staff members made oral presentations to many scientific society meetings.

LaWAYNE R. STROMBERG, MD.
Colonel, USA
Director

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EXPERIMENTAL HEMATOLOGY DEPARTMENT

The Experimental Hematology Department conducts research on the effects of ionizing radiation upon the blood cell forming system in the bone marrow. Personnel exposed to radiation doses up to 1000 rads are injured or die because of damage to the blood cell precursors in the bone marrow. The objective of the Department is to develop biological diagnostic indicators for the early measurement of the extent of radiation damage and therapeutic means to ameliorate the sustained injury.

Primarily because the mechanism of blood cell production is poorly understood there exists at present no efficient biological indicator for the prediction of radiation injury. Consequently, the hematologists of the Department are presently engaged in research to determine what initiates white cell production. To date several studies appear to support the contention that the organization governing white cell production is controlled by a combination of positive and negative humoral feedback systems. The next step will involve the purification and concentration of humoral inhibitor and stimulatory substances and study of their precise involvement in the cell production process. Utilizing ultrastructural studies, tissue culture and in vivo testing should permit the elucidation of the processes controlling white cell production. Once this has been established, it should be possible to develop diagnostic tools and means for the enhancement of white cell production after exposure to radiation doses which do not completely destroy the hematopoietic system.

Personnel exposed to radiation doses which damage primarily the blood cell forming system usually become sick or die of bacterial infection. The departmental microbiologists are involved in extensive studies to delineate the route of postirradiation infection and the breakdown of normal defense mechanisms. Based on their studies the following therapeutic approaches were suggested for patients with radiation-induced decrease in the numbers of circulating white cells: (1) control of endogenous flora; (2) white cell transfusion; (3) antibiotics; and (4) attenuation of the inflammatory response. The microbiologists are aided by the efforts of the physiologists who are making progress in the development of methods for the cryopreservation and storage of typed white cells. This is a joint effort with the U. S. Naval Unit at Chelsea, Massachusetts.

Once all bone marrow tissue is destroyed the only life saving procedure is the transplantation of compatible bone marrow stem cells. Unfortunately, basic immune mechanisms interfere with this procedure. The immune system remains intact up to lethal doses of 600 rads and any noncompatible transplant is rejected. At higher doses immunological active donor cells attack vital

organs of the host and initiate the fatal graft versus host disease. Efforts of the departmental immunologists have resulted in the mechanical removal of immune killer cells with the net result of increased survival of lethally irradiated mice., The physiologists are engaged in an effort to freeze-store specifically typed bone marrow stem cells which could be used for postirradiation therapy.

Shortly, the Department will initiate studies on the effect of additional or combined injuries in radiation casualties. Initial efforts by the toxicologists, who are developing models for the detection of toxic products in radiation contaminated environments, are described.

****

STIMULATION OF STEM CELL RELEASE BY HUMORAL AGENTS PRODUCED IN INFLAMMATORY EXUDATES

Principal Investigators: *S. J. Baum, T. J. MacVittie, R. T. Brandenburg and S. G. Levin*

Personnel subjected to ionizing radiation which will not induce immediate mortality may become ill or die 2 to 3 weeks later due to the diminished number of white cells in the circulation to combat bacterial infections. The decrease in the number of white cells is caused by the injury to the blood cell forming system in the bone marrow which must repair itself before it is capable of producing the necessary number of functional white cells. The present study was designed to discover means for enhanced recovery of the blood cell forming system which would initiate an earlier production of white cells.

The precise way multipotential stem cells (CFU-s) are stimulated for differentiation into leukocytic progenies is not known. Various hypotheses have been suggested which consider either a cell to cell or a humoral agent to cell interaction. Production of humoral substances could occur either in the bone marrow leukocytic storage pool, in the circulating leukocytes or in inflammatory exudates. The present study is based on the hypothesis that a humoral agent produced in inflammatory exudates is transported to the bone marrow where it stimulates CFU-s to differentiate into leukocytic precursors.

Inflammatory exudates were produced by the implantation of an acrylic cup filled with bacteria free Hanks' solution into a subcutaneous pouch of B6D2F1 mice. The cups were removed 24 hours later, the cells separated from the supernatant and both were pooled. Thereafter, normal mice received via tail vein either 2×10^7 exudate cells, 0.2 ml supernatant, 0.2 ml plasma from cup implanted or from normal mice, 2×10^7 bone marrow cells or 2×10^7 splenic cells from normal animals or 0.2 ml Hanks' solution (Figure 1). Another 24 hours later bone marrow was removed from all mice and 2.5×10^4 cells were injected into previously lethally irradiated mice (900 rads ^{60}Co gamma irradiation), while 5×10^4 cells were suspended in vitro in agar supported media. Nine days later the mice were euthanatized, the spleens removed and colonies (CFU-s) were counted. At the same time colonies (CFU-c) were enumerated from the agar supported media.

The results clearly indicate (Table 1) that the bone marrow obtained from mice stimulated with either exudate cells or supernatant or plasma obtained from cup implanted mice contained a significantly greater number of CFU-s than did either one of the four control groups. A similar trend was observed in the colonies produced in vitro.

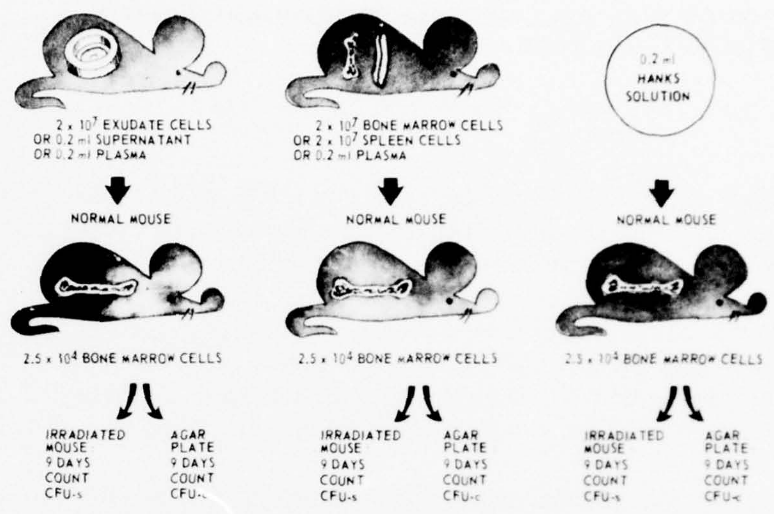

Figure 1. Schematic presentation of the experimental design

Table 1. Number of CFU-s Per 2.5×10^4 Bone Marrow Cells in Mice Stimulated With Cells or Humoral Agents

	Total Number of Colonies	Total Number of Spleens	Mean	Standard Error	Number of Cells Injected	Volume of Plasma or Supernatant Injected (ml)
Exudate[1] Supernatant (ES)	640	95	6.7[3]	.28	--	.2
Exudate Plasma (EPL)	570	90	6.3[3]	.29	--	.2
Exudate Cells (EC)	529	92	5.8[3]	.24	2×10^7	--
Normal[2] Plasma (NPL)	467	95	4.9	.18	--	.2
Hanks' Solution (H)	436	91	4.8	.21	--	.2
Normal Bone Marrow (NBM)	417	95	4.4	.21	2×10^7	--
Normal Spleen (NS)	351	79	4.4	.22	2×10^7	--

[1]Connotes mice implanted with acrylic cups.

[2]Mice without implanted cups.

[3]Significant difference from controls ($P \leq 0.05$).

It is therefore postulated that cells which had migrated into the exudates produced a humoral substance which is transported via the circulation to the bone marrow where it stimulates the release of multipotential stem cells into the leukocytic cellular compartment. This humoral substance will be analyzed further in order to determine under what physiological condition it will enhance white cell production in personnel after radiation exposure.

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ENHANCEMENT OF IN VITRO COLONY FORMATION BY HUMAN SERUM

Principal Investigator: *T. L. Weatherly*

The present work is part of a combined effort to study regulators of bone marrow cell growth. An understanding of this regulation may provide knowledge for useful therapeutic manipulation in cases of bone marrow dysfunction associated with disease states and radiation injury.[1]

Normal human serum enhanced in vitro colony formation by human, murine, canine, and simian bone marrow cells at optimal concentrations of colony stimulating activity. The number and size of colonies were increased. Murine colony formation was induced by pregnant mouse uterus extract, mouse L-cell conditioned medium, or human peripheral blood leukocytes. Canine colony formation was induced by 15 percent dog plasma in an underlayer of 1 percent agar and culture medium. Sources of colony stimulating activity for human and simian colony formation were peripheral blood leukocytes and human activated (phytohemagglutinin-P, Difco Laboratories) lymphocyte conditioned medium. Activated lymphocyte conditioned medium stimulated murine colony formation only if normal human serum was present. At optimal colony stimulating activity concentrations, fetal calf, dog, horse, and murine sera either did not enhance colony formation or did so to a lesser degree than normal human serum. Maximal enhancement was obtained with serum concentrations of 7.5 to 10 percent (v/v); at higher concentrations colony formation decreased below maximum number. Preincubation of murine marrow with normal human serum in liquid culture for 1 hour did not result in subsequent enhancement. Rather, for enhancement to take place serum was required to be present during the culture period. Human bone marrow was cultured over peripheral blood leukocytes with or without a nonhuman source of colony stimulating activity (pregnant

mouse uterus extract). The presence of pregnant mouse uterus extract in human bone marrow cultures did not inhibit enhancement by normal human serum in the concentrations used. In the presence of normal human serum, the curve describing colony number versus colony stimulating activity concentration appeared sigmoid in shape and was shifted to the left and to a higher plateau. The slope of the exponential portion of the curve increases slightly.

Normal human serum enhances colony formation by bone marrow of several species. Our data suggest that serum modifies the activity of colony stimulating activity without, however, reacting or combining with it irreversibly outside the cell. The net enhancing effect of normal human serum is probably a function of other variables including the levels of inhibitors of colony formation.

These results suggest the possibility of enhancing bone marrow cell growth *in vivo* in personnel exposed to ionizing radiation. Further work is required to elucidate the mechanism of action of serum enhancing factors and their biochemical properties.

REFERENCE

1. Weatherly, T. L. Enhancement of *in vitro* granulocyte-macrophage colony formation by normal human serum or plasma. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR76-43, 1976.

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CHARACTERISTICS OF THE IN VITRO MONOCYTE-MACROPHAGE COLONY FORMING CELLS WITHIN MOUSE LYMPHOID ORGANS

Principal Investigators: *T. J. MacVittie and T. L. Weatherly*

The goal of these studies is to minimize the deleterious effects of radiation by development of preventive or therapeutic measures. Knowledge of the mechanisms which control proliferation and differentiation of stem and progenitor cells of the white blood cell is essential for the development of such measures.

The development of an in vitro assay for granulocyte-macrophage progenitor cells of man and animal has resulted in considerable knowledge concerning the regulation of granulopoiesis. In the mouse, the colony forming cell has been detected in fetal yolk sac, liver, spleen and marrow as well as the marrow, spleen, peripheral blood and peritoneal exudate of the adult with the notable exception, however, of the thymus and lymphoid organs. Here we report the detection of in vitro monocyte-macrophage colony forming cells within the thymus and lymphoid organs of the mouse. Cell suspensions were prepared from thymuses, cervical and mesenteric lymph nodes, spleens, peripheral blood leukocytes and bone marrow which were obtained from adult, male and female mice of strain B6D2F1 Cum. The standard double layer agar culture technique was employed. Extracts of pooled mouse placentae, membrane, and gravid uteri, mouse L-cell conditioned medium and mouse sera collected after injection of endotoxin were used as sources of colony stimulating activity. The specific factor(s) necessary to induce colony formation from thymus and lymph node cells was present only in placentae, membrane and uteri extract and not in mouse L-cell conditioned medium or mouse sera collected after injection of endotoxin. The relative concentrations of colony forming cells detected within hematopoietic and lymphoid organs were bone marrow 1802 ± 122, spleen 63 ± 9, thymus 32 ± 3, mesenteric lymph nodes 22 ± 7, cervical lymph nodes 4 ± 2 and peripheral blood leukocytes 10 ± 5 per 10^6 B6D2F1 cells cultured. Colony formation was linearly related to the number of thymus and lymph node cells cultured. Morphologically, all of the cells examined within thymus- and lymph node-derived colonies have been mononuclear, monocyte-macrophage type. Colony formation from thymus and lymph node cells, however, began only after an approximate 8- to 12-day lag period, respectively, and reached maximum numbers after 21 days of culture. Respective population doubling times were 57.6 hours and 43.2 hours. Colony formation from bone marrow suspensions reached plateau values within 10 days of culture with the population doubling every 17.6 hours.

The enigmatic presence of these colony forming cells within the thymus and lymph node organs prompted us to examine additional characteristics of this cell population in an effort to determine their relationship to colony forming cells detected within hematopoietic organs. Their cluster to colony ratio, survival in the absence of colony stimulating factor, radiosensitivity, drug sensitivity, fraction in cell cycle and response to the enhancing factor in normal human sera were compared to the same parameters from bone marrow colony forming cells. Several changes were observed: (1) Cluster to colony ratios were bone marrow 6-8:1, spleen 4:1, thymus and lymph node 1:1. (2) Thymus colony forming cells were markedly less sensitive than bone marrow colony forming cells to the absence of placentae, membrane and uteri extract in culture. A 7-day absence of placentae, membrane and uteri extract in thymus cultures was required to reach the 50 percent decrease in colony formation noted in bone marrow cultures after 1 day absence of placentae, membrane and uteri

extract. (3) Normal human sera enhanced thymus and lymph node colony formation by respective factors of 2.55 and 9.94 while bone marrow, spleen, and peripheral blood leukocyte colony formation were enhanced by factors of 1.82, 2.2 and 0.72, respectively. Similarities were observed in (4) radiosensitivity (bone marrow, $D_0 = 90$ rads; thymus, $D_0 = 85$ rads; cervical lymph nodes, $D_0 = 80$ rads); (5) sensitivity to cytoxan; and (6) the fraction in cell cycle (thymus and bone marrow colony forming cells were both inactivated 40-50 percent of control by hydroxyurea). These data indicated the presence of a factor(s) in placentae, membrane and uteri extract capable of inducing proliferation in a heretofore undetected population of monocyte-macrophage progenitor cells within the mouse lymphoid organs. Data have been provided on the nature of the ubiquitous colony forming cell and the possible effect of organ microenvironment on determination of progenitor cell heterogeneity.

These experiments provided comparative data on a heretofore undetected white blood cell progenitor located in the thymus and lymph nodes of the mouse. Knowledge of the regulation of this ubiquitous cell and the production of its essential product, the white blood cell, will enable us to define better and understand man's hematological response in a nuclear weapons environment.

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HEMATOPOIETIC STEM CELL POPULATIONS IN Sl/Sl^d MICE

Principal Investigator: *K. F. McCarthy*

Knowledge of the mechanisms initiating and regulating regeneration of hematopoietic tissues in the postirradiated animal would aid immensely in the design of new and more effective postirradiation therapy in man. A novel approach to acquiring such knowledge is the study of hematopoietic mechanisms in animals carrying mutations that compromise blood cell forming tissue regeneration in the postirradiation state.[1] One such animal is the Sl/Sl^d mouse.

The Steel gene mutation (Sl) interferes with the regeneration of postirradiated hematopoietic tissue, especially splenic, to such an extent that Sl/Sl^d mice are characterized by an $LD_{50/30}$ of 130 R as compared to an $LD_{50/30}$ of greater than 600 R for normal mice. Apparently, the gene product of the Steel locus regulates, in normal mice, colony forming unit growth in some unknown manner. Therefore, in the present study a comparison of colony forming unit population sizes in Sl/Sl^d and normal +/+ mice was undertaken.

As presented in Table 2 it was found that colony forming unit populations in Sl/Sl^d mice, with the exception of the splenic colony forming unit population, were reduced in size. The finding of a normal size splenic colony forming unit population in Sl/Sl^d mice was unexpected. However, it was reasoned that this colony forming unit population in Sl/Sl^d might be maintained by an auxiliary long range mechanism possibly humoral in nature generated in response to the macrocytic anemia suffered by these mice. This hypothesis was tested by hypertransfusing the animals and determining their colony forming unit population sizes. In Table 3, it can be seen that this treatment drastically reduces the size of the splenic colony forming unit population and to a lesser extent the marrow population of Sl/Sl^d but not +/+ mice.

Table 2. Colony Forming Unit Population Sizes in WCB6-Sl/Sl^d and +/+ Mice

CFU	Sl/Sl^d	+/+
Femur	19,500 (9.7%)*	39,800 (15.0%)
Splenic	39,000 (5.7%)	36,000 (13.8%)
Blood	23 (-)	51 (-)

*Value in parentheses is the two hour seeding efficiency.

Table 3. Colony Forming Unit Population Sizes in Hypertransfused WCB6-Sl/Sl^d and +/+ Mice

CFU	Sl/Sl^d	+/+
Femur	12,400 (15.0%)	71,500 (8.2%)
Splenic	2,500 (14.7%)	71,000 (4.2%)
Blood	--	--

The conclusion drawn from this study is that splenic stromal tissue in normal mice elaborates a colony forming unit proliferation factor in response to depleted erythrocytic differentiation and maturation compartments. This factor acts over a very short range, i.e., is "local" in nature and occupies a central role in the regeneration of hematopoietic tissue in the postirradiated mouse. It is further suggested that knowledge gained from studying this animal model may be applied to develop therapeutic means for postirradiation personnel.[1]

REFERENCE

1. McCarthy, K. F., Ledney, G. D. and Mitchell, R. G. A deficiency of hematopoietic stem cells in Steel mice. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR76-51, 1976.

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SECONDARY DISEASE, MITOGEN AND COLONY FORMING UNIT RESPONSES IN MICE GRAFTED WITH ALLOGENEIC BONE MARROW CELLS ADHERENT TO GLASS BEADS

Principal Investigators: *M. R. Gambrill and G. D. Ledney*

The only treatment available to soldiers and civilians exposed to lethal irradiation is to engraft blood cells from other individuals. This work was initiated with the objective of obviating a lethal reaction, graft versus host disease, which develops as a consequence of this treatment.[1]

It was previously observed in this laboratory that acute graft versus host disease in lethally irradiated mice may be obviated by engraftment with allogeneic spleen cells adherent to glass beads. Consequently, it was hypothesized that this technique might be of value in mitigating delayed secondary disease (CBA→C57BL/6) by the removal of graft versus host disease producing cells or their precursors from mouse bone marrow. CBA or C57BL/6 bone marrow cells were separated on glass beads (350-500 μm range) into nonadherent and adherent (eluted with 0.02 percent EDTA) cell fractions. The following tests were performed on the separated marrow: (1) mitogenic stimulation with phytohemagglutinin and lipopolysaccharide, (2) in vitro and in vivo colony forming cells and (3) murine survival studies.

It is known that mouse bone marrow cells contain fewer phytohemagglutinin reactive cells than do mouse spleen cells. Separated bone marrow cells were tested in vitro for their response to phytohemagglutinin as well as to lipopolysaccharide. A twofold increase in the phytohemagglutinin response was usually seen in the unseparated and nonadherent bone marrow populations when compared to the adherent cell population (Figure 2). On the other hand, the adherent cell population generally maintained a twofold or greater response to lipopolysaccharide over that of whole bone marrow and the nonadherent cell population.

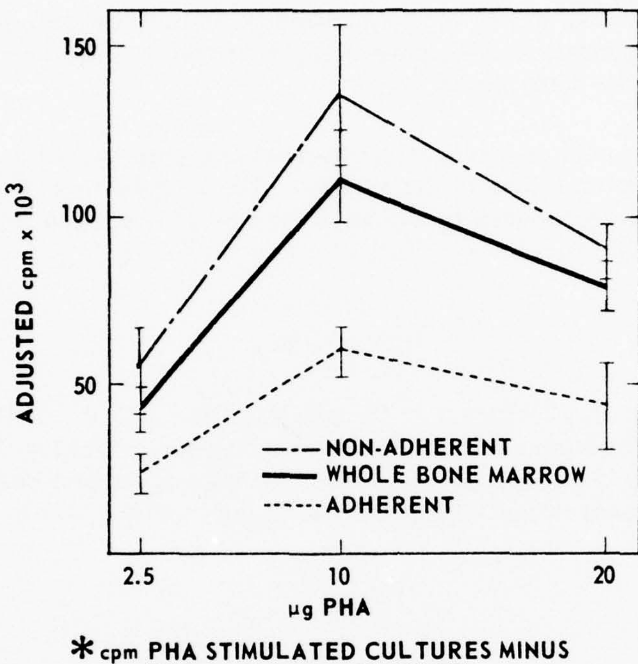

Figure 2. Adjusted counts per minute* of C57BL/6 bone marrow (2×10^6/culture) labeled with ^3H thymidine after incubation with phytohemagglutinin (PHA) for 3 days

The in vivo spleen colony forming unit assay (CFU-s) and the in vitro colony forming unit assay (CFU-c) were performed on the separated bone marrow. Based on total cell yield of each fraction, the greatest numbers of CFU-s and CFU-c were found in the adherent population. The adherent cell contents (per 10^5) of CFU-s and CFU-c were similar to the unseparated marrow cell population. The nonadherent cell content (per 10^5) of CFU-c was similar to the unseparated marrow cell population while there was an approximate twofold increase in CFU-s.

In preliminary experiments we found an increased survival in mice grafted with adherent cells. However in subsequent experiments we were unable to reproduce our initial findings. Even though myeloid cells were present in the adherent cell fraction and the phytohemagglutinin response decreased, mice engrafted with this cell population died with symptomatology of graft versus host disease as did mice given unseparated cells. Thus, while the technique of differential adherence of cells to glass beads may be of value in obviating acute graft versus host disease, it did not significantly obviate delayed secondary

disease in mice. The utility of this procedure on marrow cell populations containing greater quantities of immune competent cells, i.e., human bone marrow, remains to be determined.

This work is part of a research program designed to find a successful therapy for radiation injury in the soldier. The long-term goal of such work is to utilize such procedures to assist in therapy of combined injury and collateral damage.

REFERENCE

1. Gambrill, M. R., Ledney, G. D. and MacVittie, T. J. Mitigation of graft versus host disease in lethally irradiated mice grafted with spleen cells adherent to glass beads. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR76-5, 1976.

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INHIBITION OF LETHALITY IN ENDOTOXIN CHALLENGED MICE TREATED WITH ZINC CHLORIDE

Principal Investigators: *S. L. Snyder and R. I. Walker*

Wounds, burns and radiation injuries sustained by military personnel are usually aggravated by gram-negative bacteria which produce endotoxin. Labilization of lysosomal membranes resulting in the release of hydrolases and other mediators of inflammation is considered a central event in the pathogenesis of endotoxin shock. Therefore, agents known to have a stabilizing effect on the lysosomal membrane were tested for potential benefit in protecting patients against endotoxic shock. Previous investigators have demonstrated that zinc effectively stabilizes lysosomal membranes. This finding prompted us to investigate the use of zinc as a protective agent in endotoxic shock. We have found that zinc chloride effectively protects against the lethal effects of Salmonella typhosa endotoxin in B6CBF1 mice (Figure 3). Thus, when mice are injected intraperitoneally with zinc chloride at a dose of 0.40 mg/mouse 1 hour prior to challenge with endotoxin (0.75 mg), the 24-hour mortality was only 3 percent. This compares with 100 percent mortality found for the untreated controls. The circulating levels of β-glucuronidase

at 20 hours after endotoxin challenge are significantly depressed (approximately 50 percent) in mice protected with zinc relative to the group not protected with zinc. These results are consistent with the notion that zinc protects against the lethal effects of endotoxin by stabilizing lysosomal membranes and preventing the release of their harmful contents. The present research may lead to the development of better prophylaxis and more efficient treatment against endotoxemia.[1]

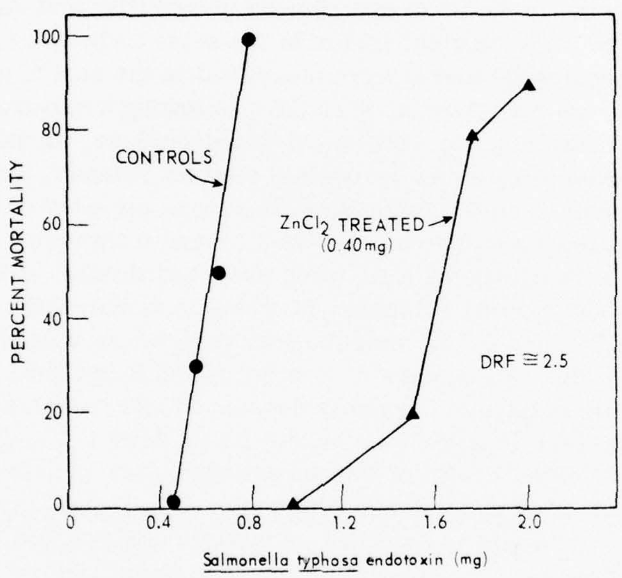

Figure 3. Mortality of control and zinc treated mice versus endotoxin dose

REFERENCE

1. Snyder, S. L. and Walker, R. I. Inhibition of lethality in endotoxin challenged mice treated with zinc chloride. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR76-14, 1976.

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RADIATION-INDUCED ALTERATIONS IN SERUM AND SPLENIC LYSOSOMAL HYDROLASES OF RATS

Principal Investigator: *S. L. Snyder*

Exposure to ionizing radiation may cause military personnel to experience periods of severe stress, nausea and debilitation. The onset of these symptoms as well as other manifestations of radiation sickness could be related to the release of certain proteolytic enzymes, called cathepsins, from lysosomes. In order to examine the possibility that lysosomal proteases might be an etiological factor in the acute radiation syndrome, the levels of cathepsins B1 and D were measured in the serum and spleen homogenates of rats for a period of 22 days following exposure to 1000 rads ^{60}Co gamma radiation. In addition, β-glucuronidase, an enzyme frequently employed to measure lysosomal enzyme release, was determined. The median level of serum β-glucuronidase was elevated only on day 4; significant decreases occurred on days 1, 2 and 9 through 22. Dramatic elevations in serum cathepsin B1 were observed through most of the investigation. The median cathepsin B1 value in serum was significantly elevated on days 3-6 and 9-15. Splenic β-glucuronidase was increased on day 1, and greatly elevated on days 3-7, after which it declined toward normal values. Splenic cathepsin D rapidly decreased and remained depressed. A biphasic increase in splenic cathepsin B1 on days 1-4 and 10-22 was also observed. The results of this investigation are consistent with the notion that activation and release of lysosomal hydrolases may be an important pathologic event in the later as well as early states of the acute radiation syndrome. Three possible mechanisms of injury evoked by radiation-induced changes in lysosomal hydrolases are suggested. The results of this investigation suggest that the release of lysosomal proteases may be a significant factor in radiation-induced tissue injury and stress. Therefore, the use of agents designed to suppress the release of lysosomal hydrolases and/or inhibit their action offers promise in the clinical management of radiation sickness.[1]

REFERENCE

1. Snyder, S. L. Radiation-induced alterations in serum and splenic lysosomal hydrolases of rats. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR76-50, 1976.

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CONTRIBUTION OF CIRCULATING BLOOD CELLS TO SURVIVAL OF MICE EXPERIENCING ENDOTOXEMIA

Principal Investigators: *R. I. Walker, S. L. Snyder, J. V. Moniot, AFRRI; and P. Z. Sobocinski, U. S. Army Medical Research Institute of Infectious Diseases*

Gram-negative bacterial infections are a major cause of death in individuals whose resistance has been compromised by wounds, irradiation, burns and other stresses. The response of circulating blood cells to gram-negative infections is hypothesized as a critical mechanism in survival.[1] The hypothesis that platelets and granulocytes play a significant role in mediating the lethal consequences of endotoxemia associated with gram-negative infections was evaluated by studying responses to endotoxin challenge in mice made leukopenic and thrombocytopenic by irradiation. Sensitivity to endotoxin entering the circulation from the intestine was increased in animals deficient in granulocytes and platelets. Alterations in blood enzyme levels measured during endotoxemia were different in nonirradiated and irradiated mice. The administration of 0.4 mg $ZnCl_2$ or 5 mg cortisone acetate prior to challenge with a lethal dose of Salmonella typhosa endotoxin provided significant protection against the toxin in nonirradiated mice, whereas only cortisone protected the irradiated animals. Failure of zinc to protect irradiated animals may be due to the absence of leukocytes and platelets. This phenomenon requires further study. Protection obtained with zinc may be due to attenuation of membrane-related activities of platelets and granulocytes. Zinc protection against endotoxin challenge in nonirradiated mice correlated with high blood levels of the ion. Administration of zinc increases the number of circulating leukocytes available at the time of endotoxin challenge, but cortisone promotes a later recovery of leukocyte numbers. Plasma glucose was elevated in zinc treated mice, but this elevation did not correlate with survival. Cortisone prevented increased levels of plasma urea ordinarily associated with endotoxemia. Zinc may enhance early damage to hepatocytes in endotoxin challenged animals. Cortisone reduced late damage in the liver. Cortisone apparently protects sites of endotoxin action other than blood components. Zinc, in contrast, appears to enhance survival primarily through action on circulating blood cells. Therefore, we believe that platelet and granulocyte transfusions may be useful in combating endotoxemia in personnel exposed to ionizing radiation.

REFERENCE

1. Walker, R. I., Snyder, S. L., Moniot, J. V. and Sobocinski, P. Z. Contribution of circulating blood cells to survival of mice experiencing endotoxemia. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR76-52, 1976.

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PROTECTION AGAINST ENDOTOXIN-INDUCED MORTALITY IN MICE TREATED WITH TRANSITION METAL SALTS

Principal Investigators: *S. L. Snyder, R. I. Walker and J. V. Moniot*

When a person's natural resistance is compromised by various traumas, he becomes extremely susceptible to life-threatening infections from his own resident flora or bacteria from exogenous sources. The toxic agent in many of these infections is a bacterial cell wall component called endotoxin. Irradiated individuals are particularly sensitive to this toxin. As part of an overall program for developing better prophylaxis and treatment for radiation injury, the protective effect of selected divalent and trivalent cations against the lethal effects of endotoxin from gram-negative bacteria was determined in mice. Manganese or chromium chloride salts given intraperitoneally 1 hour prior to challenge with a lethal dose of Salmonella typhosa endotoxin completely protected against death at 48 hours; other metal salts such as iron, copper, and cobalt were not effective (Table 4). It is possible that the mechanism of protection by manganese chloride is related to the attenuation of the release of lysosomal mediators. Chromium chloride, on the other hand, may directly attenuate the toxicity of the endotoxin molecule. These metals may be useful in determining the mechanisms associated with the action of endotoxin and could also be useful as therapeutic agents against the toxin. Thus, studies with manganese and chromium can lead to better treatment of the endotoxemia associated with gram-negative infections in human patients.

Table 4. Mortality of B6CBF1 Mice, at 48 Hours, Treated With Various Metal Salts and Challenged With Salmonella typhosa Endotoxin[a]

Metal Salts	% Mortality at dose (μmoles) specified[b]					
	0.74	1.47	2.94	4.41	5.88	8.82
NaCl (control)	--	90	--	--	--	--
$CrCl_3$	--	100	50	10	0[c]	--
$MnCl_2$	--	100	70	100	40	0[c]
$NiCl_2$	40	40[c]	70	80	--	--
$SnCl_2$	--	100	75[b]	100	--	--
$CoCl_2$	--	100	100[b]	100	--	--
$CuSO_4$	--	100	100	100	--	--
$FeCl_3$	--	80	100,	70	80	90
$FeSO_4$	--	100	100	100	--	--
$AuCl_3$	--	100	80	80	80	--

[a] Each mouse received 0.85 mg endotoxin intraperitoneally.
[b] In general, 10 mice were employed at each concentration, except where noted.
[c] Two separate experiments employed 10 mice per experiment.

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MITOGENIC AND COLONY FORMING UNIT RESPONSES OF SPLEEN CELLS FROM MICE ENGRAFTED WITH LEWIS LUNG CARCINOMA CELLS

Principal Investigators: *G. D. Ledney, J. V. Moniot, M. R. Gambrill and T. J. MacVittie*

The induction of malignant disease by radiation either alone or in combination with other severe stresses is a critical collateral damage problem. The mechanisms underlying the host's responses to malignancy are not well known. Thus an integrated study of the host's hematocytopoietic responses to malignancy was undertaken with the aid of an animal model.[1] Understanding and interpreting these responses is of critical value in determining (1) treatments that might obviate malignant cell induction or (2) treatments that will assist in the cure of the malignancy.

Certain nonleukemic, solid animal and human tumors not directly involved with the hematopoietic system are known to affect indirectly the various cellular compartments of the system. One such tumor, the Lewis lung (3LL) transplantable carcinoma, produces splenic enlargement in mice engrafted subcutaneously with a tumor cell inoculum. Thus, we hypothesized that the splenic enlargement was associated with myelocytopoietic and lymphocytopoietic changes that were dependent on (1) initial 3LL cell load, (2) time after engraftment with 3LL cells and (3) engrafted mouse strain.

The hypothesis was tested by engrafting both C57BL/6 male and B6CBF1 male mice with either 10^4, 5×10^4 or 2×10^5 viable 3LL tumor cells. Control treated mice received either no tumor cells or 2×10^5 irradiated 3LL cells. On days 3, 7 and 14 after subcutaneous engraftment, the following responses were measured simultaneously in replicate experiments: splenic enlargement and histologic appearance; spenic lymphocyte responses to phytohemagglutinin and to lipopolysaccharide; and splenic cell colony forming unit potentials as measured by the CFU-s and CFU-c assays.

In all tumor cell engrafted mice, the splenic weight was greater than that seen in control untreated mice. In each strain, the splenic weight increase was dependent on the number of engrafted tumor cells and the time after engraftment. Hyperplasia of the reticuloendothelial system was observed at all tumor-cell doses at all time intervals. Reticuloendothelial system hyperplasia tended to increase as a function of time and tumor-cell dose.

The incorporation of tritiated thymidine into splenic lymphocytes stimulated with phytohemagglutinin was reduced in all mice engrafted with tumor cells (Table 5). There was a strain difference in response to this mitogen that was dependent upon the tumor-cell load and time after tumor-cell engraftment.

Table 5. Counts Per Minute (x 10^3) of PHA-Stimulated Spleen Cells Obtained From Mice After Engraftment With 3LL Carcinoma Cells*

DAYS AFTER ENGRAFTMENT WITH 3LL TUMOR CELLS	NUMBER OF 3LL CELLS INJECTED S.C. INTO MICE					AVERAGE CPM PER DAY OF ENGRAFTMENT
	0	IRRADIATED 2×10^5	1×10^4	5×10^4	2×10^5	
3	534 ± 47 (4)	374 ± 37 (4)	303 ± 74 (4)	323 ± 56 (4)	246 ± 67 (4)	356 ± 32 (20)
7	494 ± 61 (4)	440 ± 57 (4)	405 ± 38 (4)	414 ± 27 (4)	360 ± 66 (4)	423 ± 23 (20)
14	510 ± 39 (4)	389 ± 60 (4)	481 ± 50 (4)	402 ± 45 (4)	242 ± 40 (4)	405 ± 29 (20)
AVERAGE CPM PER ENGRAFTED TUMOR-CELL DOSE	513 ± 26 (12)	401 ± 28 (12)	396 ± 37 (12)	379 ± 28 (12)	283 ± 35 (12)	

* EACH VALUE IS THE MEAN ± S.E. OF THE MEANS OF TWO TRIALS EACH WITH C57Bl/6 AND B6CBF1 MICE. NUMBER IN ( ) IS THE NUMBER OF TRIALS.

Tritiated thymidine incorporation into splenic lymphocytes stimulated with lipopolysaccharide was increased in all mice engrafted with tumor cells. There was a difference in mouse strain responses to lipopolysaccharide, but no differences were associated with the various tumor-cell doses and times after engraftment.

The number of CFU-s was marginally increased and was dependent upon the dose and time of tumor-cell administration and the mouse strain engrafted. CFU-c growth of spleen cells obtained from tumor-cell engrafted mice was increased at all time intervals and tumor-cell doses tested. Generally, the CFU-c growth of the spleen cells of both mouse strains injected with tumor cells increased as the tumor cell dose and time increased (Table 6).

Myelocytopoietic and lymphocytopoietic changes observed in the splenic cell populations of mice engrafted with the 3LL carcinoma were dependent on (1) initial tumor-cell load, (2) time after engraftment with tumor cells and (3) engrafted mouse strain. The data support the contention that investigators using 3LL transplantable carcinoma systems designed to evaluate curability of primary tumors and/or lung metastasis by either chemotherapy, radiotherapy, or adoptive immunotherapy should consider the above observations when interpreting their data.

Table 6. Agar Colonies (CFU-c) Formed Per 10^6 Spleen Cells Obtained From Mice After Engraftment With 3LL Carcinoma Cells*

DAYS AFTER ENGRAFTMENT WITH 3LL TUMOR CELLS	NUMBER OF 3LL CELLS INJECTED S.C. INTO MICE					AVERAGE NO. CFU-c PER 10^6 SPLEEN CELLS PER DAY OF ENGRAFTMENT
	0	IRRADIATED 2×10^5	1×10^4	5×10^4	2×10^5	
3	24 ± 3 (2)	61 ± 33 (2)	37 ± 1 (2)	40 ± 0 (1)	83 ± 26 (2)	50 ± 10 (9)
7	20 ± 9 (3)	66 ± 31 (3)	31 ± 13 (3)	60 ± 32 (3)	81 ± 40 (3)	51 ± 12 (15)
14	19 ± 5 (4)	100 ± 15 (4)	24 ± 6 (4)	78 ± 13 (4)	102 ± 17 (4)	65 ± 10 (20)
AVERAGE NO. CFU-s PER 10^6 SPLEEN CELLS PER DOSE OF ENGRAFTED TUMOR CELLS	20 ± 3 (9)	80 ± 14 (9)	29 ± 5 (9)	67 ± 13 (9)	91 ± 15 (9)	

* EACH VALUE IS THE MEAN ± S.E. OF THE MEAN OF THE NUMBER OF EXPERIMENTS LISTED IN ( ) AND IS BASED ON AT LEAST ONE TRIAL WITH C57Bl/6 AND B6CBF1 MICE.

This work is part of a collateral damage research program designed to understand the blood-forming tissue alterations produced by substances released from nonblood cell formed malignancies. The long-term practical goal of such work is to utilize such substances to promote blood-cell recovery in soldiers after exposure to radiation or cytotoxic drugs.

REFERENCE

1. Ledney, G. D., Moniot, J. V., Gambrill, M. R., MacVittie, T. J. and Levin, S. G. Mitogenic and colony forming unit responses of spleen cells from mice engrafted with Lewis lung (3LL) carcinoma cells. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR76-49, 1976.

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MATURATION AND DIFFERENTIATION OF IMMUNE COMPETENCE IN MOUSE RADIATION CHIMERAS

Principal Investigators: *R. M. Crawford and G. D. Ledney*

Military personnel exposed to lethal doses of radiation may only be rescued from death by bone marrow cell replacement. Such replacement therapy, when successful, results in recovery of the immune system that is not similar to that found in the host prior to exposure to radiation. This research then is part of a program to identify the problems of immune restoration in individuals exposed to radiation alone or in combination with other stresses.

Radiation chimeras, lethally irradiated animals transplanted with hemopoietic cells, will survive indefinitely if donor and host animal are genetically similar (syngeneic). However, genetic differences between donor and host animal usually lead to complications, such as graft versus host disease and infection, which culminate in the death of the host. Immune recovery, important to the health and survival of these animals, may be impeded by such complications. Therefore, we hypothesized that recovery of a fully competent immune system in the host animal is dependent on donor and host histocompatibility.

Reconstitution of immune competence was studied in CBA ($H\text{-}2^k$) and (C57BL/6 x CBA) F1 ($H\text{-}2^b$ x $H\text{-}2^k$) mice following lethal irradiation and engraftment of 5×10^6 CBA bone marrow cells. At monthly intervals, for three successive months, following engraftment, immune reactivity of spleen cells harvested from chimeras was measured by (1) *in vitro* responsiveness to phytohemagglutinin and lipopolysaccharide, (2) *in vitro* mixed lymphocyte reaction, (3) *in vivo* graft versus host reaction, and (4) skin allograft rejection.

Maturation of immune reactivity occurred faster in syngeneic chimeras than in semiallogeneic chimeras as observed by the higher levels of phytohemagglutinin and lipopolysaccharide stimulation 1 month after transplantation (Figure 4). However, 3 months after transplantation, the two groups were indistinguishable in their responses to mitogens and third party (BALB/C $H\text{-}2^d$) antigens. Still, responses were only 60-70 percent of normal CBA controls by 3 months. Spleen cells from semiallogeneic chimeras were unresponsive, or tolerant, to host (B6CBF1) C57BL/6 H-2 antigens when tested in the mixed lymphocyte reaction and graft versus host reaction. Likewise, when these spleen cells were tested against parental C57BL/6 H-2 antigens, the mixed lymphocyte reaction and skin allograft rejection test supported the idea that these cells were tolerant to C57BL/6 H-2 antigens. However, the graft versus host reaction supported the idea that these cells were immunologically responsive to C57BL/6 H-2 antigens. To summarize, the reconstitution of immune competence and ultimate immunological status of both types of chimeras

appeared to be similar, except for the specific unresponsiveness of host (B6CBF1) C57BL/6 H-2 antigens induced in the semiallogeneic combination.

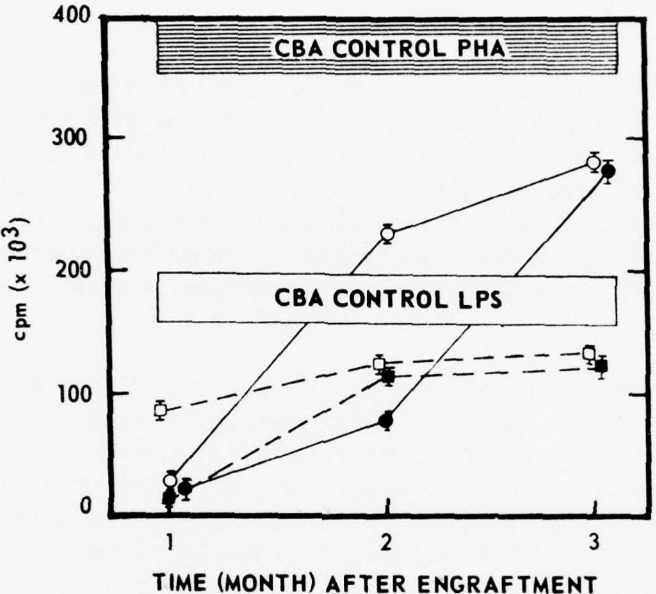

Figure 4. Counts per minute (cpm) of normal CBA or chimeric spleen cells (SPC) stimulated with either phytohemagglutinin (PHA) or lipopolysaccharide (LPS). The symbols and lines used are (1) o——o chimeric CBA SPC and PHA, (2) •——• chimeric B6CBF1 SPC and PHA, (3) □----□ chimeric CBA SPC and LPS and (4) ■----■ chimeric B6CBF1 SPC and LPS. Normal CBA spleen cell responses to PHA ▤ and LPS □ are presented in the figure. Each point represents the mean of two replicate experiments ± S.E. Triplicate cultures were performed for each point in each replicate.

This work is part of a research program designed to investigate, understand and resolve the immunological problems associated with accidental or intentional exposure to ionizing radiation in military personnel. The long-term practical goal of this work is to use bone marrow replacement therapy to abate immune dysfunction and its precipitating complications in the radiation injured soldier.

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PERITONEAL MACROPHAGE CHEMOTAXIS AND ACCUMULATION IN MICE INJECTED WITH LEWIS LUNG CARCINOMA CELLS

Principal Investigators: *J. M. Sheil, R. M. Crawford and G. D. Ledney*

Tumor development, especially in the lung, is one of the likely complications of radiation injury in the soldier. We used the in vitro macrophage chemotaxis assay to investigate changes in macrophage migration capability which occur in the host undergoing malignancy. In neoplasia, the inhibition of chemotaxis may be responsible in part for establishment of a tumor in the host. We hypothesized that peritoneal macrophages of tumor cell injected mice were less capable of chemotaxis than normal macrophages, despite an overall increase in number.

This hypothesis was tested by using the Lewis lung (3LL) carcinoma, maintained in C57BL/6 mice. Experimental groups, each containing 12 C57BL/6 male mice aged 10-12 weeks, were inoculated subcutaneously with either 2×10^5 viable 3LL cells, 1×10^4 viable 3LL cells, or 2×10^5 irradiated 3LL cells. On days 3, 7, and 14 after injection with tumor cells, we measured the following: (1) in vitro chemotaxis and (2) number of peritoneal macrophages recovered. The calculated chemotactic response (the number of macrophages per mouse capable of chemotaxis) was estimated by dividing the number of macrophages collected by the number used in the assay and multiplying that figure by the in vitro chemotactic response.

The in vitro chemotactic response was dependent on the dose of 3LL cells injected into mice and the time of macrophage collection after injection. The response was depressed in all groups on day 3. On day 7, the response remained depressed in each group but to a lesser extent than on day 3. On day 14, chemotaxis was enhanced in mice given 2×10^5 viable or irradiated 3LL cells, while that of the low dose tumor cell injected group remained near that seen on day 7.

We observed a slight increase in peritoneal macrophage accumulation, which was most noticeable in mice given 2×10^5 viable 3LL cells (Table 7). This increase, however, did not significantly vary from normal.

We combined the in vitro chemotaxis measurement with peritoneal macrophage accumulation to determine the calculated chemotactic response. This reflects more accurately changes in macrophage function in the host. In mice given 2×10^5 viable 3LL cells, the response was depressed 37 percent on day 3 and 21 percent on day 7, but was enhanced 53 percent by day 14. In mice given 1×10^4 viable 3LL cells, the response was depressed 54 percent on day

3, 44 percent on day 7, and 27 percent on day 14. In mice given 2 x 10^5 irradiated 3LL cells, the response was inhibited 35 percent on day 3, 5 percent on day 7, and was enhanced 23 percent on day 14 (Table 8).

Table 7. Peritoneal Macrophage Accumulation in C57BL/6 Mice Engrafted With Lewis Lung (3LL) Carcinoma Cells*

Group	Number of macrophages collected/ mouse (x 10^5)*		
	Day 3	Day 7	Day 14
2 x 10^5 viable 3LL cells	2.80 ± 0.17	2.60 ± 0.20	2.55 ± 0.17
1 x 10^4 viable 3LL cells	2.53 ± 0.34	2.30 ± 0.10	2.57 ± 0.07
2 x 10^5 irradiated 3LL cells	2.33 ± 0.39	2.45 ± 0.25	2.27 ± 0.18
Normal, untreated	2.23 ± 0.24	2.20 ± 0.10	2.17 ± 0.13

* Represents the mean S.E. per mouse from three replicates; determined from a sample of peritoneal fluid pooled from three mice per group for each replicate

Table 8. Peritoneal Macrophage Chemotactic Response: A Comparison Between the *In Vitro* Chemotaxis Measurement and the Calculated Chemotaxis Determination

Group	Days	*In vitro*	CRI*	Calculated†	CRI
2 x 10^5 viable 3LL cells	3	255 ± 19	0.51	1779	0.63
	7	373 ± 5	0.66	2457	0.79
	14	843 ± 191	1.42	5422	1.53
1 x 10^4 viable 3LL cells	3	204 ± 20	0.41	1294	0.46
	7	318 ± 38	0.56	1845	0.56
	14	370 ± 27	0.62	2368	0.73
2 x 10^5 irradiated 3LL cells	3	312 ± 18	0.63	1841	0.65
	7	477 ± 62	0.84	2948	0.95
	14	713 ± 56	1.20	3990	1.23
Normal, untreated	3	497 ± 14	1.00	2825	1.00
	7	566 ± 26	1.00	3102	1.00
	14	595 ± 53	1.00	3243	1.00

* CRI - chemotactic response index, determined by dividing the experimental chemotactic response by the normal chemotactic response.

† The calculated chemotactic response reflects the total number of peritoneal macrophages on a per mouse basis that are capable of responding in the chemotaxis assay, as determined by combining the accumulation response and the *in vitro* chemotaxis response.

In conclusion, we observed a significant inhibition of peritoneal macrophage chemotaxis at the initial stages of tumor development, followed by an increased chemotactic capability in later stages of tumor growth. Thus, while normal or enhanced chemotactic responses may be measured during later stages of tumor growth, the earlier suppression of this macrophage function may allow for the initial establishment and continued growth of a tumor in the host.

The long-term complications of radiation injury in the soldier are frequently characterized by the development of various malignancies, especially lung tumors. Thus, one of our primary objectives in this study was to monitor one aspect of macrophage involvement in the host's defense against neoplasia and to apply this information to the detection and treatment of these radiation injury complications in the soldier.

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CYCLOTRIMETHYLENETRINITRAMINE-INDUCED ULTRASTRUCTURAL CHANGES IN RAT LIVER AND KIDNEY

Principal Investigators: *J. E. French, S. L. Bradley, N. R. Schneider, AFRRI; M. E. Andersen and L. J. Jenkins, Jr., Naval Medical Research Institute*

Cyclotrimethylenetrinitramine (RDX) is used as an explosive. The manufacture and formulation of RDX poses an occupational hazard and is a potential environmental contaminant when it is used.

The toxicity, tissue distribution, and metabolism of RDX have been studied. Yet little information is available on morphological changes that might be attributed to it. Because RDX is a potential occupational or environmental contaminant, such information is necessary for the overall evaluation of RDX toxicity. The ultrastructure of rat liver and kidney was examined 24, 48 and 120 hours after dosing with 100 mg of RDX/kg per os. Conventional transmission electron micrographs revealed hepatocytes with dilation of the rough endoplasmic reticulum, mitochondrial swelling, and the presence of concentric membrane arrays 24 hours after RDX administration. Renal ultrastructural alteration was apparently restricted to the distal convoluted tubular cells. No consistent variation in ultrastructure was observed in glomerular cells, proximal convoluted tubular cells, and collecting tubular cells. The presence of

erythrocytes in the nephron tubules indicated hematuria. By 48 hours, hepatocyte alteration was similar and of the same magnitude, except for the proliferation of smooth endoplasmic reticulum. No consistent effects were observed on renal ultrastructure at 48 or 120 hours. At 120 hours, the characteristic hepatocyte alterations as described above persisted and smooth endoplasmic reticulum proliferation had increased dramatically. RDX effects on renal tissues at this dose level were minimal and transient, whereas hepatocyte alteration persisted, with the proliferation of smooth endoplasmic reticulum a dominant feature, indicating the possible induction of the mixed function oxidase system.

In order to develop diagnostic tests and methods of treatment it is necessary to understand the mechanism of toxicity and pathogenesis. In order to meet these goals and to aid in establishing allowable environmental levels, the RDX-induced liver and kidney pathology was studied in this animal model system.

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TOXICITY OF POLYCHLORINATED BIPHENYLS TO EUGLENA GRACILIS: CELL POPULATION GROWTH, CARBON FIXATION, CHLOROPHYLL LEVEL, OXYGEN CONSUMPTION, AND PROTEIN AND NUCLEIC ACID SYNTHESIS

Principal Investigators: *W. G. Ewald, J. E. French, AFRRI; and M. A. Champ, American University*

Combined damage resulting from irradiation and/or chemical exposure is of utmost concern to the military. Accidental introduction of chemicals into our environment can cause both ecological damage that is severely felt and undesirable toxic reactions and/or body burdens in both military personnel and collaterally involved civilian populations. It is important to monitor the effects of potential contaminants and to develop model systems in order to study and establish threshold levels for such contaminants.

Populations of *Euglena gracilis* in exponential growth under light were exposed to 2.5, 5.0, 7.5 and 10 ppm of Aroclor 1221 (a mixture of polychlorinated biphenyls). The $ID_{50/48}$ of Aroclor 1221 was estimated to be 4.4 ppm, while Aroclor 1232 tested at 20, 35, 50 and 100 ppm resulted in an $ID_{50/48}$ of 55 ppm. With Aroclor 1242, no inhibition of growth was observed with up to 100 ppm exposure. Cell cultures exposed to 4.4 ppm of Aroclor 1221 for 48 hours had a significantly reduced rate of carbon fixation and reduced levels of chlorophyll

after correction for cell density. Oxygen consumption was not affected at the ID_{50} level of the Aroclor. Uptake of [^{3}H]-leucine in treated culture was twice that of controls, and [^{3}H]-uridine uptake was significantly lower. Uptake of [^{3}H]-thymidine and incorporation of [^{3}H]-leucine, [^{3}H]-thymidine, and [^{3}H]-uridine were not significantly different in treated and control cultures. These results suggest that at the ID_{50} level, polychlorinated biphenyls reduce cell population growth in Euglena gracilis by inhibition of photosynthesis and/or chlorophyll production.[1]

An inexpensive and rapid screening method for potential toxic environmental chemical compounds used by military organizations is of great importance in case such compounds are accidently introduced into the environment. To meet these requirements, such a method was developed using the aquatic protozoan Euglena gracilis as a sensitive indicator.

REFERENCE

1. Ewald, W. G., French, J. E. and Champ, M. A. Toxicity of polychlorinated biphenyls to Euglena gracilis: cell population growth, carbon fixation, chlorophyll level, oxygen consumption, and protein and nucleic acid synthesis. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR76-33, 1976.

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EFFECTS OF DDT AND POLYCHLORINATED BIPHENYLS ON CELLULAR METABOLISM AND ULTRASTRUCTURE OF CRITHIDIA FASCICULATA, A FLAGELLATED PROTOZOAN

Principal Investigators: *J. E. French, AFRRI; and J. F. Roberts, North Carolina State University*

The morbidity due to body burdens of environmental chemical contaminants in stressful situations is of potential importance but has received little attention. Since the mechanism(s) of toxicity to most chemical contaminants is unknown, this information must be acquired in order to test and evaluate potential toxic chemicals.

In a previous report, DDT and polychlorinated biphenyls (PCBs) have been shown to be toxic to Crithidia fasciculata by inhibition of cell population growth.[1] These results suggested that further studies were required for understanding the cell population growth inhibition. Energy metabolism, protein and nucleic acid biosynthesis, and ultrastructure were chosen as indices for determining the metabolic site(s) of this inhibition. DDT had no effect on [^{14}C] carbon dioxide evolution, whereas PCB exposure caused a transient inhibition and then stimulation after 6 and 24 hours, respectively. After 6 hours exposure to DDT or PCB, inhibition of both uptake and incorporation of thymidine and uridine, but not of L-leucine, was observed. By 24 hours of PCB exposure, uptake and incorporation of these three radioprecursors was two to three times greater than control. After 24 hours DDT exposure, only thymidine uptake and incorporation exhibited such an increase. Ultrastructural changes induced by these compounds included cellular and mitochondrial swelling disruption of the mitochondrial genophore, and failure of the mitochondrion to replicate in synchrony with cell division. It is concluded that the initial DDT or polychlorinated biphenyl inhibition of nucleic acid biosynthesis and subsequent loss of cell regulatory capacity causes the decrease in cell population growth.[2]

An inexpensive and rapid screening method for toxic chemical compounds used by military organizations is of great importance. To meet these requirements, such a method was developed using the protozoan Crithidia fasciculata. These studies provide necessary information for recognizing toxic agents and a basis for studying morbidity due to toxic reactions.

REFERENCES

1. French, J. E. and Roberts, J. F. Effect of DDT and polychorinated biphenyls on cell population growth of Crithidia fasciculata, a flagellated protozoan. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR76-21, 1976.

2. French, J. E. and Roberts, J. F. Effects of DDT and polychorinated biphenyls on cellular metabolism and ultrastructure of Crithidia fasciculata, a flagellated protozoan. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR76-31, 1976.

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VINYLIDENE CHLORIDE-INDUCED ULTRASTRUCTURAL CHANGES IN RAT LIVER

Principal Investigators: *J. E. French, AFRRI; M. E. Andersen and L. J. Jenkins, Jr., Naval Medical Research Institute*

Vinyl chloride and vinylidene chloride (1,1-dichloroethylene, DCE) are closely related chemicals and are known occupational and environmental contaminants. These toxic compounds have been identified in submarine and space craft environments and are of military importance. The pathobiological effects after oral administration of DCE (in corn oil) to fasted male Holtzman rats were determined by correlating the ultrastructural damage to the liver with changes in the serum levels of glutamic-pyruvic transaminase (SGPT) and glutamic-oxaloacetic transaminase (SGOT). In a completely randomized study, fasted rats received a single oral dose of 40 or 80 mg DCE/kg of body weight. After 0, 1, 2, 4, and 8 hours, SGPT and SGOT values were determined and liver tissue samples were immersion fixed in 3 percent cacodylate buffered glutaraldehyde, postfixed in osmium tetroxide and prepared by conventional electron microscopy methods.

SGPT and SGOT values increased dramatically according to dosage and time of exposure in a linear manner, which indicated significant hepatic damage. The appearance of myelin-like bodies occurred at a similar frequency in both control and treated rats. Control rats were also characterized by slightly dilated rough endoplasmic reticulum and perinuclear cisternae. However, DCE-exposed rats showed significant dilation of the rough endoplasmic reticulum, loss of ribosomes, mitochondrial swelling, loss of cristae and chromatinolysis in a dose and time related manner. Margination of the chromatin along the nuclear envelope (chromatinorrhexis) did not occur within this time period of exposure and dosage of DCE. DCE is a very hepatoxic xenobiotic and its pathobiology may be different according to the route of administration.

In order to develop diagnostic tests and methods of treatment, it is necessary to understand the mechanism of pathogenesis. To meet these goals the mechanisms of vinyl and vinylidene chloride toxicity and pathogenesis are being studied in this animal model system.

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METABOLISM OF [^{14}C] CYCLOTRIMETHYLENETRINITRAMINE IN THE RAT

Principal Investigators: *N. R. Schneider, S. L. Bradley, AFRRI; and M. E. Andersen, Naval Medical Research Institute*

Cyclotrimethylenetrinitramine (RDX) is a high explosive widely employed by the military. Exposure of humans to this compound (field personnel, formulators or noncombatants) can produce a toxic reaction. It is therefore important to determine the possible routes and mechanisms of metabolism of this compound in order to evaluate its occupational and environmental danger and potential long-term effects.[1]

RDX toxicity and tissue distribution were reported previously, but little data are available concerning its metabolism. In the rat, [^{14}C]-RDX (50 mg RDX/kg) administered per os in dimethylsulfoxide was slowly absorbed from the gastrointestinal tract: 36.8 ± 9.0 percent(3) of the total radioactivity was still present at 24 hours, but less than 3 percent was present at 48 hours. In the first 6 days after dosing rats with 50 mg unlabeled RDX/kg, however, only 0.69 ± 0.05 percent(8) of the dose was excreted in the feces. The total fecal dry mass per day from rats dosed per os with 50 mg RDX/kg was only 25 percent of the normal value for the first 2 days after administration. During the 4 days following per os administration of 40 mg [^{14}C]-RDX/kg, 42.7 ± 2.2 percent(10) of the radioactivity was excreted via the lungs as $^{14}CO_2$, 2-3 percent in the feces, and 34.0 ± 0.9 percent(10) in the urine; 9.5 ± 0.3 percent(9) remained in the carcass (Figure 5). The majority

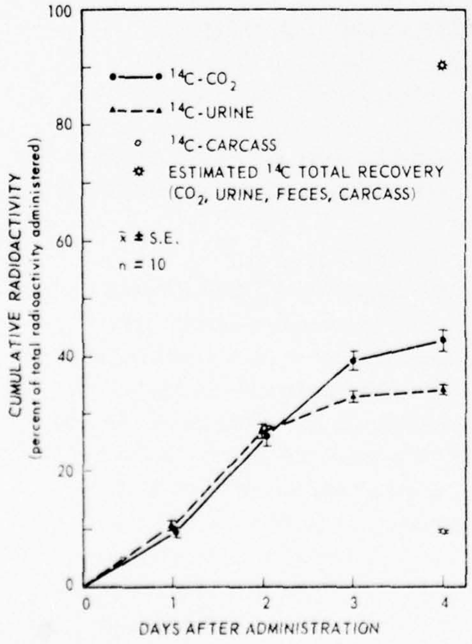

Figure 5.
Recovery of radioactivity from rats dosed by gavage with 50 mg ^{14}C-RDX/mg

of the label found in the feces, urine and carcass was not associated with unmetabolized RDX. At 24 hours after per os administration of 50 mg [^{14}C]-RDX/kg, both the liver and urine contained larger amounts of ^{14}C than could be accounted for on the basis of the amount of RDX in these samples. Furthermore, phenobarbital-induced rats metabolized RDX more rapidly than did noninduced rats. It is clear then that RDX, contrary to earlier findings, is extensively metabolized by the rat and probably also by humans presumably by enzymes of the liver, and is predominantly excreted via the lungs and urine. It does not appear that a cumulative body burden of this compound would be established.

REFERENCE

1. Schneider, N. R., Bradley, S. L. and Andersen, M. E. Toxicology of cyclotrimethylenetrinitramine (RDX): distribution and metabolism in the rat and the miniature swine. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR76-34, 1976.

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USE OF CENTRIFUGAL ELUTRIATION IN BLOOD CELL SEPARATIONS

Principal Investigators: *M. P. Grissom, J. E. French and W. G. Ewald*

The principle of centrifugal elutriation offers promise as a new method for collection of granulocytes for transfusion therapy in combating microbial infections.

Centrifugal elutriation was used to separate and collect granulocytes and monocytes from normal canine blood. The Beckman JE-6 elutriator rotor system with modifications was used in these studies. A vessel acting as a flow integrator was added to reduce pulsatile flow patterns produced by the peristaltic pump. In order to operate accurately at low flow rates (4-10 ml/min), a rheostat and narrow diameter (I.D. = 4 mm) tubing were used. The blood sample was mixed in a 50-ml siliconized glass flask with a stirring magnet. The elutriation medium was composed of Hanks' balanced salt solution minus Ca^{++} and Mg^{++}, Hepes buffer, 15 percent acid citrate dextrose and 0.1 percent basal serum albumin. The medium was adjusted to pH 7.4 and final osmolarity determined to be 340 mosm. This medium

has been found to reduce the adherence of polymorphonuclear granulocytes to tubing and vessel walls within the system. Experiments to date have shown the system to be effective in the separation of phagocytes. For 13 samples, the phagocyte to lymphocyte ratio went from 3:1 for whole blood to 18:1 for the elutriated fraction. The differential counts of the elutriated cells were compared with the original whole blood samples. It was determined through paired single tailed t-tests that enrichment in polymorphonuclear granulocytes ($p = .995$), depletion in lymphocytes ($p = .995$) and relative depletion in monocytes ($p = .995$) were all highly significant. Injection of whole blood volumes of less than 30 ml had significantly greater ($p = .975$) polymorphonuclear granulocyte enrichment in the elutriate than did whole blood volumes greater than 40 ml. To examine the handling of larger volumes (~125 ml) of blood with the system without significantly increasing the time required as compared to whole blood of less than 30 ml volume ($p < 0.80$), red blood cells were sedimented with Hypaque-Dextran and the white blood cell concentrate was elutriated. A significant decline in percent polymorphonuclear granulocyte recovery resulted in sedimented samples ($p = 0.95$) as compared with small whole blood samples. However, a much larger absolute number of phagocytes were recovered ($\bar{x} = 1.5 \times 10^8$ cells, $n = 4$) as compared to nonsedimented whole blood ($\bar{x} = 4.4 \times 10^7$ cells, $n = 5$). Total percent polymorphonuclear granulocytes recovered for whole blood (<30 ml) was 47.5 ± 8.8 and for presedimented blood (<125 ml) was 24.7 ± 10.0. The mean running time for the presedimented samples was 70.8 and for whole blood (<30 ml) 94.6 min. Maximum percent recoveries of elutriated cells to date have been 77.7 percent of polymorphonuclear granulocytes and 69.9 percent of all white blood cells. Minimum lymphocyte contamination was 0.09 percent.

Improvement of granulocyte collection methods for purity and viability would increase the chances for minimizing freeze-thaw damage and increase the clinical effectiveness of granulocyte transfusions. In working toward these goals, the centrifugal elutriation system is being studied for use in granulocyte and stem cell collection.

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LIQUID PRESERVATION OF DOG GRANULOCYTES AT 4°C IN VARIOUS STORAGE MEDIA

Principal Investigators: *J. E. French, W. J. Flor and M. P. Grissom*

Transfusion of sufficient numbers of freshly isolated granulocytes has been shown to be effective in granulocytopenic crises arising in patients with radiation and/or drug-induced bone marrow aplasia, leukemia or aplastic anemia. Few studies have investigated the loss of viability with time or the optimal liquid storage media after cell isolation and prior to use.

The feasibility of short-term liquid storage of dog granulocytes was investigated. Although liquid stored (4°C) leukocytes are thought to have a short shelf life (2-3 days), recent evidence suggests a considerably longer in vitro viability[1,2] when maintained under certain conditions.

Dog granulocytes were isolated from whole blood by defibrination or anticoagulation with 15 percent acid citrate dextrose (NIH, Formula A) and sedimentation on 2:1 Dextran-Isopaque (6 percent and 33.9 percent, respectively). Leukocytes were either washed (Hanks' balanced salt solution minus Ca^{++} and Mg^{++}) by centrifugation two times and resuspended in 40 percent Eagle's minimum essential medium, 40 percent Hanks' balanced salt solution and 20 percent autologous serum (4:4:2 medium) or remained in autologous serum (after defibrination) or acid citrate dextrose plasma without centrifugation. Leukocytes were stored at 4°C from 0 to 7 days.

The cell loss, bactericidal activity, stimulated O_2 consumption and fine structure were determined at various intervals. Polymorphonuclear granulocyte loss was greatest in defibrinated or acid citrate dextrose-anticoagulated and washed preparations (up to 62 percent after 7 days storage). Cell loss of polymorphonuclear granulocytes held in acid citrate dextrose plasma was never greater than 20 percent after 7 days storage. The percent polymorphonuclear granulocytes in the leukocyte population remained high in all preparations (53-64 percent) over the 7-day period. Bactericidal activity was greatest in polymorphonuclear granulocytes held in acid citrate dextrose plasma. Inhibition of intracellular viable growth of Escherichia coli decreased only slightly in freshly isolated cells after 7 days storage (from 99.8 percent to 97.7 percent). Polymorphonuclear granulocytes isolated by defibrination or acid citrate dextrose anticoagulation decreased significantly ($p < 0.01$) to 80 percent inhibition over the 7-day period. Oxygen consumption associated with phagocytosis in polymorphonuclear granulocytes isolated by defibrination, and acid citrate dextrose anticoagulation with washing and without washing were 84.0, 79.4 and 65.9 percent of the time zero (T_0) control values after 7 days storage. After 14 days, the respective values were 49.0, 37.2 and 63.5

percent of T_O control values. Polymorphonuclear granulocytes isolated by defibrination and stored in serum showed the greatest magnitude of response, but the percent control values were of a similar magnitude. The ultrastructure of polymorphonuclear granulocytes isolated and stored as described was best preserved in the acid citrate dextrose plasma held cells. Many polymorphonuclear granulocytes held in serum after defibrination or 4:4:2 medium after acid citrate dextrose anticoagulation and washing were within a normal range of morphologic appearance, but a greater number showed advanced pyknosis, perinuclear swelling, degranulation and vacuolation.

In conclusion, in vitro viability and structure analysis indicate that dog granulocytes can survive liquid storage for 7 days and that their structure and in vitro function remain intact to a remarkable degree. Acid citrate dextrose anticoagulation and storage at 4°C in acid citrate dextrose plasma is concluded to be the best method for dog polymorphonuclear granulocyte liquid preservation tested to date.

An effective protocol for maintaining clinical effectiveness of granulocytes after isolation and prior to use or freeze preservation would enable standardized procedures to be formulated. It would increase the availability of this postirradiation therapy by minimizing logistical problems (cell typing, matching, transportation, etc.). In order to meet these requirements, this model system was developed for studying granulocyte and stem cell preservation methods.

REFERENCES

1. Crowley, J. P. and Valeri, C. R. Recovery and function of granulocytes stored in plasma at 4°C for one week. Transfusion 14:574-580, 1974.

2. McCullough, J., Carter, S. J. and Quie, P. G. Effects of anticoagulants and storage on granulocyte function in bank blood. Blood 43:207-217, 1974.

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BIOCHEMISTRY DEPARTMENT

The Biochemistry Department was formed on 1 July 1976 to provide more intensive management for and centralization of the increasing biochemical based programs. Personnel from the Biochemistry Division of the disestablished Radiation Biology Department became the core of a new Physiological Chemistry Division, and the Neurochemistry Division of the Neurobiology Department was the basis of a new Molecular Biology Division. For completeness, all studies that were done under the previous organization are presented here, with the exception of the old Neurochemistry Division.

The primary effort of the Biochemistry Department is concerned with developing a better understanding of damage mechanisms in biological systems exposed to all types of radiation, providing more effective methods for detecting and evaluating changes in physiological systems and organ functions, and relating the physical factors of radiation to subsequent injury in man. A number of approaches, models and techniques are utilized with special emphasis upon determining the biochemical indicators of radiation injury.

Programs in the Department are divided into studies of biological changes following exposure to either ionizing or nonionizing radiation. In the ionizing radiation field, investigations continue on elevations in serum glycoproteins and protein-bound carbohydrates as significant indicators of radiation injury, trauma and other disease states. Collaborative studies with the National Cancer Institute and the National Naval Medical Center have yielded substantive findings showing a direct relationship of tumor burden with protein-bound carbohydrates as well as increased levels of acute-phase proteins. Further studies using an animal tumor model are underway to determine the responsible mechanism and to aid in relating the changes to possible radiation injury applications. Initial studies on the effect of radiation exposure on gallium-67 binding in serum indicate a dose dependent relationship over a wide range; these will be expanded to include a number of normal trace metals. Additionally, studies on the use of organometallic liquids as potential dosimetric materials for pulsed ionizing radiation were completed.

Two operationally oriented research projects funded by the U. S. Navy concern the bioeffects as a result of exposure to nonionizing radiation. The initial study of the effects of exposure to extremely low frequency (45 Hz) electric fields with emphasis on serum biochemical parameters, growth rates and histopathological changes has been completed. Research on the possible biochemical indicator of subclinical alterations due to microwave radiation is continuing. Goals include the determination of the threshold for cataract

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formation, a molecular model for the lens cataract and exploitation of this model in ionizing radiation exposures.

Based upon the operational requirement in military nuclear medicine to provide more effective methods for evaluating changes in physiological and organ functions in various forms of trauma, the former Radiological Sciences Division continued its efforts in radiopharmaceutical development and evaluation, instrumentation and isotope applications and pathophysiologic investigations. In collaboration with the National Institutes of Health, the Washington University Medical Center, St. Louis, Missouri, and the North Carolina Medical School, excellent progress and results were obtained utilizing ^{99m}Tc diphosphonate in studies on acutely injured muscle, scanning and imaging of soft tissue (muscle), regional blood flow and skeletal uptake of bone tracers and in developing improved methods of assessing splenic damage. Mathematical support was also provided for a study of regional kinetics of indium-111 in cerebral spinal fluid.

Additional collaborative effort with the Naval Medical Research Institute developed a technique that was sensitive, noninvasive, and quantitative in assessing the healing of mandibular bone grafts in a canine model. This improved method provides a better means to evaluate a combatant who has sustained a facial wound which has disrupted the mandible.

The research projects conducted in the Biochemistry Department are interrelated and complementary. Determination of accurate and valid indicators of damage using biochemical markers in a variety of organ systems in which different disease conditions exist and the application of nuclear medicine procedures for improvement of diagnostic and therapeutic techniques will contribute to a better understanding of the patterns of response, the organs at particular risk and the underlying mechanisms of damage. Effort is directed toward applying these techniques to diagnosis and treatment of conditions peculiar to the battlefield environment.

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SERUM PROTEIN-BOUND CARBOHYDRATES AND OTHER GLYCOPROTEIN ASSAYS AS INDICATORS OF TUMOR BURDEN

Principal Investigators: *J. F. Weiss, W. P. Bradley, A. P. Blasco, AFRRI; J. C. Alexander, Jr., N. A. Silverman and P. B. Chretien, National Institutes of Health*
Technical Assistance: *M. J. Ryan, M. L. Nelson, W. W. Wolfe and L. R. Wooldridge, Jr.*

Elevations in serum glycoproteins and protein-bound carbohydrates are significant consequences of radiation damage, trauma and certain disease states such as cancer. Thus it is important to determine the diagnostic, prognostic, and functional significance of the glycoprotein elevations in various injuries and diseases.

Levels of glycoprotein associated carbohydrates (neutral hexoses, hexosamine, sialic acid, and fucose) were determined in the serum of patients with either local, regional or metastatic cancer, patients clinically cured of cancer, and controls (smokers and nonsmokers). Total protein-bound carbohydrates were compared to levels of 17 normal serum glycoproteins, carcinoembryonic antigen, and to lymphocyte reactivity to phytohemagglutinin. Tumor burden was directly related to protein-bound carbohydrate levels in patient groups. Levels of bound carbohydrates reflect the sum of all the changes in serum glycoproteins, but primarily changes in the acute-phase proteins (α_1-acid glycoprotein, α_1-antitrypsin, haptoglobin, ceruloplasmin) found in the α-globulin fraction of serum. Serum protein-bound carbohydrates, such as sialic acid, appear to be better tumor markers than carcinoembryonic antigen in patients with various types of solid malignancies. Increases in sialic acid in tumor-bearers do not appear to correlate with increases in carcinoembryonic antigen, suggesting that a "nonspecific" tumor marker and a "tumor-derived" marker may complement each other in assessing patient status (Figure 6). Increased levels of the acute-phase proteins and decreased levels of α_2HS-glycoprotein occur in individuals with depressed in vitro lymphocyte reactivity to phytohemagglutinin, but the relationship between lymphocyte reactivity and specific serum glycoproteins needs further investigation. The present study increases our understanding of the relationship of serum glycoproteins to disease and injury.

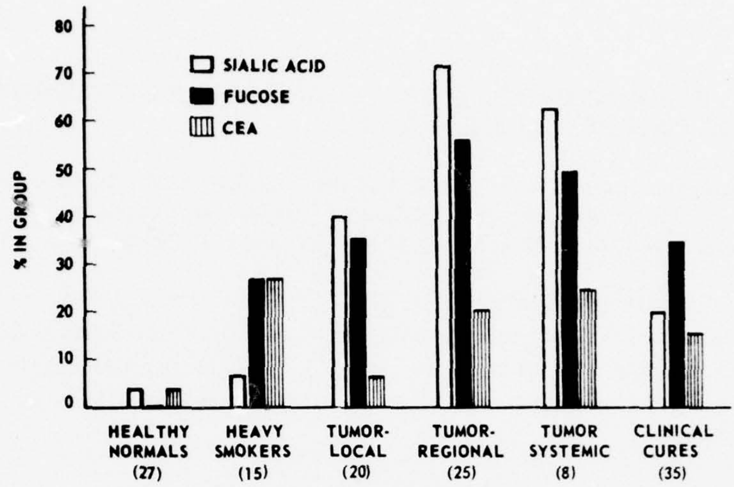

Figure 6. Percent of individuals with elevated tumor markers (above normal mean + two standard deviations). CEA = carcinoembryonic antigen.

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ORGANOMETALLIC LIQUIDS AS POTENTIAL DOSIMETRIC MATERIALS: A PULSE RADIOLYSIS STUDY OF THE TETRA-ALKYL COMPOUNDS OF Si, Sn AND Pb

Principal Investigator: *J. L. Hosszu*
Collaborator: *A. P. Blasco*

A continued requirement exists to develop dosimetry systems suitable for use in pulsed fields of ionizing radiation, particularly in the low-energy x-ray region. The desirable characteristics of such dosimeters should include both a sensitivity to the spectral distribution of the incident radiation and a capability of live time readout to follow pulse profiles over a time scale of several nanoseconds.

The organometallic compounds of the Group IVA metals have been shown to produce optically detectable changes after exposures to ionizing radiation.[2] Theoretically, the K-shell energy edges of their metal atoms serve as windows to determine the spectral distribution of the radiation pulse.

The methods of kinetic optical spectroscopy have been employed to observe the very short-lived transients produced by pulse radiolysis of some of the tetra-alkyl compounds of Si, Sn, and Pb doped with naphthalene and toluene.[1] The feasibility of utilizing such bimolecular compounds for time resolved dosimeters in the low-energy x-ray region has been demonstrated (Figures 7 and 8). This represents an additional method for refinement of the pulsed radiation profiles such as might be encountered in nuclear weapon uses.

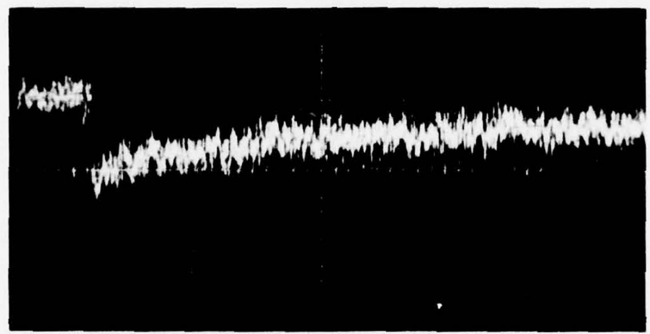

Figure 7. Long-lived optical absorption transients at 320 nm of pure Et_4Si. Vertical scale 50 mV/cm; horizontal scale 50 μsec/cm.

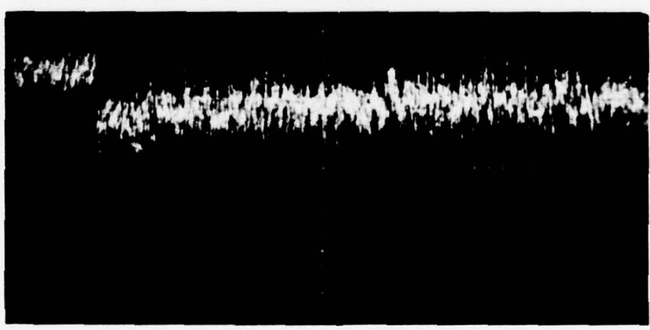

Figure 8. Long-lived optical absorption transients at 320 nm of naphthalene-doped Et_4Si. Vertical scale 50 mV/cm; horizontal scale 100 μsec/cm.

REFERENCES

1. Hosszu, J. L. Organometallic liquids as potential dosimetric materials: a pulse radiolysis study of the tetra-alkyl compounds of Si, Sn and Pb. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Technical Note TN76-3, 1976.

2. Meaburn, G. M., Hosszu, J. L. and Kiker, W. E. Organometallic liquids as potential dosimetric materials: a pulse radiolysis study of the tetraethyl compounds of Si, Ge, Sn and Pb. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR75-5, 1975.

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GROWTH EFFECTS FROM 45-Hz VERTICAL ELECTRIC FIELDS

Principal Investigators: *N. S. Mathewson, G. M. Oosta, S. G. Levin, M. E. Ekstrom, S. S. Diamond and A. P. Blasco*
Technical Assistance: *W. E. Jackson III, C. A. McIntire III, J. E. Egan, H. B. Cranford, A. L. Miller, A. E. Cummings, Jr. and P. W. Jones III*

As early as 1959 the U. S. Navy began considering a new communications system to operate in the extremely low frequency portion of the electromagnetic spectrum. This system is designed to allow submarines to receive vital communications during strategic emergencies while remaining at ocean depths which maintain their tactical invisibility and survivability. The survivability of the U. S. Fleet Ballistic Missile Submarine System is a key element of our national security as a nuclear deterrent. The Navy is completing the design of such an extremely low frequency communications system to ultimately increase the tactical safety of these submarines.

The design and placement of a transmitter site for this communications system is not complete, but one current estimate calls for twenty 50-mile long buried cables to be laid out orthogonally in a square grid. These cables are placed 5 miles apart occupying an area of 2500 square miles or twice the size of Rhode Island. This transmitter is planned to operate near 45 or 75 Hz and will typically require 100 megawatts of electric power.

The Navy has for several years supported considerable research into the existence of biological or environmental problems that may be associated with this type of antenna. Within the last several years two research efforts have led to reports that vertical electric fields, in one case at 60 Hz and the other at 45 Hz, reduced the growth of young rats during 1-month exposures.

This report summarizes the attempt by this laboratory to both observe and verify the reported reduced growth of young rats exposed to 45-Hz fields. A

total of 384 young (100-200 g) male Sprague-Dawley rats was used in four 1-month, irradiation experiments (Table 9). For each animal the growth (or change in body weight), the food consumption, and water consumption were measured three times a week during the 28-day exposure. Postexposure, a complete blood count (consisting of red blood cells, white blood cells, polymorphonuclear cells, lymphocytes, monocytes, eosinophils, hematocrit and hemoglobin) and plasma metabolites (consisting of levels of total protein, globulin, glucose, triglyceride, cholesterol and total lipid) were obtained for each animal. To further establish the general health of these animals, randomly selected rats from each field level were necropsied and 16 organ systems were preserved for histopathologic examination.

Table 9. Experimental Design and Animal Usage

Experiment	Electric Field Strength (V/m) (Control) 0	2	10	20	50	100
	Number of animals used					
E	16	16	16	16	16	16
F	16	16	16	16	16	16
G	48	--	--	48	--	--
H	16	16	16	16	16	16
	Total number of animals = 384					

We are acutely aware of the importance of observing an effect from this type of exposure, detrimental or not, since virtually everyone using an electric appliance or standing near electric wires is exposed to extremely low frequency fields. However, none of our 45-Hz data, summarized in Table 10, can support or even indicate that rat growth is lowered from exposure to 45-Hz vertical electric fields. The examination of our data for exposure to vertical electric fields up to 100 volts per meter at 45 Hz leads to the conclusion that the growth and gross metabolism as indicated by the plasma biochemical analyses and general health of these rats were not changed. That some significant differences are found in Table 10 is clearly normal when it is noted that 15 six-group analysis-of-variance type significance tests were performed and that many of the variables are correlated. The complete description and analysis of these experiments is the subject of impending reports, and two preliminary reports of these experiments have been presented[1,2] within the past year.

Table 10. Statistical Summary

ELF Vertical Electric Field Data at 45Hz				
Variable	Experiment			
	E	F	G	H
Growth	-	*	-	-
Food Con.	-	*	-	-
Water Con.	-	-	-	-
T. Prot.	-	-	-	•
Glob.	-	-	-	•
Glu.	*	•	-	•
T. Lipid	*	-	-	-
Chol.	-	-	-	-
Trig.	-	-	-	-
RBC	*	*	-	-
WBC	-	-	-	-
Poly	-	-	-	-
Lyhs.	-	-	-	-
Mono.	within normal limits			
Eo.	within normal limits			
HCT.	*	-	-	-
HGB	•	-	-	-
Adrenal wt.		-	-	-
Spleen wt.		-	-	-
Gross path.	no significant differences			
Histopath.	no significant differences			

- Implies no significant differences
• Significant difference p<.05
* Significant difference p<.01
blank Not performed

REFERENCES

1. Fourth Report on Program for Control of Electromagnetic Pollution of the Environment: The Assessment of Biological Hazards of Nonionizing Electromagnetic Radiation. Appendix D, pp. 27-29. Washington, D.C., Office of Telecommunications Policy, June 1976.

2. Mathewson, N. S., Oosta, G. M., Oliva, S. A., Levin, S. G. and Blasco, A. P. Effects of 45 Hz electric field exposure on rats. Biologic Effects of Electromagnetic Waves in special issue of Radio Science (in press).

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LENS PROTEINS: A POSSIBLE INDICATOR OF SUBCLINICAL LENS ALTERATIONS AFTER MICROWAVE EXPOSURE

Principal Investigators: *G. M. Oosta, N. S. Mathewson and F. H. Henry*
Technical Assistance: *K. M. Hartley and C. J. Morrisey*

Microwave exposures of sufficient intensity and duration are known to result in cataract formation. However, the threshold for cataract formation is not well documented as a result of the long latency periods that exist for low level microwave exposures. If a biochemical indicator of microwave damage were available, determination of the threshold could be more accurate. We are investigating the possibility that lens protein alterations can be used as indicators of subclinical, microwave-induced lens changes. In addition, it is anticipated that the results of these experiments will provide information helpful in specifying a suitable molecular model for the microwave cataract.

Cataract production is initiated as a result of multiple head only exposures at 300 mW/cm^2 for 20 min and 2.45 GHz using the elliptical reflector antenna system in the Microwave Facility, Department of Microwave Research, Walter Reed Army Institute of Research. Lens alterations are apparent within 4 weeks.

There are many models for cataractogenesis. All eventually involve lenticular protein alterations. In these experiments, data are collected based on two models. The first model specifies an increase in the fraction of total protein found in the pellet after low-speed centrifugation of a lens extract. The second specifies a shift in the state of aggregation of lens proteins eventually leading to aggregates with high molecular weight. Protein alterations are monitored in lens extracts using gel filtration and polyacrylamide, pore-limit electrophoresis. Extraction parameters such as buffer composition, ionic strength and extraction time have been studied. Experiments and data collection are now in progress.

Success in establishing proteins as indicators of lens damage after microwave exposure might allow the threshold for microwave damage to be accurately determined even for low level exposures. Such determinations would play a large role in helping to establish safety standards for human microwave exposure.

In addition, observed alterations could be exploited to develop assays suitable for estimating lens damage after ionizing radiation exposures.

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LOCALIZATION OF ^{99m}Tc DIPHOSPHONATE IN ACUTELY INJURED MUSCLE: RELATIONSHIP TO MUSCLE CALCIUM DEPOSITION

Principal Investigator: *B. A. Siegel*
Collaborators: *W. K. Engel and E. C. Derrer, National Institutes of Health*
Technical Assistance: *E. L. Barron, J. K. Warrenfeltz, M. E. Flynn, N. L. Fleming and J. J. Jozsa*

There is an operational requirement in military nuclear medicine to provide more effective methods for evaluating changes in physiological and organ function in trauma. In rats with experimental ischemic myopathy, there was a significant correlation ($r = 0.778$, $p < 0.001$) between muscle uptake of ^{99m}Tc diphosphonate (EHDP) and tissue calcium concentration.[1] In addition, the accumulation of both calcium and ^{99m}Tc EHDP in acutely injured muscles was further increased in rats with vitamin D-induced hypercalcemia. Histologic studies demonstrated staining of damaged muscle fibers with alizarin red, indicating the presence of microcrystalline or ultramicrocrystalline calcium salts. Staining of muscle fibers was most intense in the outer marginal zones of individual microscopic infarcts. Our results suggest that the uptake of ^{99m}Tc EHDP in acutely damaged skeletal muscle is directly related to the deposition of calcium salts within the injured muscle fibers. This work increases our understanding of the basic mechanisms of the pathogenesis of ^{99m}Tc diphosphonate localization in damaged muscle. The results can be applied toward improved clinical interpretations to benefit patients (soldiers) with trauma, acutely injured muscle or other conditions which can be detected with these techniques.[2]

REFERENCES

1. Siegel, B. A., Engel, W. K. and Derrer, E. C. ^{99m}Tc-diphosphonate uptake in skeletal muscle: a quantitative index of acute damage. Neurology 25:1055-1058, 1975.

2. Siegel, B. A., Engel, W. K. and Derrer, E. C. Localization of ^{99m}Tc diphosphonate in acutely injured muscle: relationship to muscle calcium deposition. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR76-45, 1976.

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^{99m}Tc DIPHOSPHONATE SCANNING OF SOFT TISSUE IN NEUROMUSCULAR DISEASES

Principal Investigator: *R. W. Kula, National Institutes of Health*
Collaborators: *B. R. Line, National Institutes of Health; B. A. Siegel, AFRRI; A. E. Jones, G. S. Johnston and W. K. Engel, National Institutes of Health*

Nuclear medicine may provide an improved means for analyzing physiology and organ function following trauma. This may be of importance in evaluating injured soldiers. Our previous studies of acutely damaged skeletal muscle in rats have demonstrated close correlation between muscle uptake of ^{99m}Tc diphosphonate by scintillation counting, increase of muscle Ca^{++}, loss of muscle K^+ and increase of plasma creatine phosphokinase. In an attempt to use the isotopic method clinically to seek and quantitate muscle damage, we have scanned limbs of more than 60 patients (various neuromuscular diseases and controls) 6 hours after intravenous administration of 10 mCi ^{99m}Tc diphosphonate. Computer-stored and analyzed images provided a soft tissue to bone ratio of radioactivity uptake. Ten patients with dermatomyositis-polymyositis had moderate to high serum creatine phosphokinases and very high soft tissue uptake per soft tissue to bone ratios. Eleven patients with X-linked muscular dystrophy all had very high creatine phosphokinases but surprisingly normal soft tissue to bone ratios. Six "other" myopathy patients and six amyotrophic lateral sclerosis patients had normal or slight-to-moderately increased creatine phosphokinases and soft tissue to bone ratios, and both usually correlated in individual cases. Four patients with "ragged-red"-fiber muscle diseases and three with myasthenia gravis had normal creatine phosphokinases but, surprisingly, slight to moderately increased soft tissue to bone ratios. Accordingly, ^{99m}Tc diphosphonate localization in soft tissue of neuromuscular disease patients does not appear to be a simple function of creatine phosphokinase-evidenced muscle fiber "damage-leakage" Other possible factors include: positive -- muscle-interstitial or subcutaneous Ca^{++}, and cellular proliferative activity; negative -- chronic fat and connective tissue replacement. The high uptake in dermatomyositis-polymyositis is likely to be due at least partly to the first and perhaps also the second factor (both known to occur in dermatomyositis-polymyositis); the third may possibly help explain the perplexing normal uptake in X-linked dystrophy. The results of these clinical studies suggest that muscle imaging with ^{99m}Tc diphosphonate may be useful for evaluating soldiers who sustain soft tissue injuries of the extremities.

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SKELETAL UPTAKE OF ^{99m}Tc DIPHOSPHONATE IN RELATION TO LOCAL BONE BLOOD FLOW

Principal Investigators: *B. A. Siegel, R. L. Donovan and P. O. Alderson*
Collaborator: *G. R. Mack, National Naval Medical Center*
Technical Assistance: *E. L. Barron, J. K. Warrenfeltz, M. E. Flynn, N. L. Fleming, H. E. Bourne and J. J. Jozsa*

Bone imaging with the ^{99m}Tc labeled phosphates has great potential for improving our abilities to evaluate bone healing after trauma, presence of stress fractures or osteomyelitis not visible radiographically, and healing of bone grafts. To make maximum use of this tool in military patients it is pertinent to attempt to increase our understanding of the basic mechanisms involved in skeletal uptake of the ^{99m}Tc labeled bone tracers.

The regional distribution of bone-seeking radiopharmaceuticals in the skeleton is potentially dependent on a number of factors including local alterations in blood flow, metabolic activity, osteogenesis, surface area for tracer exchange, capillary permeability, and the volume of interstitial fluid.[1] Several investigators have suggested that increased local blood flow is the major physiologic determinant of increased tracer deposition in skeletal lesions, but this viewpoint has been challenged. The present study was undertaken to evaluate the relationship between the skeletal uptake of ^{99m}Tc diphosphonate (ethane-1-hydroxy-1,1-diphosphonate (EHDP)) and relative bone blood flow measured with labeled microspheres under conditions of normal, decreased, and increased regional blood flow.

The right-left (R-L) ratios for tibial uptake of ^{99m}Tc diphosphonate (EHDP) and relative blood flow (from microsphere distribution) were determined in control rats and rats with right femoral artery ligation or healing right tibial fractures. There was highly significant correlation ($r = 0.917$; $p < 0.0001$) between the ^{99m}Tc EHDP uptake and relative blood flow for relative flow ratios < 1.7 (Figure 9). When relative tibial blood flow was > 1.7, there was little further increase in ^{99m}Tc EHDP uptake. In addition, femoral artery ligation in rats with healing fractures resulted in a more marked reduction of flow than of ^{99m}Tc EHDP uptake (Table 11). Our results suggest that regional bone blood flow is a major determinant of ^{99m}Tc EHDP uptake, but changes in regional tracer extraction efficiency are also important. This work increases our understanding of the basic mechanisms of ^{99m}Tc phosphate bone imaging. The results can be applied toward improved clinical interpretations to benefit patients (soldiers) with trauma, bone grafts, osteomyelitis or other conditions which can be detected with these techniques.

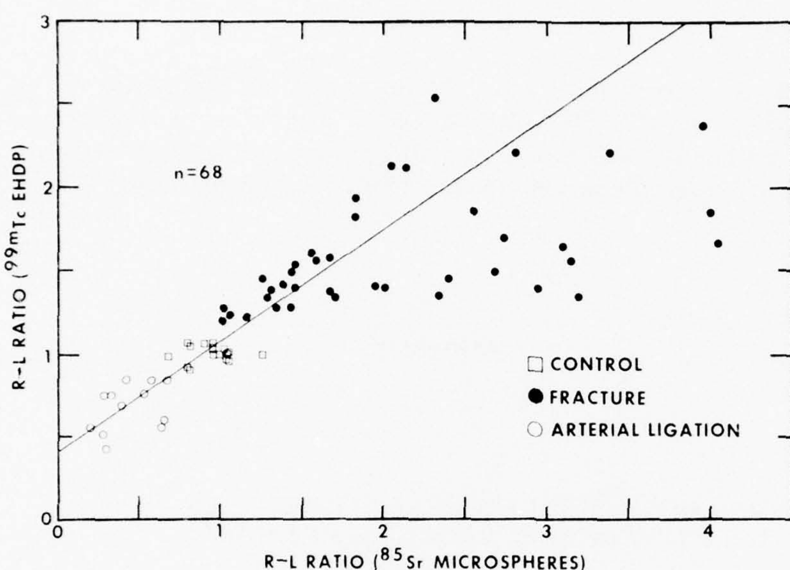

Figure 9. Relationship between tibial R-L ratios for ^{99m}Tc EHDP and ^{85}Sr microspheres. Each point represents the results for a single animal. The line shown is the calculated regression line based on the results of the 47 animals in which the ^{85}Sr microspheres R-L ratio was less than 1.7.

Table 11. Tibial R-L Ratios

Group	n	^{85}Sr Microspheres	^{99m}Tc EHDP
Control	17	0.94 ± 0.14	1.00 ± 0.05
Arterial Ligation	12	0.44 ± 0.17*	0.67 ± 0.14*†
Fracture	39	2.10 ± 0.87*	1.60 ± 0.34*†
^{85}Sr R-L ratio <1.7	18	1.37 ± 0.22*	1.38 ± 0.13*
^{85}Sr R-L ratio >1.7	21	2.72 ± 0.70*	1.78 ± 0.36*†
Fracture + Ligation	21	0.86 ± 0.32	1.29 ± 0.20*†

Results presented as mean ± 1 standard deviation

* Significantly different by unpaired t-test compared to control value at P<0.0001.

† Significantly different by paired t-test compared to corresponding microsphere value at P<0.001.

REFERENCE

1. Siegel, B. A., Donovan, R. L., Alderson, P. O. and Mack, G. R. Skeletal uptake of ^{99m}Tc diphosphonate in relation to local bone blood flow. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR76-35, 1976. Radiology 120:121-123, 1976.

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SPLENIC IMAGING WITH ^{99m}Tc-LABELED ERYTHROCYTES: A COMPARATIVE STUDY OF CELL DAMAGING METHODS

Principal Investigators: *R. G. Hamilton and P. O. Alderson*
Collaborators: *J. F. Harwig, Washington University School of Medicine; and B. A. Siegel, AFRRI*
Technical Assistance: *E. L. Barron, J. K. Warrenfeltz, M. E. Flynn, N. L. Fleming, H. E. Bourne and J. J. Jozsa*

The objective of this research was to develop improved methods for assessing splenic damage after blunt abdominal trauma. Our specific goal was to develop an agent which would localize in the spleen to a much greater extent than the liver. This would minimize problems of image interpretation that can be caused by liver overlying the spleen. We wished to develop an agent which had the high photon yields and low radiation dose of ^{99m}Tc labeled tracers and the splenic specificity of ^{51}Cr red blood cells or ^{203}Hg red blood cells. Therefore we chose to investigate ^{99m}Tc red blood cells.[1]

Several methods of damaging red blood cells for splenic imaging have been compared to determine the optimum approach. Red blood cells from donor animals were labeled with $^{99m}TcO_4^-$ and damaged by heat, excess acid citrate dextrose, excess Sn(II) ion, or the sulfhydryl inhibitors N-ethylmaleimide or p-hydroxymercuribenzoate. The organ distribution of undamaged and damaged red blood cells was determined in rats, and splenic imaging studies were performed in rabbits. Splenic deposition and spleen to liver ratios with heat and sulfhydryl damaged ^{99m}Tc red blood cells were significantly greater ($p < 0.001$) than the values obtained using acid citrate dextrose or Sn(II) ion methods (Figures 10, 11, Table 12). Heat damaging produces good splenic localization of ^{99m}Tc red blood cells but requires rigidly controlled incubation conditions. N-ethylmaleimide damaging provides an excellent and preditable alternate approach.

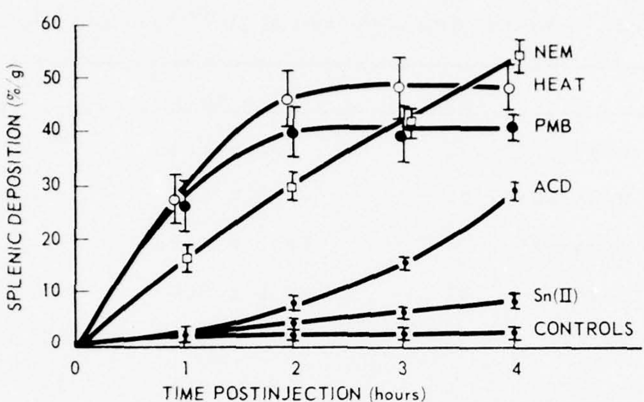

Figure 10. ^{99m}Tc RBC splenic deposition (percent/g ± S. D.) obtained using several methods of damaging. Each ACD and Sn(II) ion data point comprises 5 animal studies. All other data points comprise at least 12 animal studies.

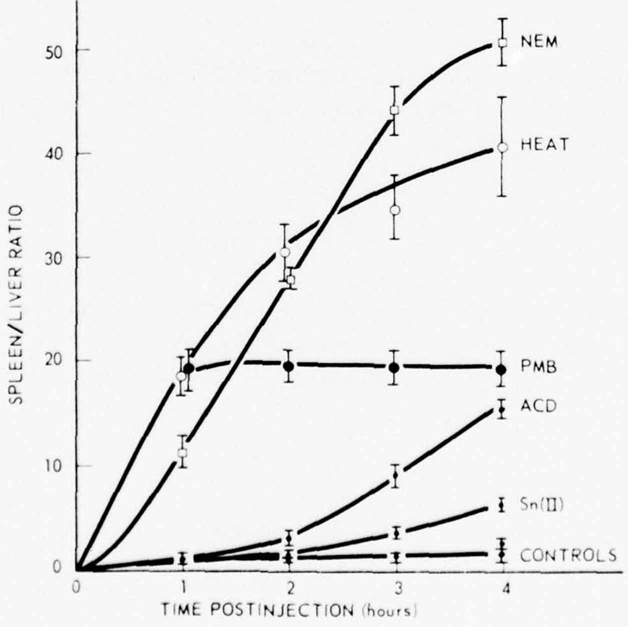

Figure 11. Spleen to liver ratios (S/L ± S. D.) obtained using several methods of damaging. The data were obtained from the same animal population referred to in Figure 10.

Table 12. Total Splenic Deposition of ^{99m}Tc Red Blood Cells*

	total N	Two-Hour	Four-Hour
Controls	36	2.1 ± 0.3	2.2 ± 0.2
Heat Damaged†	24	46.3 ± 4.2	47.5 ± 5.0
Heat Damaged††	24	19.1 ± 0.9	21.2 ± 1.5
NEM Damaged	24	30.1 ± 3.4	55.2 ± 2.0
PMB Damaged	24	38.5 ± 3.2	38.0 ± 2.6
Excess ACD	10	8.3 ± 0.6	26.7 ± 2.3
Excess Sn(II)	10	4.8 ± 0.3	8.0 ± 1.5

* Total splenic activity expressed as % injected dose per total organ ± S.D.

† Heat damaging performed in a constant temperature water bath (49-50°C).

†† Heat damaging performed in a variable temperature water bath (48-51°C).

The results of this study can be applied in military hospitals to provide improved detection of splenic injury after abdominal trauma that might be sustained on the battlefield.

REFERENCE

1. Hamilton, R. G., Alderson, P. O., Harwig, J. F. and Siegel, B. A. Splenic imaging with ^{99m}Tc-labeled erythrocytes: a comparative study of cell damaging methods. J. Nucl. Med. (in press).

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REGIONAL KINETICS OF INDIUM-111 DTPA IN CEREBROSPINAL FLUID IMAGING OF NORMAL VOLUNTEERS

Principal Investigators: *C. L. Partain, University of North Carolina School of Medicine; P. O. Alderson and R. L. Donovan, AFRRI*

Collaborators: *N. Rujanavech, Washington University School of Medicine; and B. A. Siegel, AFRRI*

The objective of this research project was to obtain specific information about cerebrospinal fluid tracer kinetics so that improved calculations of cerebrospinal fluid radiation dosimetry could be performed. The tracer chosen was ^{111}In-DTPA, a chelate widely used for clinical studies of cerebrospinal fluid kinetics (cisternography). The clinical data were obtained by studying volunteers at the Washington University School of Medicine, St. Louis, Missouri. The project was approved by the Radiation Safety and Human Research committees of that institution. The regional kinetics of ^{111}In-DTPA was determined in these six volunteers following the intrathecal administration of 500 microcuries of ^{111}In-DTPA. Gamma camera images of the head and lumbar regions were obtained every 2 to 4 hours, and digitized data were obtained simultaneously by a small laboratory computer. In addition, sequential blood and urine samples were obtained from the patients. The digitized data were made available to AFRRI, and a simple, three-compartment bidirectional flow model describing radiopharmaceutical kinetics in the lumbar regions, basal cisterns and parasagittal region was developed (Figure 12). The resulting model parameters are listed in Table 13. These data may be used as a basis for improved cerebrospinal fluid radiation dosimetry calculations as well as providing an improved basis for clinical assessment of the results of cisternography with ^{111}In-DTPA.[1,2] (AFRRI provided mathematical support for this clinical research project.)

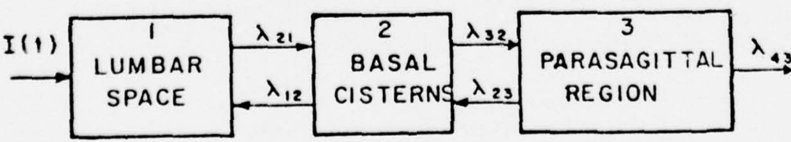

Figure 12. Improved compartmental model for cerebrospinal fluid kinetics

Table 13. Mathematical Model Parameters

Parameter*	Volunteers 1	3	5
λ_{21}, hours^{-1}	.47	.48	.59
λ_{12}, "	.035	.087	.14
λ_{32}, "	.096	.16	.19
λ_{23}, "	.0064	.018	.017
λ_{43}, "	.29	.098	.041
τ_{1}, hours	0.0	0.0	0.0
τ_{2}, "	0.5	0.8	0.4
τ_{3}, "	2.8	3.0	5.0

*Refer to Figure 12 for symbol definition

REFERENCES

1. Partain, C. L., Alderson, P. O., Donovan, R. L., et al. Regional CSF kinetics of ^{111}In-DTPA in normal volunteers. ERDA Symposium Series (in press).

2. Partain, C. L., Staab, E. V., WuHp, et al. Cerebrospinal fluid kinetics in normal human volunteers via radionuclide imaging and mathematical modeling. IAEA Symposium 210/166, 1976 (in press).

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QUANTITATIVE RADIONUCLIDE EVALUATION OF ALLOGENEIC AND COMPOSITE MANDIBULAR BONE GRAFTS

Principal Investigator: *J. F. Kelly, Naval Medical Research Institute*
Collaborators: *J. D. Cagle, Naval Medical Research Institute; G. J. Adler and R. L. Donovan, AFRRI*
Technical Assistance: *H. E. Bourne, E. L. Barron, J. K. Warrenfeltz and M. E. Flynn*

Bone grafting is one means of hastening repair of disrupted skeletal tissue after trauma. We have attempted to improve methods for evaluating the healing of bone grafts using radionuclides. The evaluation of osseous healing of

bone grafts in clinical practice is dependent on qualitative parameters including radiographs and empirical judgment of the surgeon concerning the stability of the graft. These parameters are unreliable in the early phases of healing, and other laboratory techniques are invasive which involve tissue destruction and usually require termination of the procedure. There is a need for a method of bone graft evaluation which will provide a quantitative measure of osseous repair by means of data that can be acquired sequentially and by noninvasive techniques. For the past 3 years studies have been proceeding in our laboratory to evaluate various bone graft systems. Autologous and allogeneic systems were studied and evaluated by a number of parameters including sequential radiographs, radionuclide imaging and end point histologic documentation. A direct correlation between histological activity and radionuclide image profile has been demonstrated. A radionuclide image profile was found to be a reliable instrument for assessing the fate of bone transplant in the mandible of an animal model and preceded by several months the positive correlation of radiographic interpretation.

The image profile is developed for the various graft systems by interfacing the gamma camera with a minidigital computer. The radionuclide ^{99m}Tc-Sn-diphosphonate is utilized as the imaging agent, and the resulting data are transmitted from the gamma camera to a computer for display and analysis. By the use of special localization techniques, specific locations in the graft and adjacent host bone could be reproducibly visualized on successive images. A ratio of activity was developed between grafted and ungrafted mandible in order to determine the progress of activity; this activity is then expressed as a normalized ratio in the various zones of the grafted bone. The activity is then plotted for the period of study. Variations in osteogenic activity between individual grafts can be determined and verified by subsequent radiographic and histological evaluation. This technique provides sensitive, noninvasive, quantitative data that portray alterations in bone physiology perceived as a result of osseous repair.

This method may prove useful in the evaluation of soldiers who have sustained skeletal trauma and require bone grafting.

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SEQUENTIAL QUANTITATIVE RADIONUCLIDE EVALUATION OF MANDIBULAR BONE GRAFT REPAIR

Principal Investigator: *J. F. Kelly, Naval Medical Research Institute*
Collaborators: *J. D. Cagle, Naval Medical Research Institute; G. J. Adler and R. L. Donovan, AFRRI*
Technical Assistance: *H. E. Bourne, E. L. Barron, J. K. Warrenfeltz and M. E. Flynn*

Methods for evaluating healing of bone grafts are of importance in treating patients who have sustained bone injuries which require grafting. A particularly important group is the soldier who has sustained a facial wound which has disrupted the mandible.

We have previously shown that radionuclide bone imaging using a gamma camera interfaced to a digital computer is a feasible method to evaluate repair in mandibular bone grafts.[1,2] However, our prior analysis of the radionuclide studies was based on qualitative judgments because of the inability to reproducibly localize specific areas on sequential images. To develop quantitative data it is necessary to measure the radionuclide activity from selected regions in the bone in successive examinations.

The mandibles of five dogs were partially dedentated bilaterally, leaving the two most distal molars and the incisors in the jaw. Three months later, a surgical defect was produced in the midportion of the right mandibular body by resection of a 20-mm full thickness bone segment after lateral application of Sherman bone plates. Lyophilized 20-mm segments of allogeneic mandible as "U" shaped trays were filled with allogeneic crushed cortical bone and transplanted to the mandibular defects. Submento-occlusal radiographs and radionuclide bone images were obtained at 1, 2, 4, 6 and 8 weeks and at monthly intervals thereafter. The imaging system consisted of a gamma camera interfaced to a digital minicomputer. Digitized gamma camera images were collected in a 64 x 64 array and stored on magnetic tape. The results of the study provide quantitative, sequential evidence of a prolonged but constant repair process in the grafted mandible. Corresponding radiographs did not reveal the sustained repair activity and could not quantitate repair in any fashion. The radionuclide method is sensitive and noninvasive and provides quantitative assessment of the progress of osseous repair in healing bones. This may be useful in the follow-up care of soldiers who need bone grafting to repair osseous injuries.

REFERENCES

1. Cagle, J. D., Kelly, J. F., Stevenson, J. S. and Adler, G. J. Radionuclide, radiographic, and histologic evaluation of mandibular bone grafts. J. Maxillofac. Surg. 2:179, 1974.

2. Kelly, J. F., Cagle, J. D., Stevenson, J. S. and Adler, G. J. Technetium-99m radionuclide bone imaging for evaluating mandibular osseous allografts. J. Oral Surg. 33:11, 1975.

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A MULTIPLE ANIMAL ARRAY FOR EQUAL POWER DENSITY MICROWAVE IRRADIATION

Principal Investigators: *S. A. Oliva and G. N. Catravas*

Microwave research on biological subjects has in the past experienced problems in illuminating significant numbers of animals with a uniform power density electromagnetic field. The problem of generating such a uniform field has led some investigators to present new techniques for producing microwave exposure systems which can illuminate large volumes using parabolic reflectors and a minimum of anechoic material. While such techniques are certainly useful in increasing the area over which power density can be maintained to within a $\pm$ 3 dB-variation, they cannot help to reduce the perturbations which are created in the field by the introduction of biological subjects, which are capable of reflecting in random directions large percentages of the microwave energy incident upon them. The interference patterns created by these reflections have made it impossible to predict with accuracy the exact power density at any particular location within a closely spaced multiple animal array. In addition, many exposure facilities which do not utilize techniques for broadening the uniform field as described above have been constructed and are in use. The arrays used in multiple animal exposures have typically been of the "checkerboard" variety, with closely adjacent cubicles of Styrofoam lined up in a compact plane perpendicular to the axis of the transmitting antenna. Such an exposure facility allows significant variation in the power received by the animals, due to some animals being off the axis of the transmitting antenna, and to the interference patterns set up by the animals themselves.

In the past, the above techniques have been adequate in determining the gross effects of microwave exposure on biological subjects. The necessity today, however, is not to be sure of the power within a few decibels, but to be sure within a few percent.

An exposure array has been devised that negates these disadvantages and enables simultaneous irradiations of multiple animals at uniform average power density (± 5 percent).[1-3] The array consists of microwave transparent cages positioned in accordance with the natural characteristics of the microwave field and separated sufficiently to insure minimum interaction between animals due to microwave reflection. The array has been tested in an anechoic chamber at a frequency of 2450 MHz using an isotropic field probe.

REFERENCES

1. Oliva, S. A. and Catravas, G. N. Microwave exposure facility: multiple animal exposure at equal power density. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR76-12, 1976.

2. Oliva, S. A. and Catravas, G. N. Microwave exposure array: improved field measurements (in preparation).

3. Oliva, S. A. and Catravas, G. N. A multiple animal array for equal power density microwave irradiation. IEEE Trans. Microwave Theory Tech. (in press).

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MODIFICATION OF A MINICOMPUTER DATA ACQUISITION SYSTEM FOR MULTIPLE USE UTILIZING DIRECT MEMORY ACCESS

Principal Investigators: *S. A. Oliva, R. L. Donovan, AFRRI; and A. H. Shimer, Digital Equipment Corporation*

Minicomputers, with their low initial cost, small size, and relative simplicity of design, have become very popular in data acquisition systems for use in biological research, as well as other areas. In addition, the growth in capability of minicomputer systems has led to the development of complex

multiprogramming operating systems designed for them. To use a minicomputer for both real time data acquisition and batch job processing is therefore very attractive. To maximize system throughput in such a system environment, the acquisition of analog data should utilize direct memory access (DMA).

Many minicomputer systems have the capability of DMA on one data channel, with the intent of maximizing the data acquisition rate for that channel. In a multiprogramming environment, however, the reduction of processor time necessary to sustain a given data acquisition rate for multiple data channels (usually not under DMA) becomes important, even at rather low throughputs. In situations where high data acquisition rates are not the primary purpose, but rather multichannel data acquisition with minimal effect on the processor performing other tasks, the solution is multiple channel DMA. A modification of a multiplexed 16-channel data acquisition system originally capable of DMA on only one channel has been developed to allow DMA on one, two, four, eight, or sixteen channels.[1]

REFERENCE

1. Oliva, S. A., Donovan, R. L. and Shimer, A. H. Put all input a/d channels under DMA control. A simple modification to the data-acquisition unit unburdens your system's computer. Electronic Design 1:100-102, 1977.

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A METHOD OF MEASURING THYROID BURDEN OF ^{125}I

Principal Investigators: *W. R. Webber and R. L. Donovan*

Laboratory workers producing ^{125}I labeled compounds may ingest or inhale small quantities of radioactive iodine which is deposited preferentially in the thyroid. Measurement of the thyroid burden is required to quantify the radiation dose to the individual and to provide early warning of possible health hazards. We have developed a rapid, accurate method of measuring thyroid burden using an Anger camera and pulse height analysis techniques.[1] The procedure requires 2 to 3 minutes, equipment commonly available in a nuclear

medicine department, and causes no discomfort for the subject. The minimum detectable amount of ^{125}I has been found to be $\simeq$1.0 nCi with a counting time of 100 seconds. The linearity of the method has been measured using an Oak Ridge Institute of Nuclear Studies thyroid phantom and sources traceable to the National Bureau of Standards. The method is in regular use to accurately evaluate the thyroid burden of our personnel.

REFERENCE

1. Webber, W. R. and Donovan, R. L. A method of measuring thyroid burden of ^{125}I. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Technical Note TN76-5, 1976.

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BEHAVIORAL SCIENCES DEPARTMENT

The Behavioral Sciences Department is concerned with developing a better understanding of the acute effects of exposure to radiation, chemicals and drugs on behavior, trained performance, neurological integrity and physiology of experimental animals, and extrapolation of these data to man. Research projects in support of these objectives are conducted by the three divisions of the Behavioral Sciences Department, and in collaboration with other departments of the Institute, the Naval Medical Research Institute, the Food and Drug Administration, the National Institutes of Health, and several universities within the area.

On 1 July 1976, the scientific base of the Behavioral Sciences Department was broadened by the addition of two investigative teams from the Neurobiology Department. Dr. W. A. Hunt and his group became members of the Physiological Psychology Division, providing the capability of organic pharmacological research. Dr. M. E. Goldberg and his team form the core of the new Experimental Neurology Division and provide expertise in basic neurological sciences. The research efforts of these two teams for the past year are thus reported within this section.

The primary research activities of the department during the past year have been to determine the effects of tactical nuclear weapon radiation on behavior and performance of man in a combat situation. Emphasis has been on evaluating the effects of high-energy neutrons, mixed gamma-neutron fields of varying ratios, and physical activity vis-a-vis cognitive task in relation to response to radiation.

Research studying the bioelectric changes in the central nervous system is providing clues to the possible development of methods to prevent or modify performance decrements caused by high dose radiation exposure.

Behavioral toxicology screening methodologies for neonatal pigs (FDA studies) and for rodents and primates (Naval Medical Research and Development Command studies) continued to be evaluated. These unique methodologies are providing information for establishing maximum permissible occupational levels for industrial and military environments and have promise of developing into models for use in collateral and combined injury studies.

Results of the Behavioral Sciences Department research programs are forwarded to the services and appropriate agencies by informal reports, incorporated into committee and working group reports, discussion and correspondence as well as being made available to the scientific community through AFRRI and open literature scientific reports and presentations.

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PRIMATE PHYSICAL ACTIVITY FOLLOWING EXPOSURE TO A SINGLE 4600-RAD PULSED DOSE OF MIXED GAMMA-NEUTRON RADIATION

Principal Investigator: *C. G. Franz*
Technical Assistance: *L. Clark, C. H. Avila, J. E. Crawford, Jr. and P. E. Heinig, Jr.*

The majority of studies investigating primate behavior following exposure to high doses of ionizing radiation have used animals confined to restraint chairs and trained to perform a learned task requiring little physical movement. A few studies using unrestrained animals have indicated that there are differences in the postirradiation response of restrained animals and unrestrained, physically active animals. This observation is of considerable interest to the military services which have many missions requiring movement or physical activity for combat effectiveness. Thus the objective of this study was to determine the effects of high neutron ($n/\gamma = 3$) radiation on the performance of a physical activity task. This study covered the effects of a 4600-rad pulse ($n/\gamma = 3$, pulse width = 50 msec) on the performance capability of 10 male rhesus monkeys trained to operate a physical activity wheel as a nonmotorized treadmill. The pattern of postexposure incapacitation was (1) an early onset of incapacitation with 80 percent of the animals incapacitated within 8 min postexposure; (2) an apparent recovery from incapacitation with only 30 percent of the animals incapacitated between 45 and 70 min postexposure; (3) a steady increase in the number of animals exhibiting incapacitation until 60 percent were incapacitated between 105 min and the end of the test period 6 hours postexposure (Figure 13a). By 24 hours postexposure, 90 percent of the animals were incapacitated. The percent of the available work time lost to incapacitation follows closely the percent of group incapacitated (Figure 13b). Group mean performance for animals when not incapacitated was 58 ± 9 percent of baseline throughout the 6 hour test period (Figure 13c). Survival time for the group ranged from 7 to 132 hours, with a mean of 37 hours.[1]

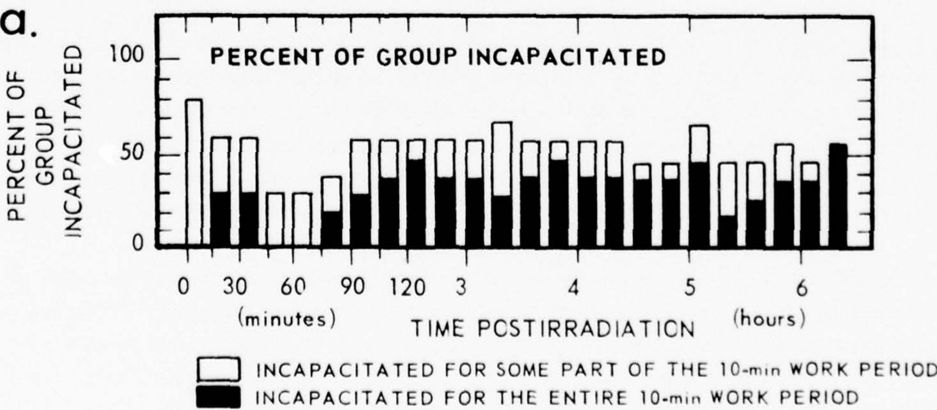
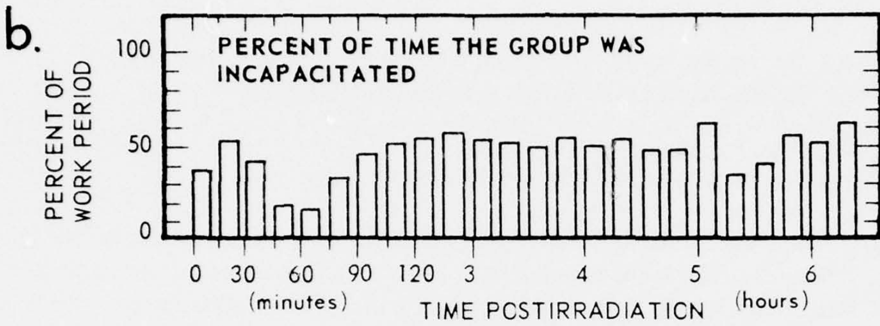


Figure 13. a. Percent of group incapacitated for 1 to 10 minutes during a work period.



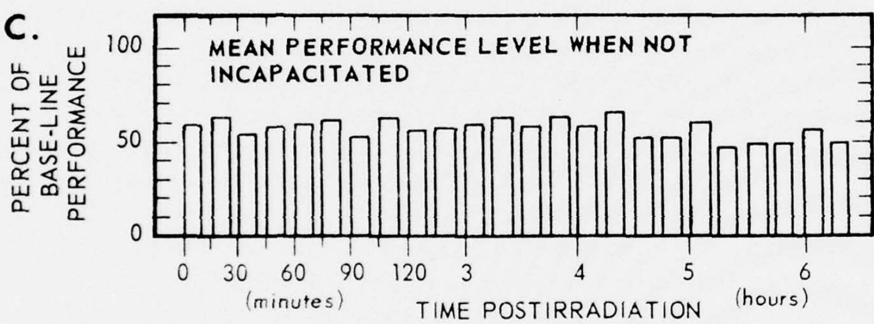


Figure 13 (continued). b. Percent of total available time the group lost to incapacitation for each work period. c. Mean performance level when not incapacitated for each 10-minute work period.

REFERENCE

1. Franz, C. G., Clark, L. and Cable, J. W. Primate physical activity following exposure to a single 4600-rad pulsed dose of mixed gamma-neutron radiation. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR76-42, 1976.

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EFFECT OF ELECTROMAGNETIC PULSE ON AVOIDANCE BEHAVIOR AND ELECTROENCEPHALOGRAM OF A RHESUS MONKEY

Principal Investigators: *J. L. Mattsson and S. A. Oliva*
Technical Assistance: *W. N. Fry, J. P. Alligood, D. N. Stevens and E. L. Ross*

Upon detonation, nuclear weapons create an electromagnetic pulse as well as blast, thermal and radiation energy. The electromagnetic pulse is known to have a deleterious effect on some types of electronic equipment, but it is not known if electromagnetic pulse has an effect on personnel. Most animal research data are negative, but the research was performed on untrained animals, with few exceptions. The objective of this experiment was to evaluate the effects of repeated, high intensity electromagnetic pulse on the electroencephalogram and performance of a highly trained rhesus monkey.[1] A 12-kg male rhesus monkey was exposed to electromagnetic pulse at 266 kV/m, 5 pulses per sec, for 1 hour (18,700 pulses). The effects of electromagnetic pulse on Sidman avoidance behavior and on postexposure electroencephalogram were evaluated, and no significant changes were detected. An analysis of an electromagnetic pulse showed that it contained various frequency components extending from 0 Hz to 10^9 Hz. However, the pulse configuration was such that its power was mainly confined to the longer wavelengths (< 39 MHz). The lack of biologic effect was attributed to the fact that the wavelengths were long relative to the size of the monkey, and little energy deposition was likely to occur. In addition, the electric field was evenly distributed across all lower frequencies so that only a very small electric field component existed at any specific low frequency.

REFERENCE

1. Mattsson, J. L. and Oliva, S. A. Effect of electromagnetic pulse on avoidance behavior and electroencephalogram of a rhesus monkey. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR76-29, 1976.

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EFFECTS OF CHRONIC INHALATION OF PROPYLENE GLYCOL 1,2-DINITRATE ON THE CONDITIONED AVOIDANCE BEHAVIOR OF PRIMATES

Principal Investigators: *R. W. Young, C. R. Curran and C. G. Franz*
Collaborator: *L. J. Jenkins, Jr., Naval Medical Research Institute*
Technical Assistance: *G. G. Kessell, AFRRI; R. A. Jones and M. E. Andersen, Naval Medical Research Institute*

Propylene glycol dinitrate has been reported to produce toxic effects at concentrations as low as 1.3 mg/m^3 for acute exposures of 8 hours or less. Since there are situations in which military personnel could be exposed to propylene glycol dinitrate, we undertook a study to assess the effects of chronic exposure to the vapors of this material. In order to accomplish this objective, a study was conducted in which four primates (Macaca mulatta), trained to a multiple avoidance schedule, were evaluated.[1] The schedule consisted of alternating sessions of discrete trial cued avoidance and free operant avoidance, separated by 3-min rest periods. Two of the animals were exposed to successive atmospheric concentrations of propylene glycol dinitrate vapors of 1.8, 5.6, 11.0 and 28.2 mg/m^3. Exposure occurred on a 23-hour per day basis in a Rochester-type inhalation chamber at the Navy Toxicology Unit. Consecutive exposures lasted 35, 56, 20 and 14 days respectively. The other two animals were housed in an identical inhalation chamber but remained in an ambient atmosphere for the duration of the testing period. These animals served as controls. Plasma concentrations of propylene glycol dinitrate appeared to increase each time the chamber concentration was increased; however, none of the four propylene glycol dinitrate concentrations had a discernible effect on avoidance behavior. There was no measurable change in the overall behavior of either test animal which could have been attributed to general debilitation, sensory deficit, or motor dysfunction. Food and water consumption remained unchanged. Necropsy and histopathological examinations were negative. This study employed behavioral performance measures as indices of the presence or absence of toxic effects that could be important in exposure situations involving military personnel, and indicates that for the concentrations tested no gross toxicity should be expected for even continuous exposures for the durations tested.

REFERENCE

1. Young, R. W., Curran, C. R., Franz, C. G. and Jenkins, L. J., Jr. Effects of chronic inhalation of propylene glycol 1,2-dinitrate on the conditioned avoidance behavior of primates. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR76-25, 1976.

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THE INCIDENCE OF RADIATION-INDUCED INCAPACITATION AS A FUNCTION OF PHYSICAL CONDITIONING AND TASK PERFORMANCE

Principal Investigators: *R. W. Young and C. G. Franz*
Technical Assistance: *J. R. Harrison, P. Mannon, G. G. Kessell, C. A. Boward and L. Clark*

Data obtained in this laboratory suggest that ionizing radiation may produce more debilitation in primates performing a physical task than in those performing a cognitive task. Thus, this study was undertaken to compare the relative incidence of incapacitation for a physical and a cognitive task after irradiation, and to evaluate the effect of preirradiation physical conditioning on the postirradiation performance of the monkey.

Twenty-five male rhesus monkeys (Macaca mulatta) were used as subjects in this study. Ten of these animals were trained to a discrete-trial, shock-avoidance visual discrimination task. These subjects were trained, maintained and irradiated in primate chairs. Ten other monkeys were trained to perform a free-operant, shock-avoidance physical activity task. These animals were maintained in cages and trained and irradiated in a physical activity wheel. The five remaining monkeys were trained to the discrete-trial visual discrimination task in the primate chair, physically conditioned in the physical activity wheel, then irradiated in the primate chair while performing the visual discrimination task. All of the subjects were irradiated with 4500 rads (midhead dose) in a single whole-body pulse of high neutron radiation (n/g = 3) from the TRIGA reactor.

The analysis of these data indicates that there was no difference in the percentage of the groups experiencing incapacitation within the first hour of irradiation (80 percent in all cases) and that the differences in percent of time incapacitated between the groups were not significant. There was a statistically significant difference in survival time ($p = .05$, Mann-Whitney U-test) between the unrestrained, physically active animals ($\bar{x} = 37$ hours) and the animals irradiated in primate chairs whether physically conditioned ($\bar{x} = 16$ hours) or not ($\bar{x} = 13$ hours).

These results suggest that for radiation doses large enough to incapacitate most animals, task differences and preirradiation physical conditioning may have less effect on performance than physical activity after irradiation. These data are potentially significant in defining the interaction of radiation, preirradiation physical condition and postirradiation activity on the postirradiation effects seen in combat personnel.

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CROSS TOLERANCE BETWEEN MORPHINE AND CHOLINERGIC BLOCKING DRUGS MICROINJECTED INTO THE ANTERIOR AMYGDALA

Principal Investigators: *H. Teitelbaum, J. F. Lee and S. G. Levin*

As an example of cellular adaption to chemical stress, the study of opiate tolerance provides a useful toxicological model for radiation research. Drug tolerance is seen after repeated treatment with opiates, barbiturates and alcohol. The mechanism for this altered sensitivity to drugs is poorly understood. One theory is that the blood-brain barrier is altered so that less drug gets into the brain in tolerant organisms.

The present experiment demonstrates that tolerance to morphine can be developed in a discrete brain region where the drug is directly injected into this region, bypassing the blood-brain barrier. Microinjections of morphine and other opiates produce epileptiform EEG patterns at injection sites in the anterior amygdala.[1] By calculating EEG power (mV^2) it is possible to quantify this effect in order to study dose-response relations and changes in sensitivity with repeated administration. When tolerance to morphine (9 μg in 0.9 μl) is manifested, cross tolerance to scopolamine (18 μg in 0.9 μl) and hemicholinium (4.5 μg in 0.9 μl) is found. A morphine response can be obtained from the tolerant injection site by increasing the morphine dosage to 27 μg in 0.9 μl. Recovery of normal sensitivity to these drugs occurs when the daily drug treatment schedule is interrupted for 18-25 days.

The experimental methods and procedures used for studies of opiate tolerance are being applied to study neurochemical and bioelectrical alterations associated with radiation-induced behavioral incapacitation.

REFERENCE

1. Teitelbaum, H., Blosser, J. and Catravas, G. Bilateral electroencephalographic response and unilateral tolerance to unilateral intracerebral morphine injections. Nature 260:158-159, 1976.

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CLASSICAL CONDITIONING OF HIPPOCAMPAL THETA PATTERNS IN THE RAT

Principal Investigators: *H. Teitelbaum, W. L. McFarland and J. L. Mattsson*

Conditioned hippocampal theta responses are time locked to a specific stimulus, and can be localized to a distinct cellular layer in the hippocampus. For these reasons, classically conditioned hippocampal theta responses provide a valuable method for studying cellular alterations that are associated with radiation damage in a distinct brain region.

Electrical stimulation (88 Hz) of the lateral hypothalamus elicits sustained theta response at hippocampal recording sites of rats immobilized with succinylcholine. By pairing this unconditioned stimulus with a 10-sec presentation of a light, the conditioned theta consistently appear after 40 trials. When the conditioned rats were tested in the absence of a neuromuscular blocking agent, the conditioned stimulus elicited a theta response that was associated with slow locomotor activity in 70 percent of the trials. The procedure provides an assay for hippocampal bioelectric activity that is useful in understanding radiation-induced behavioral incapacitation.[1]

REFERENCE

1. Teitelbaum, H., McFarland, W. L. and Mattsson, J. L. Classical conditioning of hippocampal theta patterns in the rat. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR76-46, 1976.

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INDUCTION OF MOVEMENT IN CATALEPTIC RATS WITH STIMULATION OF THE LATERAL HYPOTHALAMUS AND SUBSTANTIA NIGRA

Principal Investigators: *G. A. Mickley and H. Teitelbaum*
Technical Assistance: *J. F. Lee and B. A. Dennison*

Disorders of voluntary movement caused by administration of drugs and toxic agents provide a model for the akinesia produced by radiation. The present experiment investigates the relief of haloperidol-induced catalepsy with electrical stimulation of the brain. Since high frequency bipolar electrical

stimulation of the lateral hypothalamus produces vigorous locomotor activity in the rat, we chose to challenge haloperidol-induced catalepsy with this stimulation. Haloperidol's cataleptic effect is the result of dopaminergic receptor blockade. Since the lateral hypothalamus contains noradrenergic and serotonergic fibers in addition to dopaminergic fibers, we were interested in comparing the effects of lateral hypothalamic stimulation with stimulation of the dopaminergic nigrostriatal pathway in haloperidol treated rats.

In a preliminary experiment we evaluated the ability of various amounts of haloperidol to produce catalepsy and chose a dose which exceeded the ED_{100}. Undrugged (saline control injections) stimulation-induced activity was observed in rats with electrodes in the lateral hypothalamus and substantia nigra. Behaviors characteristic of lateral hypothalamic stimulation included exploration-like walking and sniffing, while nigral stimulation produced more stereotyped movement consisting mainly of circling, rearing and leaning to one side. These behaviors were quantified by the use of a Lafayette Instrument activity platform which measures minute vibrations. Periods of activity surveyed were: (1) 30 sec immediately before stimulation (prestimulation base line), (2) the 15 sec during stimulation, and (3) 15 sec immediately poststimulation. On a different day, haloperidol was injected and the above measures were again taken using identical stimulation parameters.

Haloperidol treated subjects reacted to lateral hypothalamic electrical stimulation with responses qualitatively similar to those produced with lateral hypothalamic stimulation in the saline control sessions. Stimulus bound movement showed a 39 percent reduction after haloperidol. Stimulation of the substantia nigra also countered the haloperidol-produced catalepsy but the stimulation response was reduced by 61 percent when compared to the undrugged base line. This large reduction is to be expected since the substantia nigra contains a higher concentration of dopaminergic neurons than does the lateral hypothalamus. Poststimulation movements were drastically attenuated in both groups.

These results suggest that the catalepsy produced by haloperidol may be overcome by electrical stimulation of the lateral hypothalamus and substantia nigra. Since this akinesia is similar to that seen during radiation-produced early transient incapacitation, it may be possible to relieve locomotor deficits with brain stimulation and ultimately mimic these electrophysiological effects through pharmacological intervention.

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A CHEST HARNESS AND POLE LEASH FOR ROUTINE TRANSFER OF RHESUS MONKEYS FROM HOME CAGE TO BEHAVIORAL TEST APPARATUS AND BACK

Principal Investigator: *J. L. Mattsson*
Technical Assistance: *J. P. Alligood and L. F. Robertson III*

A chest harness and pole leash method to transfer rhesus monkeys (Macaca mulatta) weighing up to 16 kg from home cage to primate restraint chair was designed.[1] The harness was made of leather straps that crossed the chest in an X fashion, and created a V neck. The V neck eliminated the strangulation hazard of neck collars, and minimized interference between the harness and neck hole of restraint chairs. Two pole leashes attached to two points on the harness gave the handler considerable control over the posture of the monkey, making it easier to teach the monkey to walk with a leash and to climb into its restraint chair or test apparatus. During transfer the rigid pole prevented escape and protected the handler from attack. Quick release mechanisms on the pole leash allowed prompt, complete separation from the animal when it reentered its home cage.

REFERENCE

1. Mattsson, J. L., Alligood, J. P. and Robertson, L. F. III. A simple chest harness and pole leash for routine transfer of rhesus monkeys from home cage to behavioral test apparatus and back. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Technical Note TN76-4, 1976.

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THE SUPERIOR COLLICULUS

Principal Investigators: *M. E. Goldberg and D. L. Robinson*

The superior colliculus is a laminar structure in the midbrain consisting of layers of cells separated by fiber bundles. The neurons in the more superficial layers receive afferents from cortical and subcortical areas which are most directly linked to the visual system. Afferents to progressively deeper layers are progressively more distant from the primary visual areas.

Neurons in the superficial layers respond most dramatically to visual stimuli; compared to the visual cortical neurons, collicular cells are less fastidious in their stimulus requirements and have larger receptive fields. These neurons are not rigidly locked to their visual afferents, and their activity is constantly modulated by eye movements and other nonvisual factors. Neurons in the intermediate layers discharge prior to saccadic eye movements into parts of the contralateral visual field. This relationship persists regardless of the mechanism which generates the eye movement. These same neurons also respond to afferent stimulation from other modalities.

Although the superior colliculus is clearly related to vision and eye movements, the deficits resulting from its destruction are rather subtle. Cats with collicular damage tend to neglect their contralateral visual field whereas monkeys with tectal damage tend to make fewer saccadic eye movements and the initiation of such eye movements is delayed.

These anatomical, physiological, and behavioral data have been interpreted to suggest that the superior colliculus is involved in the selection of important stimuli from the visual environment. Such a selection process is critical for various aspects of visually guided behavior. An understanding of the role of the colliculus in visual behavior is critical both to an understanding of normal vision and treatment of visual diseases of humans.

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SUPERIOR COLLICULUS NEURONS WHICH DISTINGUISH BETWEEN TYPES OF STIMULUS MOVEMENT HAVE RESPONSE ENHANCEMENT

Principal Investigators: *D. L. Robinson, AFRRI; and R. H. Wurtz, National Institute of Mental Health*

We have studied the effects of real versus self-induced stimulus movement on 231 neurons in the superficial layers of the monkey superior colliculus.[2] Most of these neurons (65 percent) respond to stimuli swept across their visual receptive field while the animal fixates; most of these same neurons (61 percent) do not respond when the animal makes a saccadic eye movement which induces comparable stimulus movement. Of the cells which make this differentiation, many have a background discharge rate high enough to enable us to observe a suppression of firing with eye movements in total darkness.

This indicates the presence of an extraretinal input. On the basis of timing, directionality and postsaccadic excitability, this extraretinal signal can account for the lack of response of cells to stimulus movement induced by an eye movement. We have found that cells which differentiate between the two types of stimulus movement and have evidence of an extraretinal input also have the enhancement effect (Figure 14).[1] These cells respond to a stationary stimulus flashed in the receptive field while the animal fixates. The response to the same stimulus is enhanced when it is to be the target for a saccadic eye movement. Thus, these cells appear to be extremely sensitive to salient stimuli in the external visual environment, since they receive facilitatory inputs to enhance their response to significant stimuli (targets for saccadic eye movements) and inhibitory inputs to suppress their response to erroneous stimulation (motion caused by eye movements). An understanding of the mechanism of processing visual information through the colliculus may aid in the prevention and treatment of human diseases of the visual system.

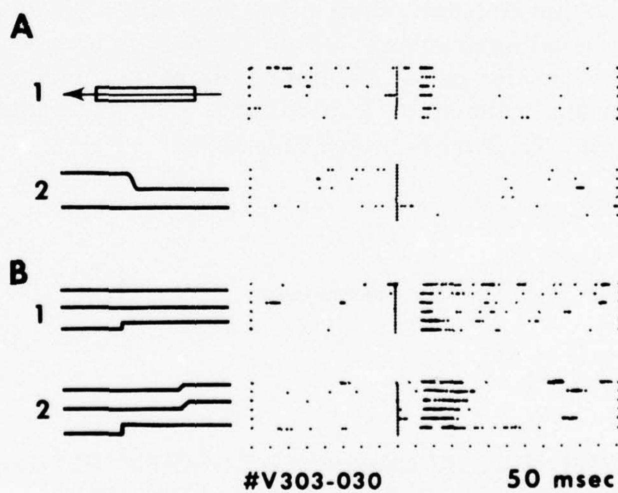

Figure 14. Demonstration of response enhancement for a superior colliculus neuron which distinguishes between real and self-induced stimulus movement. A1 illustrates the response of the cell to rapid movement of a stimulus over its receptive field while the monkey fixates. A2 shows the lack of response of the same cell when the animal makes an eye movement which causes similar stimulus movement. B illustrates the response of the cell to a stationary stimulus flashed in its receptive field while the animal fixates (B1) and the enhanced response to the same stimulus when it is to be the target for an eye movement (B2).

REFERENCES

1. Goldberg, M. E. and Wurtz, R. H. Activity of superior colliculus in behaving monkey. II. Effect of attention on neuronal responses. J. Neurophysiol. 35:560-574, 1972.

2. Robinson, D. L. and Wurtz, R. H. Use of an extraretinal signal by monkey superior colliculus neurons to distinguish real from self-induced stimulus movement. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR76-28, 1976.

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VISUAL-MOTOR INTEGRATION IN MONKEY SUPERIOR COLLICULUS

Principal Investigators: *R. H. Wurtz, C. W. Mohler, National Institute of Mental Health; and D. L. Robinson, AFRRI*

The superior colliculus is a central structure in the second visual pathway to the brain, and in this study the characteristics of collicular cells and inputs were analyzed. The monkey superior colliculus can be separated into at least two divisions according to the functional relationships of the cells to behavior. Cells in the superficial layers respond to visual stimulation and cells in the intermediate layers increase their discharge rate before saccadic eye movements. While there are separate inputs and outputs from each of these groups of layers, the frequently held assumption has been that there is a sequence of processing starting with the superficial visual cells and moving through the intermediate layer eye movement cells which results in an output from the deepest parts of the intermediate and deep layers.

We think that our data can be used to reject the notion of downward processing. First, in order for the most dorsal movement cells to drive deeper ones, the dorsal cells would have to start discharging before the deeper ones. We find in fact that the deeper movement cells discharge before the most dorsal ones. Second, for the dorsal cells to drive deeper cells, the dorsal would always have to discharge when the deeper ones do. This is not the case. We have identified a new cell type, a visually triggered movement cell at the dorsal edge of the movement related cells, that discharges before visually triggered eye movements but not before spontaneous eye movements. These more dorsal cells could not drive the deeper cells during spontaneous eye movements.

All of the movement cells appear to be better related to the monkey's readiness to make a saccade rather than the metrics of the saccade. Instead of a downward sequence of processing in the intermediate layers, these findings suggest a convergence of visual and movement related activity or a parallel sequence of processing. If there were an upward flow of information in the intermediate layers, the cells discharging earlier might activate more dorsal cells. The visually triggered movement cells would then represent a convergence of the upward directed movement information of intermediate layers and downward directed visual information of superficial layers and be a possible efferent cell from the colliculus.

In the visual cells of superficial layers there is also indication of an upward directed activity from movement cells. Some of these cells show an enhanced response to a visual stimulus when the monkey uses that stimulus as the target for a saccade. The characteristics of this enhanced response can be entirely explained by a projection from deeper movement related cells to these superficial layers (Figure 15).

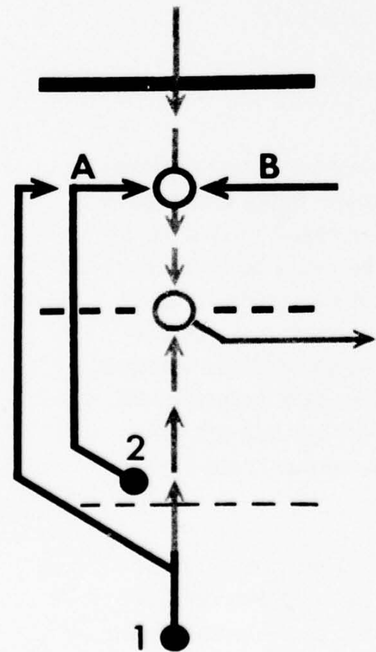

Figure 15.
Organization of inputs and outputs of superior colliculus. The solid line represents a neuron in the superficial layers of the superior colliculus which receives a visual afferent (shaded arrow). This same cell also receives a facilitatory input from eye movement related cells either in the intermediate layers (2) or the center which drives the intermediate layer cells (1). This afferent causes the enhancement effect. The same superficial layer cell also receives an inhibitory afferent from an eye movement related area (B) which prevents the cell from responding to stimulus movement caused by an eye movement. These superficial layer cells converge onto a visually triggered movement cell (shaded circle) at the junction of the superficial layers and the intermediate layers.

Some cells in the superficial layers respond to stimulus movement in the environment but do not respond to comparable stimulus movement when it is generated by an eye movement. It is unlikely that this differentiation is due to visual factors since these cells continue to differentiate when these experiments are conducted in a darkened environment. These cells also receive an extraretinal signal which is appropriately timed to eliminate the effects of

stimulus movement caused by an eye movement. Cells which differentiate also show the enhanced response; however, it is unlikely that an input from the movement cells is responsible for the differentiation. An understanding of the function of the colliculus is necessary in defining and treating human visual disorders.

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VISUAL RESPONSES OF AREA 18 NEURONS IN THE AWAKE, BEHAVING MONKEY

Principal Investigators: *J. S. Baizer, National Institute of Mental Health; D. L. Robinson, AFRRI; and B. M. Dow, National Eye Institute*

The mechanisms underlying effects of ionizing radiation on visual-motor tasks in combat are not understood but definitely involve some disruption of visual perception. The degree of involvement is not known and must be studied and quantified in animal models. It is possible to identify those parts of the central nervous system necessary for visual perception. Once these areas have been identified, their sensitivity to irradiation can be studied more precisely. Visual responses of area 18 neurons were studied in the awake, behaving monkey. Cells were divided into six different classes on the basis of their stimulus preferences and spatial characteristics. Orientation cells were sensitive to the orientation of elongated stimuli. Color cells had nonoriented receptive fields with spatially coextensive opponent color inputs. Direction cells preferred moving stimuli, giving the greatest response to movement in some direction and no response or inhibition to movement in the opposite direction. Spot cells preferred a properly positioned small spot of light and responded equally well to all directions of stimulus movement (Figure 16). Border cells responded best to a stimulus that filled an excitatory region without encroaching on a powerful suppressive flank. Light-inhibited cells had high maintained spontaneous activity that was reduced or abolished by light. Most cells responded equally well to monocular and binocular stimulation; some orientation cells greatly preferred binocular stimulation. In conclusion, independent classes of cells in area 18 perform qualitatively different analyses of incoming visual information. Our data suggest that area 18 is involved in visual perception. Since perceptual difficulties are included in the early transient incapacitation occurring postirradiation, these experiments suggest that area 18 should be very sensitive to ionizing radiation.[1]

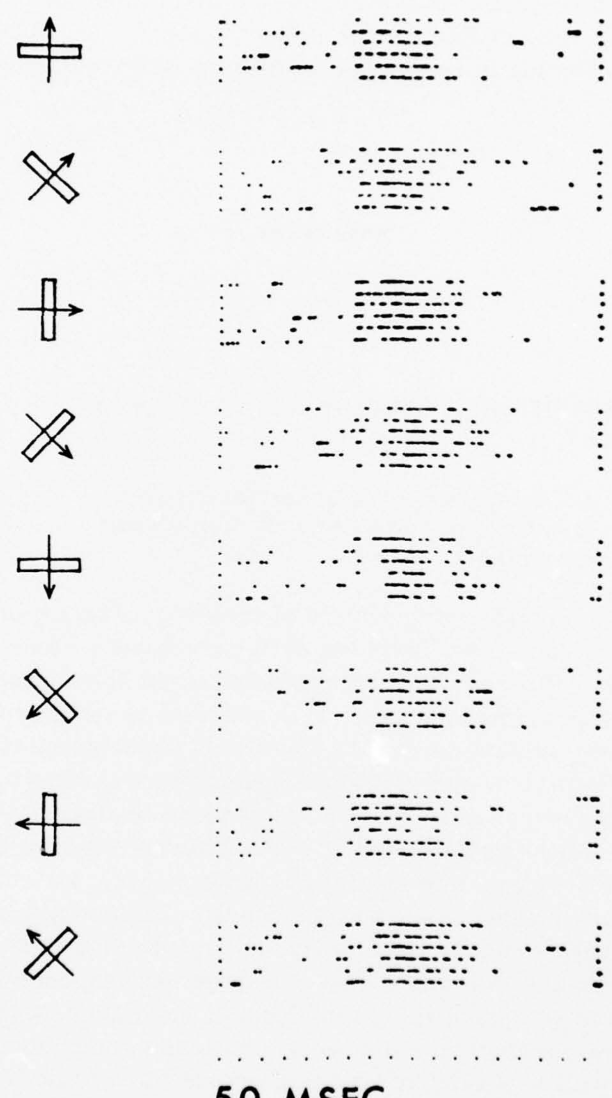

Figure 16. Response of a neuron in area 18 to eight directions of stimulus movement. A slit of light was moved in the directions illustrated at 10°/sec. The cell responded equally well to all directions of movement demonstrating that it has no directional selectivity nor is its receptive field oriented.

REFERENCE

1. Baizer, J. S., Robinson, D. L. and Dow, B. M. Visual responses of area 18 neurons in the awake, behaving monkey. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR76-44, 1976.

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BRAIN ACETYLCHOLINE LEVELS UNDER VARIOUS CONDITIONS OF ETHANOL TREATMENT

Principal Investigators: *W. A. Hunt and T. K. Dalton*

In order to develop useful pharmacologic treatments for disease- and injury-induced states, it is important to describe the changes in given chemicals, i.e., neurotransmitters, in the central nervous system. A study of brain acetylcholine levels was undertaken in an attempt to reconcile discrepancies reported on the effects of ethanol treatment on the cholinergic system.[1] Acetylcholine levels were measured in seven areas of brains of male Sprague-Dawley rats after a single dose of ethanol or after the induction of ethanol-dependence.[2] At various times after treatment, the rats were euthanatized by focused microwave irradiation. The excised brains were dissected into the following parts: cerebellum, brain stem, hypothalamus, thalamus, hippocampus, caudate nucleus and cerebral cortex. After an acute dose of ethanol, acetylcholine levels increased in most areas of the brain when blood ethanol concentrations were quite high (Table 14). As blood ethanol declined, acetylcholine levels decreased to below control values (Figure 17), with similar results observed in ethanol-dependent rats still intoxicated (Table 14). No alteration was observed in any of the areas studied during the ethanol withdrawal syndrome. These data suggest that ethanol treatment exerts multiple effects on the cholinergic system, but they do not as yet support a role of acetylcholine in the expression of the ethanol withdrawal syndrome. Further, this study provides more information on the development and exploration of models for the investigation of chronic insults to the brain, such as long-term exposure to toxic chemicals and ionizing and nonionizing radiation.

Table 14. Regional Acetylcholine Levels After Acute and Chronic Ethanol Treatment. Values represent the means ± S. E. Numbers in parentheses refer to the number of animals in each group. In acute experiments animals received 6 g/kg, per os, 2 hours prior to the end of the experiment. Blood ethanol levels in acute animals were 445 ± 25 mg/dl, while in ethanol-dependent animals still intoxicated, they were 219 ± 16 mg/dl.

	ACh levels (nmoles/g ± S.E.)			
	Controls	Acute	Chronic Intoxicated	Withdrawal Syndrome
Cerebellum	7.6±0.69(22)	8.4±0.51(5)	7.2±1.17(8)	6.3±0.50(7)
Brain stem	24.2±1.28(21)	30.9±1.42(5)*	18.5±0.99(6)*	27.0±1.69(6)
Hypothalamus	29.6±2.26(11)	26.7±1.36(5)	22.8±1.85(7)	24.1±1.03(8)
Hippocampus	24.3±0.93(19)	27.0±1.55(5)	17.2±0.85(6)+	23.0±1.68(8)
Caudate nucleus	46.0±1.48(10)	57.0±2.41(5)*	32.2±2.36(6)+	50.9±3.54(8)
Cerebral cortex	19.5±1.04(17)	22.5±1.28(5)	17.2±1.17(6)	19.3±0.90(7)

* $p < 0.05$
+ $p < 0.001$

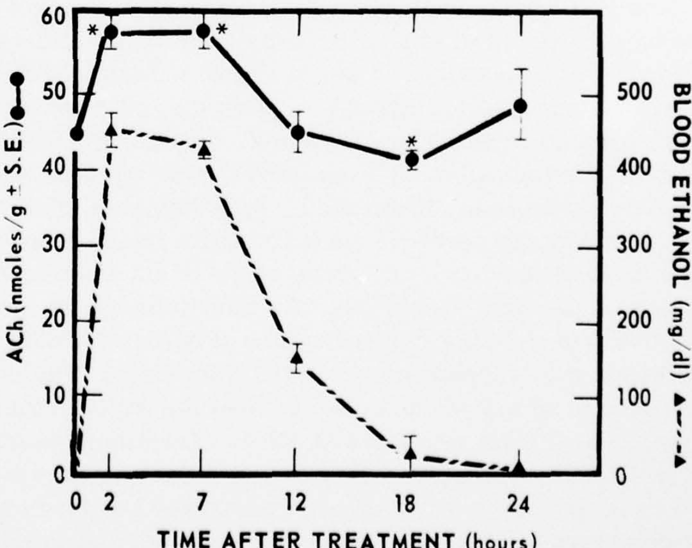

Figure 17. Striatal ACh levels after a single dose of ethanol. Each point represents the mean ± S. E. M. of five to ten animals. * denotes statistical difference from control ($p < 0.05$).

REFERENCES

1. Hunt, W. A. and Dalton, T. K. Regional brain acetylcholine levels in rats acutely treated with ethanol or rendered ethanol-dependent. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR76-41, 1976.

2. Majchrowicz, E. Induction of physical dependence upon ethanol and the associated behavioral changes in rats. Psychopharmacologia 43:245-254, 1975.

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RELATIVE INTOXICATING DOSES OF ALIPHATIC ALCOHOLS AND DIOLS

Principal Investigators: *W. A. Hunt and M. J. McCreery*

The mechanism of alcohol-induced intoxication is unknown. Previous studies using a limited number of alcohols have suggested that they exert their depressant effect by interacting with membranes and that their potency is directly related to their membrane partition coefficient. In the present effort this approach was expanded to include a number of homologous series of aliphatic alcohols and diols which were tested for their ability to induce intoxication. Male Sprague-Dawley rats were injected intraperitoneally with several doses of a given drug and observed for the most severe signs of intoxication as described by Majchrowicz.[1] From dose-response curves the dose needed to produce ataxia-2 was determined. Of over 60 compounds tested, almost all of them induced a behavioral spectrum of intoxication virtually identical to that of ethanol. Within a homologous series the potency increased as the aliphatic chain was lengthened up to 6-8 carbons. With additional carbons the potency progressively declined. A plot of [log oleyl alcohol/H_2O partition coefficient] was linear, supporting a direct relationship between potency and partition coefficient (Figure 18). Hydrocarbons, although having a high partition coefficient, were ineffective in inducing intoxication. These data suggest that the amphophilic nature of alcohols is essential for inducing intoxication and that their three-dimensional structure is unimportant to the potency except as it affects the partition coefficient. The data derived from this study give further information on how simple organic molecules can disrupt nerve function and could have practicality in the study and treatment of the effects of a wide variety of toxic chemicals on behavior.

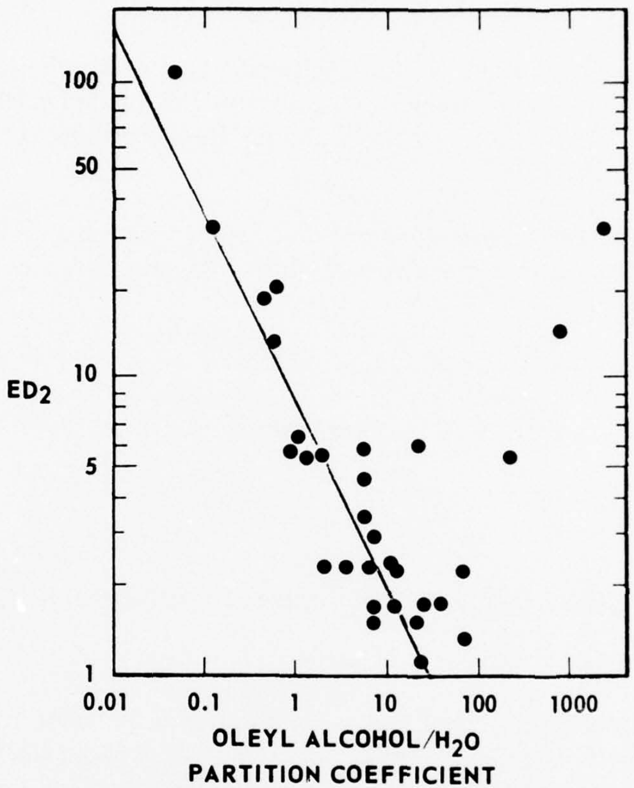

Figure 18. Potency of alcohols as a function of partition coefficient. ED_2 is the dose of alcohol capable of inducing ataxia-2. A significant correlation exists between the two parameters ($r = -0.93$).

REFERENCE

1. Majchrowicz, E. Induction of physical dependence upon ethanol and the associated behavioral changes in rats. Psychopharmacologia 43:245-254, 1975.

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TEMPORAL RELATIONSHIP OF THE INDUCTION OF TOLERANCE AND PHYSICAL DEPENDENCE ON ETHANOL AFTER CONTINUOUSLY SUSTAINED INTOXICATION WITH HIGH DOSES OF ETHANOL IN RATS

Principal Investigators: *E. Majchrowicz, National Institute on Alcohol Abuse and Alcoholism; and W. A. Hunt, AFRRI*

Any animal model for ethanol addiction must display signs of tolerance and dependence to be valid. This study was undertaken to study the time course of development of tolerance and physical dependence using a new model in the rat.[1] Animals were treated by intragastric intubation of a 20 percent ethanol solution in doses of 9-15 g/kg in three to five fractions for 1-7 days. Ethanol doses were determined individually for each animal utilizing intoxication-dose relationships. Tolerance was assessed by correlating the signs of intoxication with the descending blood ethanol concentration during the prodromal detoxication phase of the withdrawal period. The severity of intoxication was measured using a spectrum of signs which were related directly to the blood ethanol concentrations: coma, loss of righting reflex, ataxia-3, ataxia-2, ataxia-1, sedation and neutrality. As blood ethanol concentrations approached 100 mg/dl, the ethanol dependence phase emerged which was characterized by the onset of a spectrum of signs and responses of progressive severity: hyperactivity, tremors, spastic rigidity and spontaneous convulsive seizures. The severity of the withdrawal syndrome was rated using a scale of 0-3. The results of this study demonstrate that when rats are treated with the maximum tolerable doses of ethanol, both tolerance and physical dependence can be induced in only a few days. As the duration of treatment increased, the blood ethanol concentration for a given sign of intoxication also increased, when compared to blood ethanol concentrations observed after single doses of ethanol (Figure 19). A significant degree of tolerance was demonstrated for all signs of intoxication after 4 days of treatment, but did not reach the maximum level even after 7 days of treatment. The severity of the withdrawal signs and reactions intensified progressively as the period of ethanol treatment increased (Figure 20). Maximum intensity of the withdrawal syndrome was observed after 4 days of treatment, when as many as 72 percent of animals exhibited severe withdrawal signs and reactions including convulsive seizures. The different time courses of development of tolerance and physical dependence might suggest that the two phenomena are mediated through different mechanisms. The results provide further information on the use of animal models for the study of chronic insult to the brain as related to human alcoholism and its treatment and long-term exposure to ionizing and nonionizing radiation.

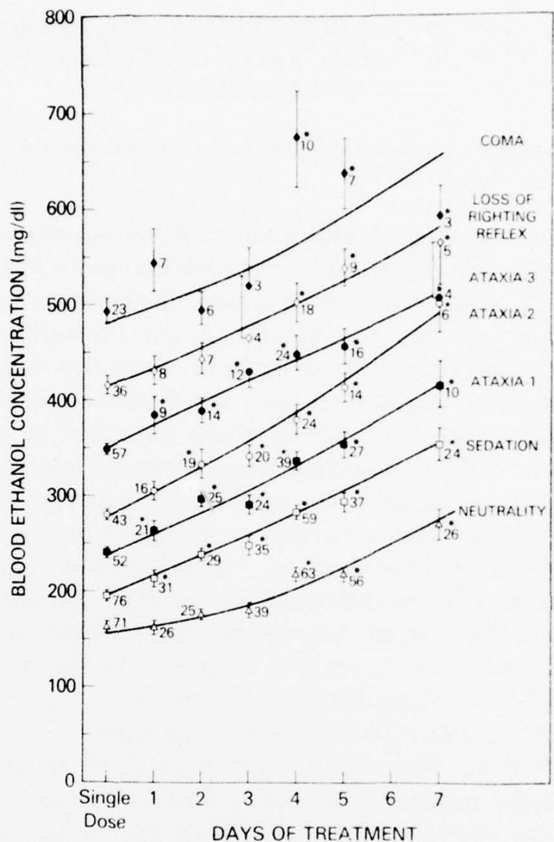

Figure 19. Time course of acquisition of tolerance to ethanol following sustained intoxication with high doses of ethanol. All observations were made during the ethanol detoxication phase of the withdrawal period which corresponds to the descending limb of the blood ethanol curve. Each point represents the mean blood ethanol concentration ± S. E. at the onset of a given sign of intoxication during the ethanol detoxication phase of the withdrawal period or after a single intubation with ethanol (5 g/kg). The numbers beside the blood ethanol points indicate the number of observations used for the assessment of the signs of intoxication. The asterisk (*) denotes statistical significance from control using Student's "t" test ($P < 0.05$). Linear regression analysis of the data for sedation, ataxia-1, ataxia-2, ataxia-3 and loss of righting reflex showed a significant upward trend ($P < 0.05$). This analysis was not performed for the data for neutrality and coma either because of insufficient data or lack of linearity.

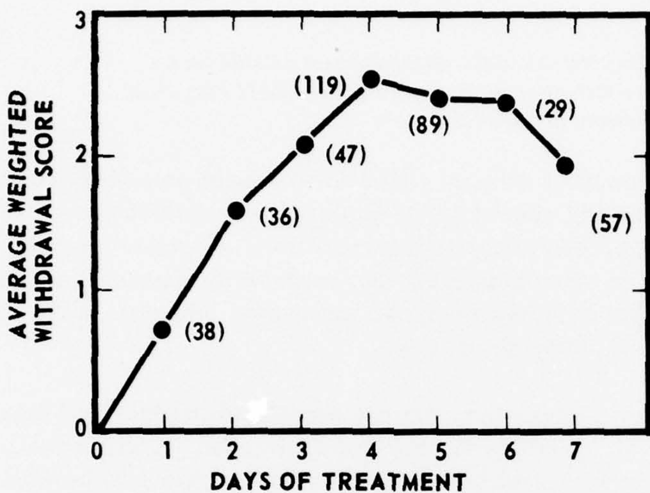

Figure 20. Time course of acquisition of physical dependence on ethanol following sustained intoxication with high doses of ethanol. The average weighted withdrawal score was obtained by multiplying the number of animals given a certain withdrawal score by that score and dividing by the total number of animals for each day of treatment. The numbers in parentheses denote the total number of animals used. X^2 analyses between each successive day of treatment demonstrated a significant upward trend in the withdrawal scores up to 4 days ($P < 0.05$).

REFERENCE

1. Majchrowicz, E. and Hunt, W. A. Temporal relationship of the induction of tolerance and physical dependence after continuous intoxication with maximum tolerable doses of ethanol in rats. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR76-48, 1976.

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SUPPRESSION BY 1,3-BUTANEDIOL OF THE ETHANOL WITHDRAWAL SYNDROME IN RATS

Principal Investigators: *E. Majchrowicz, National Institute on Alcohol Abuse and Alcoholism; W. A. Hunt, AFRRI; and C. Piantadosi, University of North Carolina*

Clinical management of ethanol withdrawal has to consider a wide variety of complications including neurological dysfunction, malnutrition, liver disease and general psychiatric and clinical deterioration. A major problem in treatment is controlling the neuromuscular and autonomic hyperactivity associated with withdrawal so that exhaustion can be prevented, thus allowing necessary treatment of other symptoms to proceed.

Presently available drugs for treating an ethanol withdrawal syndrome are often toxic and have an addiction liability of their own. A structural analogue of ethanol, 1,3-butanediol, has been known for a long time to be nontoxic and was tested for its ability to suppress an ethanol withdrawal syndrome. Male Sprague-Dawley rats were rendered physically dependent upon ethanol by intragastric administration of 9-15 g/kg of ethanol per day over a 4-day period. A nonintoxicating dose of 1,3-butanediol of 4 g/kg, per os, administered after elimination of ethanol from the blood was effective against the tremulous and convulsive components of the withdrawal syndrome in all animals for 1-5 hours (Figure 21). This period of time coincided with the time of maximum

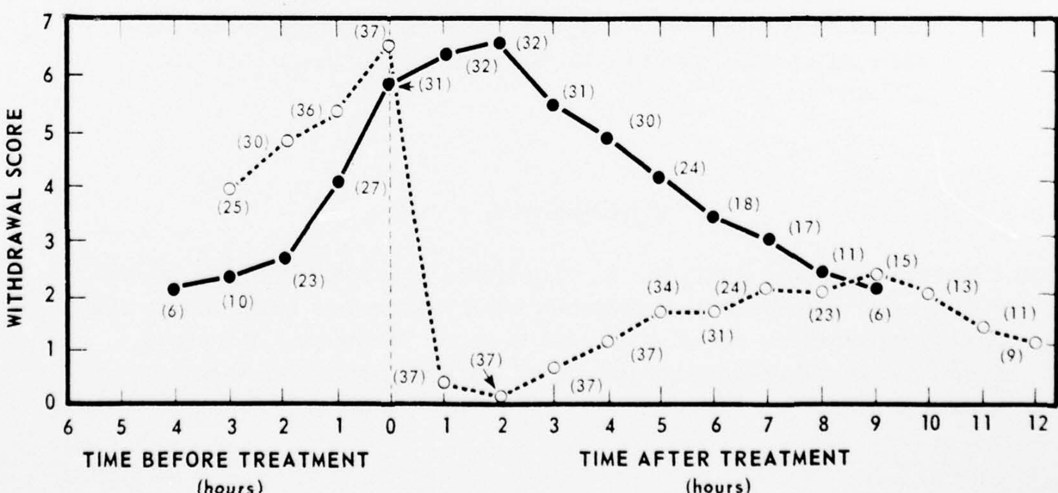

Figure 21. Effect of 1,3-butanediol (BD) (4 g/kg, per os) on the withdrawal score. The withdrawal score is defined in the text.[1] ●—● refers to untreated ethanol-dependent rats. o····o refers to BD-treated, ethanol-dependent rats. Time zero corresponds to the point at which all ethanol has been eliminated from the blood, and for BD-treated animals denotes the point at which BD was administered. Numbers in parentheses denote the number of animals observed, while each point refers to the mean of the withdrawal scores. Statistical differences were found for the period 1-5 hours after BD treatment using the median test ($P < 0.05$).

severity of the withdrawal syndrome as seen in the control animals. These results suggest that 1,3-butanediol may be useful in the treatment of the ethanol withdrawal syndrome in man. Thus 1,3-butanediol may be a clinically useful drug in the treatment of alcoholism.[1]

REFERENCE

1. Majchrowicz, E., Hunt, W. A. and Piantadosi, C. Suppression by 1,3-butanediol of the ethanol withdrawal syndrome in rats. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR76-38, 1976.

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LACK OF ALTERATION IN REGIONAL BRAIN ADENOSINE 3',5'-CYCLIC MONOPHOSPHATE LEVELS AFTER ACUTE AND CHRONIC TREATMENT WITH ETHANOL

Principal Investigators: *J. D. Redos, American University; W. A. Hunt and G. N. Catravas, AFRRI*

Proper methodology plays an important role in obtaining valid information. Recent evidence has revealed that the rapid inactivation of brain enzymes is paramount in measuring levels of 3',5'-cyclic adenosine monophosphate. Previously reported studies have suggested that acute and chronic treatment with ethanol induces alterations in cyclic adenosine monophosphate levels in the brain. Because the methods used in those studies to minimize postmortem accumulation of cyclic adenosine monophosphate are now considered to be inadequate, the effects of ethanol were reinvestigated using focused microwave irradiation to prevent postmortem cyclic adenosine monophosphate accumulation. These studies were extended to include measurements in seven areas of the rat brain after acute administration of ethanol and in animals rendered ethanol-dependent.[1] Three treatment groups were examined: acutely treated while intoxicated (6 g/kg, per os), ethanol-dependent while intoxicated, and ethanol-dependent while undergoing a withdrawal syndrome. Animals were euthanatized by a 3.5-sec focused microwave exposure to the head (1.2 kW). Tissue samples were purified by adsorption and ion exchange chromatography and quantitated using a competitive protein binding assay. No changes in cyclic adenosine monophosphate levels were observed in any of the brain areas studied after any of the ethanol treatments (Table 15). The data suggest that changes in cyclic

adenosine monophosphate levels in the brain do not play any role in the acute and chronic effects of ethanol. The results further demonstrate the importance of methodology in neurochemical studies. This has considerable relevance in studying potential alterations in brain chemistry results from chronic exposure to toxic chemicals and ionizing and nonionizing radiation.

Table 15. Brain Cyclic Adenosine Monophosphate (c-AMP) Levels in Discrete Areas After Various Conditions of Ethanol Treatment

	c-AMP Levels (pmoles/mg protein + S. E.)			
	Controls	Acute	Chronic Intoxicated	Withdrawal Syndrome
Cerebellum	3.4 ± 0.16	3.2 ± 0.08	3.5 ± 0.34	3.7 ± 0.19
Brain Stem	3.1 ± 0.17	3.0 ± 0.08	3.2 ± 0.11	3.0 ± 0.24
Hypothalamus	3.2 ± 0.32	3.2 ± 0.19	3.3 ± 0.10	3.4 ± 0.26
Thalamus	4.5 ± 0.51	4.5 ± 0.36	4.3 ± 0.30	4.3 ± 0.34
Hippocampus	3.7 ± 0.09	3.7 ± 0.39	3.8 ± 0.14	3.8 ± 0.24
Caudate Nucleus	3.8 ± 0.27	3.5 ± 0.18	3.7 ± 0.15	3.3 ± 0.24
Cerebral Cortex	7.2 ± 0.36	7.2 + 0.28	7.1 ± 0.43	6.9 ± 0.46

Each group consisted of 5-10 animals. Blood ethanol levels in acutely treated animals were 373 ± 8.9 mg/dl, while in chronic intoxicated animals they were 334 ± 22.9 mg/dl.

REFERENCE

1. Redos, J. D., Hunt, W. A. and Catravas, G. N. Lack of alteration in regional brain adenosine 3',5'-cyclic monophosphate levels after acute and chronic treatment with ethanol. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR76-23, 1976.

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ETHANOL-INDUCED DEPLETION OF CEREBELLAR GUANOSINE 3',5'-CYCLIC MONOPHOSPHATE LEVELS

Principal Investigators: *J. D. Redos, American University; G. N. Catravas and W. A. Hunt, AFRRI*

Ethanol in moderate doses induces ataxia in man and laboratory animals. This motor deficit may be cerebellar and may involve an interruption of normal excitatory input to Purkinje cells. A chemical mediator of such influence is thought to be 3',5'-cyclic guanosine monophosphate. The ability of ethanol to interact with cerebellar cyclic guanosine monophosphate was examined. Male Sprague-Dawley rats were intubated with a single dose of ethanol (1-6 g/kg) and the animals euthanatized at different times thereafter. The cerebellum was obtained and analyzed for cyclic guanosine monophosphate and cyclic adenosine monophosphate after prior purification. Ethanol induced a dose-dependent depletion of cyclic guanosine monophosphate up to 95 percent. A significant reduction was observed at blood ethanol levels found after moderate drinking (Table 16). This response was completely reversible with ethanol elimination (Figure 22). No change in cyclic adenosine monophosphate was observed. The data suggest that cyclic guanosine monophosphate depletion in the cerebellum may play a role in the intoxication properties of ethanol.[1] Also, this response may have relevance to the study of other motor and behavioral deficits such as cerebellar disease and early transient incapacitation.

Table 16. Cerebellar Cyclic Guanosine Monophosphate (GMP) and Cyclic Adenosine Monophosphate (AMP) 2 Hours After a Single Dose of Ethanol. Each value (mean ± standard error) was obtained from five to ten animals.

Dose (g/kg)	Cyclic GMP (pmole/mg of protein)	Cyclic AMP (pmole/mg of protein)	Blood ethanol (mg/dl)
Control			
None	4.0 ± 0.14	3.4 ± 0.16	
Ethanol-treated			
1	4.3 ± 0.58		31 ± 10
2	2.6 ± 0.42*		100 ± 6
3	1.4 ± 0.11*		183 ± 11
4	1.0 ± 0.16*		285 ± 24
6	0.5 ± 0.04*	3.2 ± 0.08	373 ± 9

* Denotes statistical significance ($p < .05$) as determined by Student's "t" test.

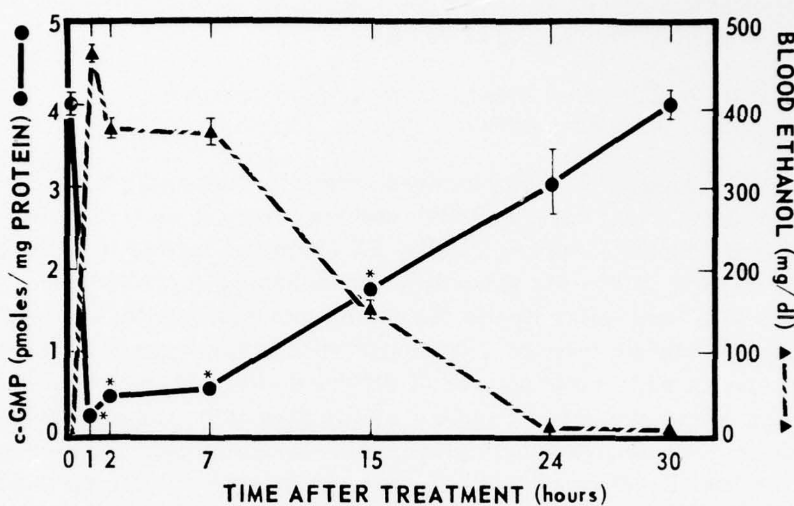

Figure 22. Concentration of cyclic guanosine monophosphate (solid line) in the cerebellum as a function of time after treatment and the concentration of ethanol (dot and dashed line) in the blood. Each value represents the mean ± standard error and was obtained from five to ten animals. The asterisks denote statistical significance ($p < .05$) as determined by Student's "t" test.

REFERENCE

1. Redos, J. D., Catravas, G. N. and Hunt, W. A. Ethanol-induced depletion of cerebellar guanosine 3',5'-cyclic monophosphate levels. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR76-24, 1976.

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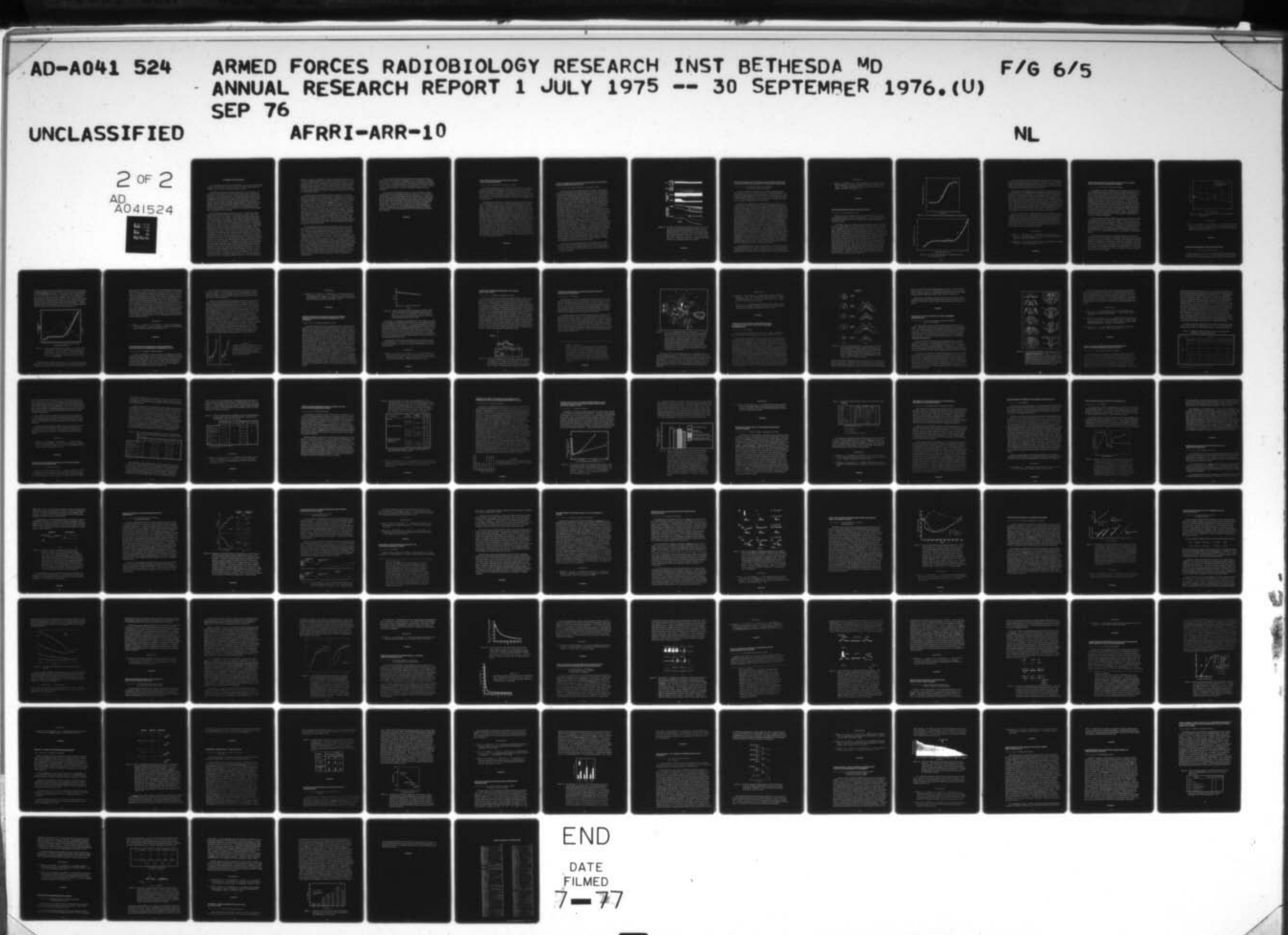
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NEUROBIOLOGY DEPARTMENT

The Neurobiology Department was established in 1973 to provide a research program with its primary activities directed at an understanding of the basic mechanisms of the effects of radiation on nervous tissue.

The Cellular Neurobiology Division, headed by Dr. W. G. Shain, Jr. is composed of a laboratory for the development of tissue culture of continuous cells lines of nerve, glia and muscle. These laboratories are primarily concerned with an understanding of the normal interaction between these various constituents of the central nervous system and target organs, as well as the mechanisms whereby the development of normal connections is altered by a variety of injurious agents, including ionizing radiation. This laboratory also utilizes a number of purified proteins obtained from snake toxins as marker substances for the study of normal receptors for neurotransmitters and, in the case of β-bungarotoxin, as a probe which may be acting at calcium binding sites of presynaptic nerve terminals.

The Neurophysics Division, headed by Dr. M. L. Wiederhold, contains laboratories involved in the study of basic properties of nerve cells in elementary nervous systems as well as a laboratory for the study of the auditory system of mammals. The primary research projects utilizing simpler nervous systems aim at an understanding of the metabolic properties determining nerve cell excitability, and an understanding of the mechanisms of interaction between nerve cells and from nerve to peripheral target cells. A considerable number of the research projects of the department involve people collaborating between the Cellular Neurobiology and the Neurophysics Divisions. This is particularly true for two areas of research that are of critical interest to the Defense Nuclear Agency. The first of these is the problem of the organization of receptors for neurotransmitter substances and in particular those receptors for histamine on nerve cells and on smooth muscle. Histamine is released by radiation from mast cell stores throughout the body and is probably the humoral agent involved in causing early transient incapacitation, a phenomenon involving a performance decrement of trained animals for a short period of time beginning some 5 min postirradiation. Treatment and prevention of such incapacitation necessitates a knowledge of the receptors for histamine and investigation into agents which could prevent this effect by blocking the receptors. We are studying the receptors for histamine in two different preparations. The first is the marine mollusc Aplysia where histamine appears to be used as a neurotransmitter within the nervous system. We have found at least two pharmacologic types of histamine receptors and at least three different ionic responses on different neurons. In addition, human smooth muscle cells have been cultured and are being investigated electrophysiologically. These smooth muscle

cells have receptors for histamine. Because smooth muscle in blood vessel walls is exceptionally difficult to record from electrophysiologically, the use of a tissue culture system to study these receptors and their pharmacological sensitivities is the most direct means of understanding how radiation-induced histamine release might cause a performance decrement in intact animals. The latter is presumably secondary to a fall in cerebral blood flow resulting from interaction of histamine with these receptors on the smooth muscle cells in cerebral blood vessels.

A second research area involving a number of people in both of these divisions is the study of how cell properties are controlled by calcium concentrations, and particularly by the intracellular concentration of free calcium. This problem appears to be central in an understanding of the primary effects of radiation on neurons. When isolated Aplysia neurons are exposed to 20 MeV electrons, they show an initial excitation by the radiation but no direct effects with the time course of early transient incapacitation. However at about 8 to 12 hours postirradiation, at a very high dose level, these neurons show signs of an increase in intracellular calcium concentration. This proceeds to a total loss of membrane resistance and finally membrane potential, suggesting that the primary effect of the radiation has been to interfere with the regulatory mechanisms of calcium concentration control. Because of these facts we are actively engaged in the study of those mechanisms which control calcium concentration at the level of the plasma membrane as well as the level of intracellular storage sites. The development of β-bungarotoxin as a tool to block the calcium entry site at the plasma membrane has aided enormously in achieving this goal.

The Neurological Sciences Division is directed by Major E. N. Gunby. The research in this division primarily utilizes primates and is directed principally at an understanding of mechanisms of injury to the nervous system. The professional staff consists of clinical neurologists and neurosurgeons, and the research projects are ones which have direct relevance to treatment of human injury and disease states. One of the laboratories in this division is headed by Colonel A. N. Martins, Chairman of the Neurosurgery Department, Walter Reed Army Medical Center. Dr. Martins' research interests include the study and manipulation of cerebral and spinal blood flow, the study of cerebral spinal fluid production and mechanisms underlying local brain injury and development of cerebral edema. Some of the research projects in this laboratory attempt to test experimentally the value of drugs which might have clinical use. Another major research project in this division is the study of experimental head injury in a primate model, and this research program is a joint one between AFRRI staff and the National Institute of Neurological and Communicative Disorders and Stroke and the Department of Transportation.

A portion of the research conducted during this reporting period by the former Neurochemistry Division, headed by Dr. G. N. Catravas, is reported in this section. This division's laboratories utilized techniques for the chemical measurement of neurotransmitters, their synthetic and degradative enzymes and other organic substances in the brain upon exposure to a variety of insults, including ionizing radiation, microwave irradiation, alcohol and opiate dependence.

The Neurobiology Department sponsors a number of activities aimed at assisting the training and scientific growth of its members. These include a weekly seminar, a weekly informal research discussion group, a weekly journal club and evening graduate seminar courses. Some of these activities are held in conjunction with the Department of Physiology at the George Washington University School of Medicine, with which the Neurobiology Department has close ties. During this period we have had four graduate students at AFRRI doing work which will lead to degrees from George Washington University. In addition we have had seven professionals working at AFRRI on NIH Postdoctoral Fellowships sponsored officially through George Washington University.

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FUNCTIONAL DISTRIBUTION OF SYMPATHETIC NERVES IN THE CYNOMOLGUS MONKEY

Principal Investigators: *W. A. Alter III, D. E. Evans and L. J. Parkhurst*

Fatalities following head injury in military personnel may be related to trauma-induced cardiac arrhythmias. This study was begun to determine the peripheral autonomic nerves which may be involved in these arrhythmias. Prior to investigating arrhythmias, the normal function of these nerves must be established. The rhesus monkey could be a suitable animal model for this study, but due to their shortage, an alternative species, the cynomolgus monkey, was chosen.

Ten cynomolgus monkeys were anesthetized and prepared for recording arterial blood pressure, heart rate, right atrial force and right ventricular force. The stellate ganglia, middle cervical ganglia and thoracic vagi were exposed and all discernible branches stimulated. The largest cardiac sympathetic trunk on the right side was the middle cervical cardiac nerve, stimulation of which resulted in a significant increase in all recorded parameters. Right stellate nerves passing directly to the heart were not observed in cynomolgus monkeys, but small stellate branches coursing into the vagus were consistently found and stimulation of these branches usually resulted in a significant increase in all recorded parameters. Sympathetic fibers also entered the vagus at the level of the right middle cervical ganglion and then emerged in the craniovagal and caudovagal cardiac nerves. After administration of atropine, stimulation of these cardiac vagal nerves resulted in significant positive chronotropic and inotropic responses without any significant changes in arterial blood pressure. The right phrenic nerve was also found to have significant effects on heart rate and right cardiac function. The major pathway on the left side affecting rate and force was the ventromedial cardiac nerve which originates in the middle cervical ganglion. These results indicate that the cynomolgus monkey is a suitable alternative to the rhesus monkey for studies of neural control of the heart.

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EFFECT OF CORONARY ARTERY OCCLUSION ON REGIONAL MYOCARDIAL BLOOD FLOW AND CARDIAC FUNCTION

Principal Investigators: *W. A. Alter III, D. E. Evans, L. J. Parkhurst and T. F. Doyle*

Chest impact frequently results in trauma to the heart. This trauma can lead to irreversible cardiac dysfunction. This study was undertaken to investigate the alterations in cardiac function which occur after production of focal areas of myocardial ischemia in an animal model. Ischemia was induced by temporary interruption of blood flow to discrete areas of myocardium. Myocardial blood flow (by the hydrogen polarographic technique), contractile force and electrical activity were detected from both the base and apex of the anterior wall of the left ventricle. Twelve minutes of distal left anterior descending coronary artery occlusion resulted in significant decreases in apical myocardial blood flow (control, 119 ± 8 ml/min/100 g of tissue; occlusion, 42 ± 31 ml/min/100 g) and contractile force (38 ± 5 percent below preocclusion value). The figure shows that distal left anterior descending coronary artery occlusion resulted in a marked decrease in contractile force and myocardial flow in the ischemic area (apex) while cardiac function and blood flow in the nonischemic area (base) were not affected (Figure 23). Additionally, local subepicardial electrograms were markedly abnormal in the ischemic region. In contrast, none of these parameters were altered at the base of the anterior wall of the left ventricle which was outside the region of occlusion. Removal of the occlusion clip resulted in a marked reactive hyperemic response 100 ± 16 percent at the apex of the anterior wall of the left ventricle. In most cases, contractile force recovered to control with six animals demonstrating a transient positive overshoot in contractile force. In all cases, electrical activity returned to the preocclusion waveform within 5 min after the removal of the occlusion clip. No change in heart rate or blood pressure or the Lead-II ECG was observed during or after occlusion. Based on these results, it was concluded that the mechanical and electrical changes which occurred during and after a transient distal coronary artery occlusion are local responses resulting from inadequate blood flow to the ischemic region.

This study has demonstrated that brief interruptions of myocardial blood flow lead to local reversible changes in cardiac function. These results in conjunction with existing data provide a better understanding of the function of ischemic heart muscle and should lead to better treatment of military personnel.

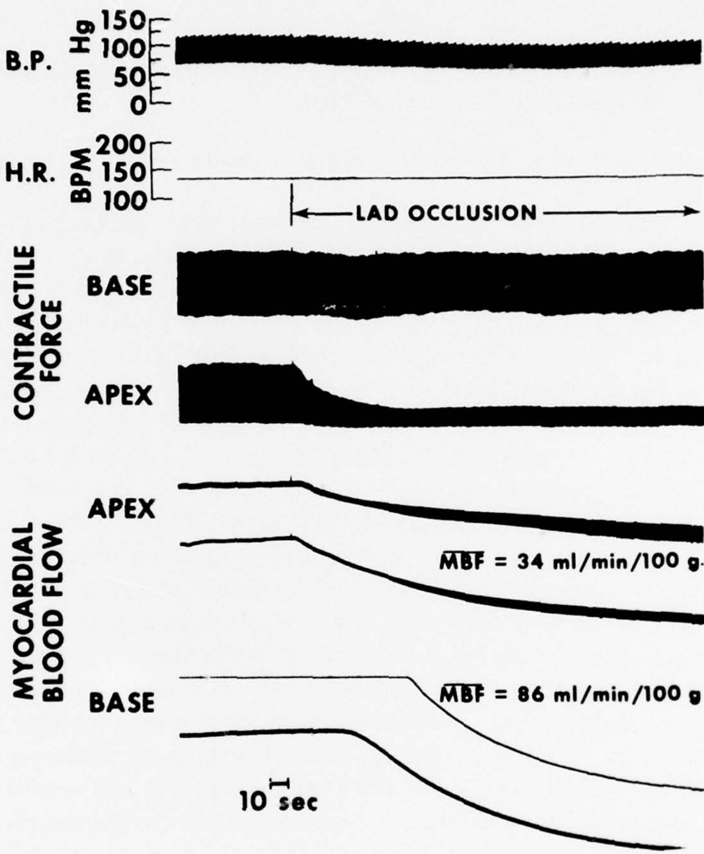

Figure 23. The effect of left anterior descending coronary artery (LAD) occlusion on myocardial blood flow and cardiac function. Both contractile force and blood flow at the apex were significantly affected. Contractile force and myocardial blood flow were not affected at the base of the left ventricle which was outside the ischemic area. B. P. - arterial blood pressure, H. R. - heart rate, and MBF - mean myocardial blood flow for two electrodes located in each region.

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EFFECT OF OUABAIN AND ITS INTERACTION WITH DIPHENYLHYDANTOIN ON CARDIAC ARRHYTHMIAS INDUCED BY HYPOTHALAMIC STIMULATION

Principal Investigators: *D. E. Evans, AFRRI; and R. A. Gillis, Georgetown University Schools of Medicine and Dentistry*

Cardiac arrhythmias resulting from myocardial infarction and head injury result in the loss of a significant number of military personnel. Appropriate and timely pharmacological treatment can restore normal, cardiac rhythm and greatly enhance the chance for recovery of victims. The drugs currently used as antiarrhythmics have been shown to also affect the central nervous system's modulation of cardiac function.

This study was undertaken to investigate the possible central nervous system effects of the antiarrhythmic drugs diphenylhydantoin and ouabain in an animal model (cat).[1] Electrical stimulation of the posterior portion of the hypothalamus resulted in alterations of cardiac rhythm, and recordings from cardiac bound sympathetic nerves revealed continuous activity during the time of arrhythmia. Intravenous administration of small doses of ouabain (i.e., 10-30 μg/kg) prevented both the arrhythmias and the associated hyperactivity on the sympathetic nerves. The antiarrhythmic effect was not present in animals with denervated baroreceptors indicating that the ouabain effects were due to sensitization of baroreceptor reflexes. Intravenous administration of large but subarrhythmic doses of ouabain (i.e., 60-80 μg/kg) converted a subarrhythmogenic hypothalamic electrical stimulus to a threshold stimulus for arrhythmias. The increased response of the heart to brain stimulation in the presence of these doses of ouabain was associated with an increased discharge of cardiac sympathetic nerves, indicating that ouabain was exerting its arrhythmogenic enhancing effect on the central nervous system rather than on the heart. Pretreatment with diphenylhydantoin prevented the arrhythmias produced by the combination of ouabain and hypothalamic stimulation. Prevention of the arrhythmia was associated with prevention of the associated sympathetic hyperactivity responsible for the arrhythmia, thus indicating a central nervous system site of action of diphenylhydantoin. These data suggest that central nervous effects of ouabain and diphenylhydantoin are important in the ability of these drugs to alter cardiac rhythm.

An understanding of the central nervous system's control of cardiac function will not only provide a basis for rational prevention of neurally induced arrhythmias following such events as head injury but may also provide important perspectives in management of the patient with arrhythmias or infarction.

REFERENCE

1. Evans, D. E. and Gillis, R. A. Effect of ouabain and its interaction with diphenylhydantoin on cardiac arrhythmias induced by hypothalamic stimulation. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR76-7, 1976.

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PHYSIOLOGIC CONTROL OF SPINAL CORD BLOOD FLOW

Principal Investigators: *A. I. Kobrine and T. F. Doyle*

Spinal cord injury is a major source of human disability for the Department of Defense and the Veterans Administration. In order to understand the mechanism of spinal injury we have investigated the effect on blood flow of local spinal trauma.

The hydrogen clearance technique was used to measure spinal cord blood flow in the rhesus monkey to test the hypothesis of autoregulation in the spinal cord.[1] Laminectomies were performed at the T9-T10 level, and 250-μm diameter platinum electrodes were placed through the intact dura, 2 mm into the spinal cord, midway between the midline and lateral borders. Flows were calculated from the desaturation curves of inhaled hydrogen. When the mean arterial pressure was kept normal and the $PaCO_2$ was varied by hyperventilation or changes in inspired CO_2, spinal cord blood flow remained normal with $PaCO_2$ values of 10-50 mm Hg. Spinal cord blood flow increased as $PaCO_2$ was raised from 50 to 90 mm Hg. Above a $PaCO_2$ of 90 mm Hg, spinal cord blood flow did not increase (Figure 24). When $PaCO_2$ was kept constant and mean arterial pressure was varied by norepinephrine infusion or bleeding, spinal cord blood flow was constant and in the normal range when the mean arterial pressure was between 50 and 135 mm Hg. Above 135 mm Hg mean arterial pressure, the spinal cord blood flow increased directly with mean arterial pressure. Below 50 mm Hg, spinal cord blood flow decreased directly with mean arterial pressure (Figure 25).

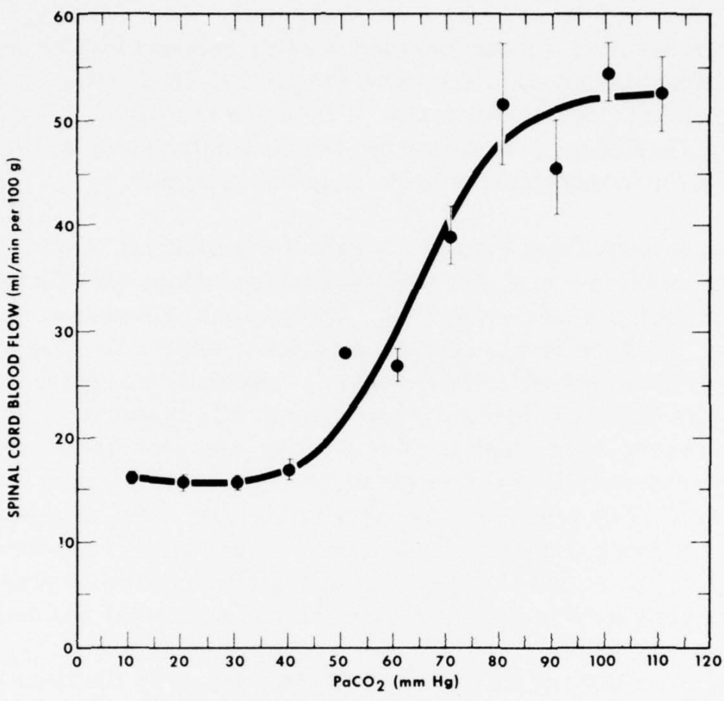

Figure 24. The relationship of spinal cord blood flow to arterial PCO_2

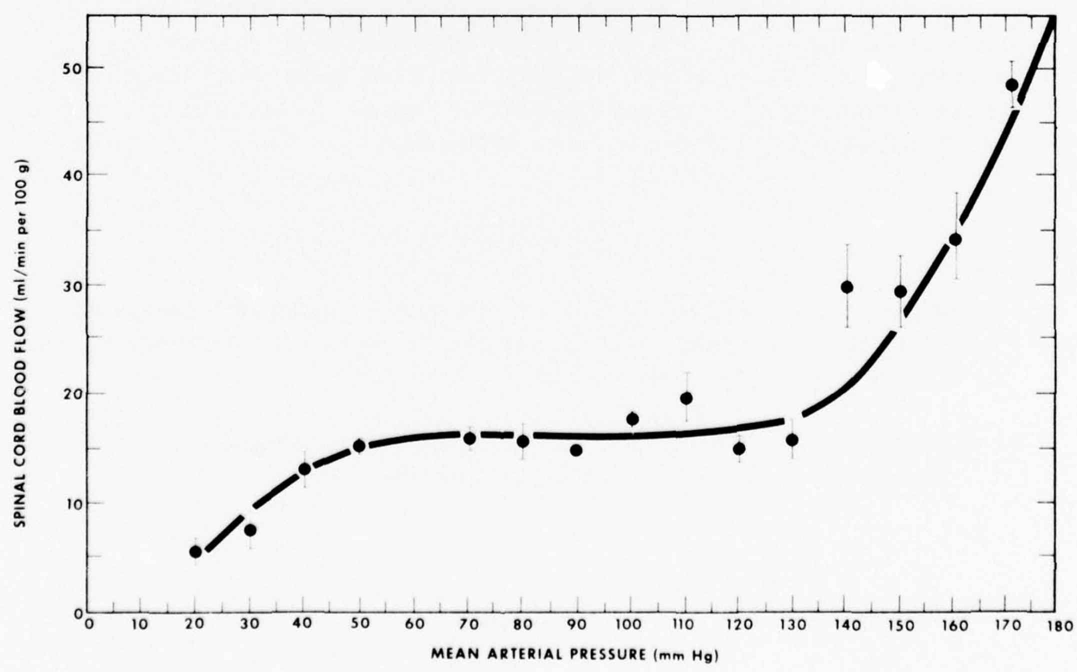

Figure 25. The relationship of spinal cord blood flow to mean arterial blood pressure

One can hypothesize that the mechanisms responsible for autoregulation in the brain and spinal cord are quite similar, if not identical, since the histology and embryological derivation of both are the same. The above model would seem therefore to be suited for the continuing study of the mechanisms responsible for autoregulation in the nervous system.

As can be seen from Figure 25, at a mean arterial pressure greater than 135 mm Hg there is a breakthrough of autoregulation, with further increases in mean arterial pressure effecting a corresponding increase in spinal cord blood flow. At these levels of mean arterial pressure the vessels are maximally constricted, vascular resistance is maximal, and spinal cord blood flow becomes passively related to mean arterial pressure. Likewise, at mean arterial pressure levels less than 50 mm Hg, the vessels are maximally dilated, vascular resistance is minimal, and again spinal cord blood flow is passively controlled by mean arterial pressure. Similarly, Strandgaard demonstrated intact autoregulatory mechanisms in the brain of baboons up to a mean arterial pressure of 120-140 mm Hg, above which cerebral blood flow increased directly with further increases in mean arterial pressure.[2]

Other investigators have addressed themselves to the question of autoregulation in the spinal cord. Using such methods as the particle distribution technique, surface flow measuring devices, and the intraparenchymal injection of tracer substances, they have generally agreed that autoregulation exists in the spinal cord and is not unlike that described for the brain. An understanding of the basic mechanisms controlling spinal cord blood flow is necessary to provide a rational approach to therapy in human injury, and these studies assist in achieving such knowledge.

REFERENCES

1. Kobrine, A. I. and Doyle, T. F. Physiologic control of spinal cord blood flow. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR75-28, 1975.

2. Strandgaard, S. The lower and upper limit for autoregulation of cerebral blood flow. Stroke 4:323 (Abstract), 1973.

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HYPEREMIA AND EDEMA IN THE MONKEY SPINAL CORD FOLLOWING INJURY: MODIFIED BY HISTAMINE ANTAGONISTS

Principal Investigators: *T. F. Doyle, A. I. Kobrine and A. N. Martins*

The mechanisms of the development of acute changes which occur in the early posttraumatic period of spinal cord injury are not fully understood. After contusion of the spinal cord, blood flow markedly decreases in the central gray region and increases in the lateral, white region. Microangiographic studies show that the capillaries in the central region become progressively occluded over the first 4 hours; however, the capillaries of the peripheral white matter remain patent and functional. The hemorrhaged and disrupted central gray tissue shows extensive leakage while the vessels of the peripheral white matter still permit blood flow, although edema soon develops. Histamine levels in the cord increase significantly after trauma. It is reasonable to suspect, therefore, that histamine might be involved in posttraumatic hyperemia and edema in the spinal cord. To test this possibility after experimentally induced myelopathy, changes in the spinal cord blood flow and edema formation were measured after the administration of H_1 and H_2 histamine antagonists.[1]

Using the hydrogen washout technique, blood flow in the lateral funiculus of the spinal cord of rhesus monkeys was measured before and after a contusion sufficient to render the animals paraplegic. In untreated animals the blood flow increased 1 hour after injury to almost twice the normal flow rate (17.6 to 31.8 ml/min/100 g). When the animals were pretreated with histamine antagonists (chlorpheniramine, an H_1 antagonist, and metiamide, an H_2 antagonist) there was no significant increase in blood flow following contusion (Figure 26). Antihistamines, however, were ineffective in preventing edema.

Metiamide when given alone or in combination with chlorpheniramine alone does not block the hyperemia completely. Since hyperemia is a function of decreased vascular resistance, it is apparent in this instance that the vasodilatation responsible for the hyperemia is blocked by metiamide. This finding is in agreement with a recent study in cats which showed that blood vessels responsible for the general blood pressure have predominantly or exclusively H_2 receptors which modulate vessel tone. This study may be of value in treating or better understanding spinal cord injury suffered by military personnel both in peacetime and combat.

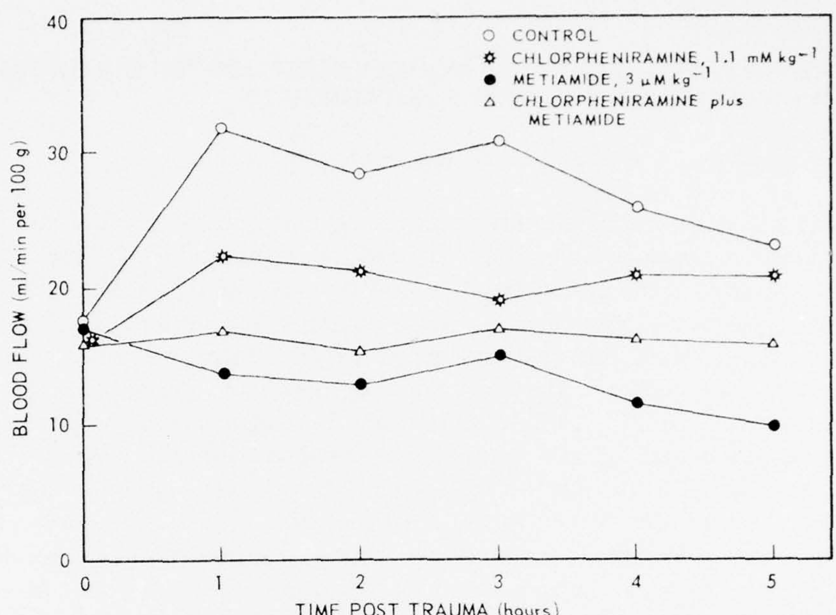

Figure 26. Spinal cord blood flow (white matter) as modified by antihistamines

REFERENCE

1. Doyle, T. F., Kobrine, A. I. and Martins, A. N. Hyperemia and edema in the monkey spinal cord following injury: modified by histamine antagonists. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR76-8, 1976.

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FURTHER STUDIES IN SPINAL CORD AUTOREGULATION

Principal Investigators: *A. I. Kobrine, T. F. Doyle and N. Newby*

Spinal cord injury is a major cause of hospitalization, and especially chronic hospitalization. This study is part of a continuing search to understand the basic mechanisms involved in spinal injury.[1] Using the hydrogen

clearance method to measure focal spinal cord blood flow in the rhesus monkey (Macaca mulatta), the effect of high cervical cord section on the phenomenon of autoregulation was studied. Laminectomies were performed at the T7-T11 as well as the C1-C2 levels. The spinal cord was completely severed at C1-C2. Under normocapnic conditions, spinal cord blood flow was then measured in the thoracic spinal cord over a wide range of mean arterial blood pressures. The mean arterial blood pressure was either lowered by bleeding or raised by the intravenous infusion of norepinephrine. Autoregulation was found to be intact between 50 and 125 mm Hg, following a pattern similar to the one observed in the intact animal (Figure 27).

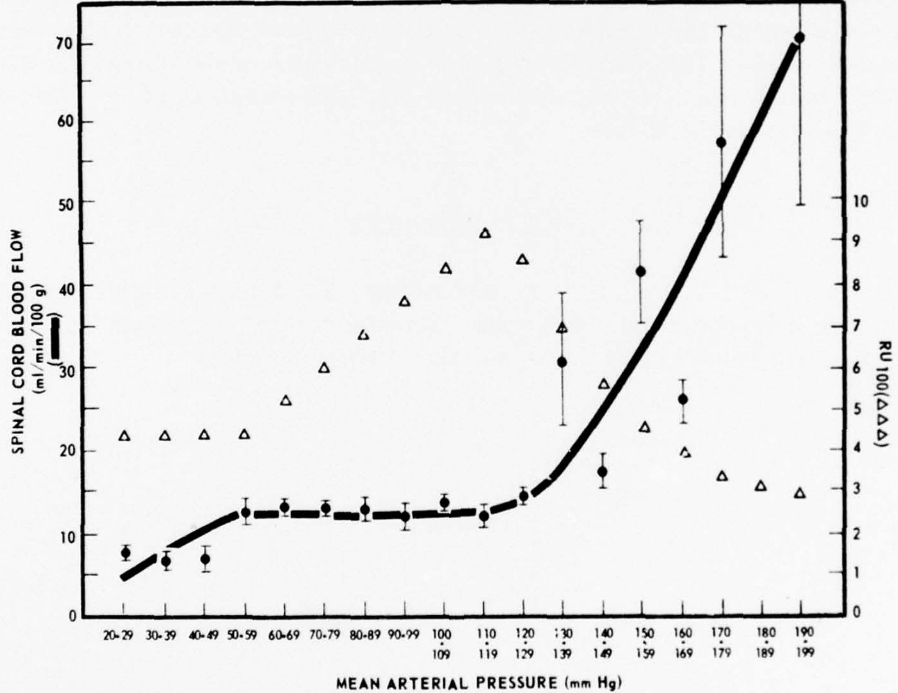

Figure 27. A best fitting line through the means (± S. D.) of spinal cord blood flow as the flow varied with changes in mean arterial blood pressure. The left ordinate is in units of blood flow (ml/min/100 g). The right ordinate is in units of vascular resistance $\left(\frac{\text{mm Hg}}{\text{ml/min/100 g}}\right)$. Spinal cord severed at C1-C2.

Studies involving cerebral autoregulation have suggested that the sympathetic nervous system may play an important role, and anatomic studies have demonstrated adrenergic nerve endings in the walls of blood vessels at the

base of the brain. Physiologic studies have also tended to implicate the sympathetic nervous system in control of the cerebral circulation. A dual mechanism has been suggested, involving the sympathetic nervous system in control of the extraparenchymal vessels and local tissue pH changes in control of the small intraparenchymal vessels. The role of the sympathetic nervous system in control of spinal cord circulation, however, remains moot. The data from this experiment tend to negate a major role of the sympathetic nervous system in the mechanism of autoregulation in the spinal cord because, in this preparation, autoregulation has remained intact after the spinal cord has been removed from the cerebral control of the sympathetic nervous system. These findings were essentially unchanged from observations of similar phenomena in intact animals and therefore provide evidence against the importance of either the sympathetic nervous system or its cerebral control in these situations. This observation is of importance in the clinical treatment of spinal injury and also increases our basic understanding of how the nervous system regulates blood flow.

REFERENCE

1. Kobrine, A. I., Doyle, T. F. and Newby, N. Further studies in spinal cord autoregulation. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR76-9, 1976.

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VENTRICULOCISTERNAL PERFUSION STUDIES IN THE MONKEY. PCO_2 AND THE RATE OF FORMATION OF CEREBROSPINAL FLUID

Principal Investigators: *A. N. Martins, T. F. Doyle and N. Newby*

The rate of formation of cerebrospinal fluid in the subprimate mammal decreases during hypocapnia. However, the effect of hypercapnia on cerebrospinal fluid formation rate (V_f) remains controversial. By direct observation of the exposed choroid plexus of the cat, it was found that hypercapnia increased V_f above normal. On the contrary, studies using the ventriculocisternal perfusion technique to measure V_f in the cat, dog, and rabbit have failed to associate hypercapnia with an increase in V_f.

In view of these conflicting data and the lack of information concerning the effect of PCO_2 on V_f in the primate, we have reexamined the question in the rhesus monkey. Our results suggest that marked alterations in PCO_2, sufficient to produce large documented changes in cerebral blood flow, have little effect on V_f in the monkey.[1]

Changes in cerebrospinal fluid formation rate with hypocapnia and hypercapnia were measured by the ventriculocisternal perfusion technique in 24 rhesus monkeys anesthetized with nitrous oxide. In addition, cerebral blood flow was measured by the hydrogen clearance method. V_f in control animals declined at 2.3 μl/min each hour during the last 4-1/2 hours of a 7-hour perfusion although parameters known to affect V_f remained stable. Three hours after perfusion began, V_f of normocapnic controls was 41.4 μl/min ± 5.4 and cerebrospinal fluid was 59 ml/100 g per min. When PCO_2 was reduced to half of control, V_f fell to 35.6 μl/min ± 6.3 and cerebrospinal fluid fell by 27 percent. When PCO_2 was doubled, V_f fell to 33.1 μl/min ± 5.3 and cerebrospinal fluid increased threefold. The difference in V_f is significant only for the hypercapnic group ($p = 0.01$). When animals were used as their own controls, there were no significant differences in V_f with hypocapnia compared to normocapnia. These results indicate that in the monkey, variations of PCO_2 within broad physiologic limits which are sufficient to cause large changes in cerebral blood flow (Figure 28) have little effect on V_f. This observation is of clinical importance in all respiratory disease states characterized by an elevated PCO_2, and also is of very practical significance to surgeons and anesthesiologists who often wish to vary PCO_2 at surgery.

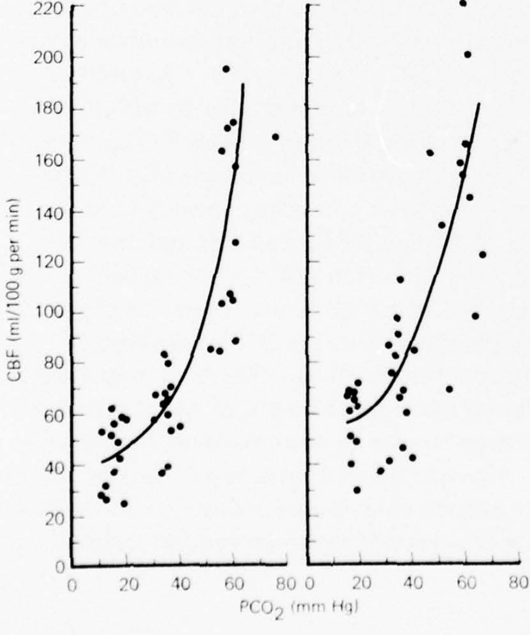

Figure 28.
Cerebral blood flow (CBF) response to step increases or decreases in PCO_2 in the experimental group of monkeys. Left: PCO_2 started low and was increased stepwise twice. Right: PCO_2 started high and was decreased stepwise twice.

REFERENCE

1. Martins, A. N., Doyle, T. F. and Newby, N. Ventriculocisternal perfusion studies in the monkey. II. PCO_2 and the rate of formation of cerebrospinal fluid. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR76-17, 1976.

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VENTRICULOCISTERNAL PERFUSION STUDIES IN THE MONKEY. SOURCES OF ERROR IN MEASURING CEREBROSPINAL FLUID FORMATION

Principal Investigators: *A. N. Martins, N. Newby and T. F. Doyle*

Ventriculocisternal perfusion is regarded as a precise method of measuring the rate of formation of cerebrospinal fluid but it possesses inherent potential sources of error not commonly appreciated. Using the technique to measure cerebrospinal fluid formation rate in the rhesus monkey, we have observed rate changes when none were expected.[1] Most puzzling has been the steady decline of cerebrospinal fluid formation rate at 4 percent each hour during the final 5 hours of a 7-hour perfusion although variables known to affect cerebrospinal fluid formation remained stable (Figure 29). In addition, artifactitious alterations in rate were observed in experiments in which craniospinal blood volume was changed by sudden changes of either PCO_2 or central venous pressure. Mobilization or sequestration of incompletely equilibrated cerebrospinal fluid is believed responsible. In other experiments, a small increase of intracranial pressure produced by increasing outflow resistance was quickly followed by an apparent reduction of cerebrospinal fluid formation. Here we speculate that when intracranial pressure is increased above normal, some newly formed cerebrospinal fluid may be diverted to drainage channels before it can mix with perfusion fluid. We have concluded that to assess accurately the effect of a variable on the rate of cerebrospinal fluid formation, one must control perfusion time and craniospinal blood volume as well as intracranial pressure. Knowledge of the normal rate of formation of cerebrospinal fluid is necessary before this model can be used to study relation of cerebrospinal fluid dynamics to central nervous system trauma and disease.

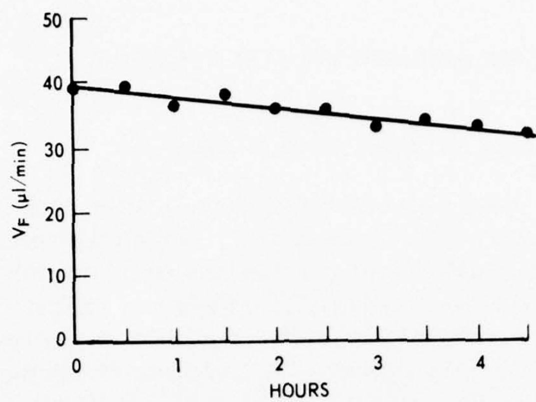

Figure 29. Linear regression analysis (least squares method) of mean CSF formation rate (V_f) plotted against duration of perfusion. $Y = -1.71x + 39.2$, in which x = time in hours with the end of 2.5 hours of perfusion taken as zero. Slope significantly different from zero ($p < .001$).

In summary, cerebrospinal fluid formation rate in the monkey, as measured by prolonged ventriculocisternal perfusion, declines spontaneously with time for reasons that remain obscure. By mobilizing unequilibrated pools of cerebrospinal fluid, sudden changes of craniospinal blood volume can lead to artifactitious changes in cerebrospinal fluid formation rate; and there is reason to believe that part of the alteration in rate observed after intracranial pressure is increased or decreased may also be an artifact of the method. These sources of error must be avoided or controlled if one is to assume correctly that a variable under study is responsible for an observed alteration in cerebrospinal fluid formation rate.

Hydrocephalus is often associated with head injuries which are treated in military hospitals. A more precise method of measuring cerebrospinal fluid formation would be of value in treating or understanding this commonly occurring injury.

REFERENCE

1. Martins, A. N., Newby, N. and Doyle, T. F. Ventriculocisternal perfusion studies in the monkey. III. Sources of error in measuring cerebrospinal fluid formation. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR76-22, 1976.

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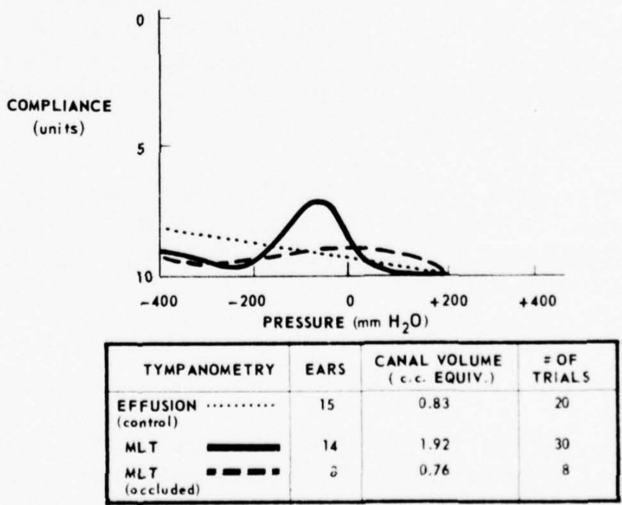
A SELECTIVELY PERMEABLE MEMBRANE FOR TYMPANIC VENTILATION TUBES

Principal Investigators: *J. B. Castelli, J. P. Murray and H. O. deFries*

We have evaluated the design and clinical application of a selectively permeable membrane for use in the lumen of tympanic ventilation tubes. The membrane is composed of polytetrafluoroethylene which is impermeable to fluid, acts as a bacterial filter, and allows middle ear ventilation under clinical conditions. An experimental cat model of middle ear effusion was obtained by eustachian tube ligation. Tympanometry and otomicroscopic inspection in 14 animals for up to 6 months revealed satisfactory middle ear ventilation (Figure 30). No recurrent middle ear effusion developed nor was purulent otitis media noted after controlled instillation of contaminated water into the external canal. An air and water tight tympanic membrane seal was demonstrated around the outside diameter of the tympanic ventilation tube. The membrane tympanic ventilation tube is proposed for clinical use, and clinical trials are under consideration. Besides their frequent use in pediatric otology, tympanic ventilation tubes are of use in relieving middle ear effusion caused by barotrauma. The selectively permeable membrane tube has the advantage that it can be used under water and prevents entry of water and bacteria into the middle ear. This may be of use to military personnel subject to middle ear effusion after diving or rapid changes in altitude while flying.



TYMPANOMETRY	EARS	CANAL VOLUME (c.c. EQUIV.)	# OF TRIALS
EFFUSION (control) ··········	15	0.83	20
MLT ▬▬▬	14	1.92	30
MLT ▪▬▬▪ (occluded)	[illegible]	0.76	8

Figure 30. Results of tympanometry on ears whose eustachian tubes have been ligated. In the control ears (dotted line) with eustachian tube ligated but no ventilation tube installed, there is very little change in tympanic membrane compliance with changing static pressure. This is the usual pattern with middle ear

A METHOD OF PREPARING THE IN SITU FROG SPINAL CORD FOR INTRACELLULAR RECORDING

Principal Investigator: *W. L. R. Cruce*

Since the pioneering work of Brookhart and his co-workers, the anuran (frog and toad) spinal cord has been used more and more frequently for physiological and pharmacological investigations of vertebrate synaptic transmission. The anuran spinal cord can be removed from the animals and kept alive for many hours in a bathing medium of oxygenated Ringer's solution.[1] This in vitro preparation has been especially useful because it is simple and because the spinal cord can be quickly infiltrated with known concentrations of chemical agents which affect synaptic transmission.

The in situ anuran spinal cord preparation has not been widely used for intracellular recording. However, this technique allows the exploration of problems which are more difficult or impossible with the excised, or in vitro, spinal cord (e.g., identification of supraspinal inputs). Further, it enables the cord to be kept in better physiological condition. Intracellular recordings have been made from the in situ spinal cord of small toads and small frogs, but few technical details were given about these preparations. More recently, Cruce has used in situ preparation of the spinal cord of a bullfrog in an intracellular study of lumbar motoneurons. Details of that experimental preparation are shown in Figure 31.[2]

Figure 30 (continued).
effusion. In the ear with ligation and tubes installed which have the selectively permeable membrane over their lumen (MLT, solid line) a normal tympanogram is obtained. This indicated that the tympanic membrane compliance changes with pressure and the peak at 0 pressure indicates normal ambient pressure within the middle ear. When the membrane on the ventilation tube was occluded with petrolatum (dashed line) the tympanogram reverted to a shape indicating a sealed middle ear.

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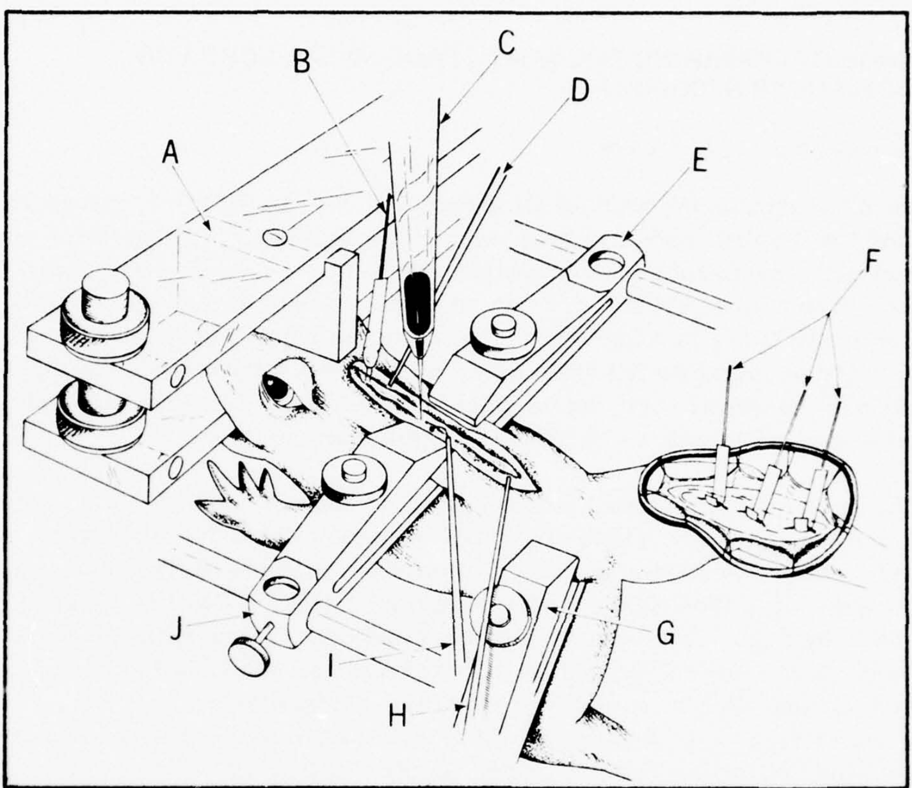

Figure 31. Overview of in situ frog spinal cord preparation. The animal was rigidly attached to a rectangular frame by clamps on the head (A), on the transverse processes of thoracic vertebrae (E, J), and on the left iliac bone of the pelvis (G). Cold, oxygenated Ringer's solution was applied to the cord (D) and sucked off (H) through small pipettes. Bipolar electrical stimulation was applied to the lower brain stem or upper spinal cord (B) and to peripheral nerves in an oil pool (F). Intracellular recording was done via a micropipette (C) while recordings were led from the dorsal surface of the cord via a platinum ball electrode (I).

The spinal cord in this preparation appeared to be healthier than in the excised, in vitro preparation. Furthermore, the preparation is especially useful for keeping peripheral nerves, as well as the brain, in connection with the spinal cord. The development of this technique will greatly assist the study of this nervous system, which is of importance because it is of a complexity intermediate between that of higher vertebrates and invertebrates. This model will be used for studies in toxicology and on mechanisms of movement.

REFERENCES

1. Brookhart, J. M. and Kubota, K. Studies of the integrative function of the motor neurone. In: Progress in Brain Research, Vol. 1, Brain Mechanisms, Moruzzi, G., Fessard, A. and Jasper, H. H., editors, pp. 38-61. New York, Elsevier, 1963.

2. Cruce, W. L. R. A method of preparing the in situ frog spinal cord for intracellular recording. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Technical Note TN76-2, 1976.

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TERMINATION OF SUPRASPINAL DESCENDING PATHWAYS IN THE SPINAL CORD OF THE TEGU LIZARD (TUPINAMBIS NIGROPUNCTATUS)

Principal Investigator: *W. L. R. Cruce*

In order to understand how the central nervous system controls movement, these studies investigated motor systems in a simple vertebrate.

Descending fiber projections to the lizard spinal cord were studied using anterograde axonal degeneration. Following hemisection of the cord at the first spinal segment, degeneration was found in the white and grey matter as far down as the 31st (caudal) segment (Figure 32). Degenerating fibers in the white matter were confined to the ipsilateral side and were found in the medial longitudinal fasciculus and the other half of the lateral and ventral funiculi. Degeneration was more intense in the dorsolateral and ventromedial funiculi than in the ventrolateral funiculus. In the gray matter, Rexed's criteria were applied to Nissl-stained material to delimit boundaries of ten laminae VII and VIII, and in medial IX. Sparse degeneration was present in the lateral parts of V, VI, and VII. Degenerating fibers were seen coursing in the dorsal and ventral commissures to ramify contralaterally in the medial part of VII, in VIII, and in medial IX. No degeneration was present in the lateral part of the spinal gray on the contralateral side. In Golgi-stained material, dendrites of lateral IX cells were seen to extend into lamina VIII, the dorsolateral part of VII, and the lateral funiculus. Thus, fibers of the ventromedial supraspinal pathway

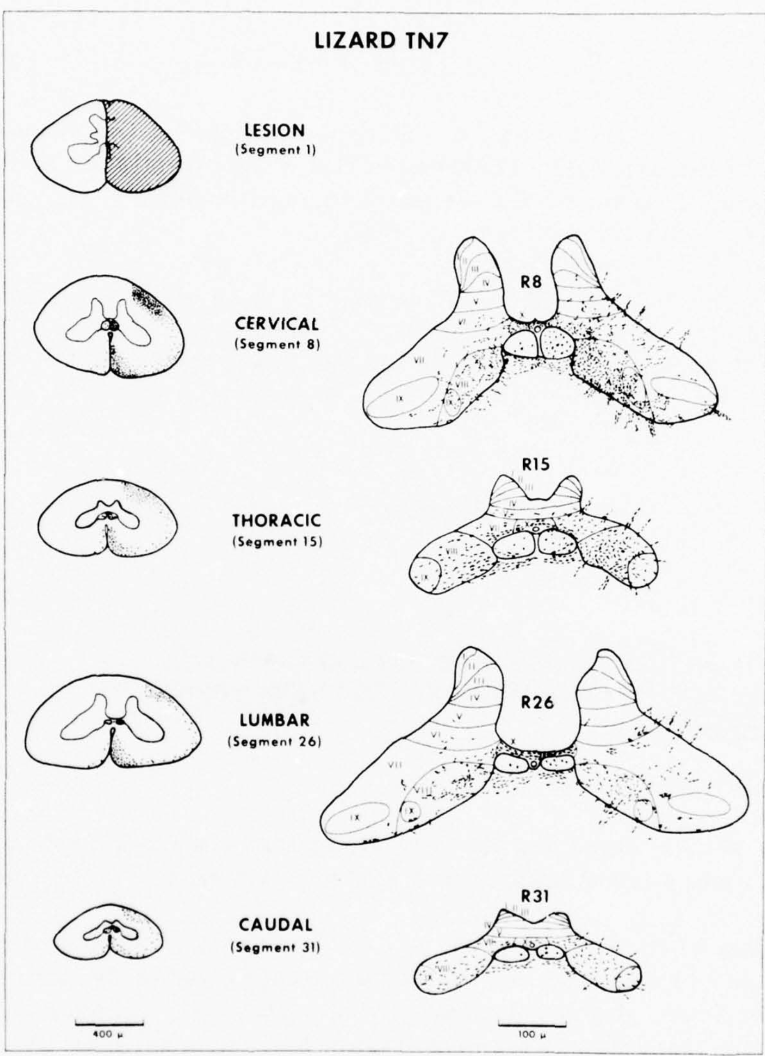

Figure 32. A plot of axonal degeneration of representative levels of the lizard spinal cord following a complete hemisection at the first cord segment. Small drawings to the left show transversely cut axonal degeneration in the white matter. The large drawings to the right depict degeneration in the gray matter. Axonal terminals are not distinguished from preterminal debris and axons of passage.

may make axodendritic contact with motoneurons of lateral IX as well as medial IX, ipsilaterally. In addition, there is a possibility of a crossed connection of contralateral motoneurons. Thus, the basic pattern of termination of all fiber systems descending to the spinal cord was like that seen in mammals

when all the noncortical descending pathways are studied. These results are consistent with the view that a corticospinal pathway does not exist in reptiles. Further studies are under way regarding the origin of the different descending pathways and the organization of their target cells in the spinal cord.

These studies of simplified models of motor systems will aid our understanding of human motor control and point out directions for possible cures of pathological conditions in motor function such as those caused by disease and injury.

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ANALYSIS OF THE VISUAL SYSTEM IN A LIZARD, TUPINAMBIS NIGROPUNCTATUS

Principal Investigators: *J. A. F. Cruce, Johns Hopkins University School of Medicine; and W. L. R. Cruce, AFRRI*

The visual system plays an important role in the behavior of all vertebrates. Through the retina of the eye visual information reaches many parts of the nervous system where the information can be analyzed, integrated and acted upon. Visual information is received by the retinal ganglion cells and transmitted via axons traveling first as the optic nerve and then as the optic tract to the brain. In the present paper, projections from retinal ganglion cells to the brain were described in one species of lizard, the Tegu lizard, Tupinambis nigropunctatus.

The pattern of connections from the retinal ganglion cells to the brain, seen in the Tegu lizard, is in agreement with the basic vertebrate plan as outlined by Ebbesson.[3] Six general regions of the brain receive retinal fibers; these areas are as follows: dorsal thalamus, ventral thalamus, pretectum, tectum, hypothalamus and basal optic nucleus. This general organization has been seen in all reptile species studied, although certain variations are present.

The existence and location of uncrossed retinal projections, as seen in this study (Figure 33), do not seem to be universally observed in the various reptiles studied. Using modern experimental techniques, ipsilateral projections have been well documented in lizards and snakes, whereas turtles and crocodiles seem to have completely crossed retinal fibers. In all of the lizards and snakes which have been studied to date, the thalamus seems to be the recipient of ipsilateral as well as contralateral retinal projections. In addition, the present study found bilateral retinotectal projections which have only been seen in three other reptiles.[2]

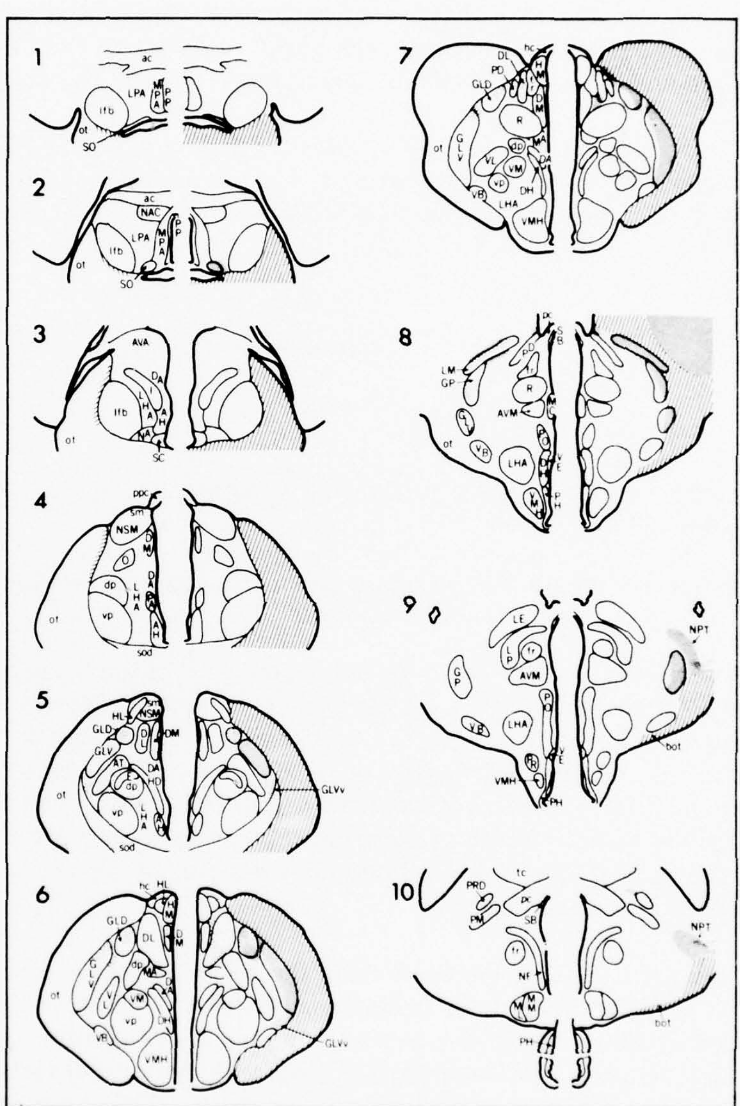

Figure 33. Line drawings of coronal sections through the brain of Tupinambis nigropunctatus. Section 1 is the most rostral level and section 10 is the most caudal. The diagonal lines indicate areas of labeled fibers and the stippling indicates areas of terminal labeling. The injection of tritiated proline was into the left eye, i.e., contralateral to the side of maximum optic tract labeling. The nomenclature follows that of Cruce.[1]

Studies of nonmammalian visual systems such as have been performed here are aimed at an understanding of how the central nervous system processes visual information. In particular, we hoped to learn what is the function of bilateral representation of the visual world in the brain. Does this function in reptiles in such a way as to give depth perception or can the information be used in some other way? This may tell us what other function bilaterality has in the human visual system and aid in curing pathologies of this system.

REFERENCES

1. Cruce, J. A. F. A cytoarchitectonic study of the diencephalon of the Tegu lizard, Tupinambis nigropunctatus. J. Comp. Neurol. 153:215-238, 1974.

2. Cruce, W. L. R. and Cruce, J. A. F. Projections from the retina to the lateral geniculate nucleus and mesencephalic tectum in a reptile (Tupinambis nigropunctatus): a comparison of anterograde transport and anterograde degeneration. Brain Res. 85:221-228, 1975.

3. Ebbesson, S. O. E. On the organization of central visual pathways in vertebrates. Brain Behav. Evol. 3:178-194, 1970.

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EFFECT OF CHRONIC ADMINISTRATION OF MORPHINE ON THE ACTIVITY OF BRAIN MONOAMINE OXIDASE IN THE RAT

Principal Investigators: *G. N. Catravas, J. Takenaga and C. G. McHale*

We have observed that during the development of tolerance to morphine a number of rats showed an activation reaction which was manifested by hyperactivity, spasmodic jumping and compulsive gnawing within the first 20-30 minutes after administration of the last dose of morphine, while other rats did not exhibit this hyperactivity reaction. This study was set up to determine if and to what extent brain monoamine oxidase activity is influenced by chronic administration of morphine to these two types of rats.[1] Monoamine oxidase activity was measured in the hypothalamus, hippocampus,

thalamus, cerebellum and cerebral cortex. The experimental animals were given morphine by injecting the drug, 40 mg/kg body weight of morphine sulfate per injection intraperitoneally, twice daily for 8 days. Control animals were injected with sterile physiologic saline in volumes corresponding to those of the morphine solution. After the 6th or 7th day of morphine administration, a number of rats (approximately 40 percent of the experimental animals) exhibited the activation response (jumping rats) for the first 20-30 minutes after each injection of the drug. The remaining rats did not show this hyperactivity reaction (nonjumping rats). At the end of the 8th day, one group of the jumping and one group of the nonjumping rats were euthanatized just before receiving the last morphine injection and were used as base-line controls. The other groups were given the last morphine dose and were euthanatized at 5, 15, 30, 60 minutes, 6 or 24 hours after the last injection. Experimental animals as well as controls were euthanatized by decapitation and their heads were instantly frozen in liquid nitrogen in a Dewar flask. The heads were later removed from the liquid nitrogen and stored at -90°C until time of assay. The frozen heads were partially thawed in a cold room and the brain regions to be analyzed were rapidly dissected out.

The effects of chronic administration of morphine on monoamine oxidase activity in the brain of rats showing the hyperactivity reaction (jumping rats) are presented in Table 17. Within minutes after the last administration of

Table 17. Changes in Monoamine Oxidase Activity in Jumping Rats Chronically Treated With Morphine

Time after last morphine injection	Brain Areas									
	Thalamus		Hypothalamus		Hippocampus		Cerebellum		Cerebral Cortex	
	Activity*	% of Control	Activity	% of Control	Activity	% of Control	Activity	% of Control	Activity	% of Control
Controls	0.49±0.02	--	0.56±0.03	--	0.52±0.03	--	0.42±0.02	--	0.44±0.04	--
Morphinized:										
0 minutes (base-line controls)	0.41±0.01	83.6	0.49±0.03	87.5	0.38±0.04	73.1	0.36±0.03	85.7	0.42±0.01	95.4
5 minutes	0.39±0.02	79.5	0.38±0.02	67.8	0.39±0.03	75.0	0.32±0.03	76.2	0.34±0.02	77.2
15 "	0.30±0.02	61.2	0.29±0.02	51.7	0.40±0.02	76.9	0.34±0.05	80.9	0.30±0.02	68.2
30 "	0.29±0.03	59.1	0.35±0.03	62.5	0.35±0.02	67.3	0.29±0.01	69.0	0.31±0.04	70.4
60 "	0.38±0.02	77.5	0.43±0.04	76.7	0.41±0.04	78.8	0.32±0.02	76.2	0.40±0.01	90.9
6 hours	0.41±0.01	83.6	0.42±0.03	75.0	0.48±0.02	92.3	0.35±0.03	83.3	0.37±0.04	84.1
24 "	0.44±0.03	89.8	0.48±0.05	85.7	0.45±0.04	86.5	0.39±0.04	92.8	0.47±0.03	106.8

*Expressed as μmoles of 4-hydroxyquinoline per 90 min per mg of protein
Values are means ± S.E.

the drug, monoamine oxidase activity decreased in all brain areas investigated, reaching lowest levels at approximately 15-30 minutes postinjection. In rats euthanatized at 60 minutes, the morphine-induced decreases in monoamine oxidase activity were less pronounced and this activity was found to return to nearly normal levels in animals euthanatized at 6 or 24 hours after the last morphine injection. No significant changes were observed in the activity of this enzyme in rats that did not exhibit the hyperactivity syndrome after morphine administration.

These observations tend to support the existence of a relationship between the appearance of the hyperactivity syndrome and the decrease in the activity of brain monoamine oxidase. These experiments add to our knowledge of opiate dependence and contribute toward the development of human treatment.

The study of morphine-induced changes in brain monoamine oxidase described above provides information on basic mechanisms of function of the mammalian central nervous system. This information is of great value to studies of effects of other toxic agents including ionizing and nonionizing radiation.

REFERENCE

1. Catravas, G. N., Takenaga, J. and McHale, C. G. Effect of chronic administration of morphine on the activity of brain monoamine oxidase in the rat. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR76-26, 1976.

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MORPHINE-INDUCED CHANGES IN INTRACELLULAR CATECHOLAMINES IN RAT CENTRAL NERVOUS SYSTEM

Principal Investigators: *J. H. Darden and G. N. Catravas*

The effects of acute and chronic doses of morphine on intracellular levels and distribution of catecholamines were studied in the rat.[1] Sprague-Dawley rats weighing 200-250 g were used. They were equally divided into control and experimental groups. Control animals were injected with 0.5 ml saline,

and morphine treated animals received either a single dose (60 mg/kg intraperitoneally) or twice daily doses of 30 mg/kg morphine sulfate for 8 days. The animals were euthanatized by decapitation 5 min to 1 hour after the last drug administration.

Brain homogenates were fractionated into crude synaptosomal pellets and supernatants. The synaptosomes were osmotically shocked, and dopamine and norepinephrine levels were measured as bound (synaptic vesicles), free (extravesicular), and extrasynaptosomal fractions in the supernatant. The rate of synthesis of catecholamine was determined by estimating the conversion of injected ^{14}C-tyrosine into ^{14}C-dopamine and ^{14}C-norepinephrine. In rats acutely treated with morphine there was an increase in both the extrasynaptosomal and free fraction of norepinephrine in each of the postmorphine testing periods (15, 30 and 60 min postinjection) (Table 18), as well as increased accumulation of extrasynaptosomal ^{14}C-norepinephrine at 60 min. An increase in endogenous levels of dopamine was seen in the extrasynaptosomal ^{14}C-dopamine at 30 and 60 min, respectively. The bound fractions demonstrated no appreciable differences at any of the testing intervals.

Table 18. Effects of Acute Doses of Morphine on Levels of Norepinephrine (ng/g) at 15, 30 and 60 Minutes After Injection. Results are the mean ± S. E. M.

	N = 8 15 min	Percent change	N = 8 30 min	Percent change	N = 8 60 min	Percent change
Control Extrasynaptosomal	187 ± 2.3		153 ± 2.5		169 ± 1.2	
Morphine treated Extrasynaptosomal	208 ± 2.0	+10	195 ± 2.5	+22*	229 ± 2.6	+26*
Control Free	38 ± 0.86		34 ± 1.0		30 ± 0.64	
Morphine treated Free	78 ± 1.9	+51*	51 ± 1.4	+33*	42 ± 0.45	+29*
Control Bound	34 ± 0.75		29 ± 0.73		27 ± 0.67	
Morphine treated Bound	34 ± 0.69		30 ± 0.67		27 ± 0.77	

* $P < 0.05$

Rats chronically treated with morphine also demonstrated an increase in the endogenous extrasynaptosomal levels of both dopamine and norepinephrine as compared to controls, while only norepinephrine was increased in the free fraction (Table 19). These data are consistent with a morphine-induced increase in the biosynthesis of dopamine and norepinephrine, resulting in increased levels of norepinephrine in the nonfunctional storage pools rather than in the more readily available pools. This is supported by the lack of

change in the levels of the bound fraction, suggesting preferential release of newly synthesized norepinephrine followed by replenishment due to increased synthesis. These conditions could result in constant equilibration of the bound fraction. An understanding of the neurochemical changes in opiate-dependent animals may provide a rational therapy for human addicts as well as an understanding of important mechanisms.

Table 19. Effects of Chronic Doses of Morphine on Levels of Norepinephrine and Dopamine 1 Hour After the Last Injection. Results are expressed as the mean ± S.E.M. in ng/g.

	Norepinephrine N = 8*	Percent change	Dopamine N = 8	Percent change
Control Extrasynaptosomal	138 ± 2.5	-	129 ± 1.3	-
Morphine treated Extrasynaptosomal	173 ± 3.4	+20+	151 ± 2.1	+15†
Control Free	34 ± 2.5	-	34 ± 1.3	-
Morphine treated Free	69 ± 3.6	+51†	34 ± 0.91	-
Control Bound	21 ± 0.63	-	8 ± 1.7	-
Morphine treated Bound	22 ± 0.57	-	7 ± 1.4	-

* N = number of experiments (four rats each)

† $P < 0.05$ $P < 0.25$

REFERENCE

1. Darden, J. H. and Catravas, G. N. Morphine-induced changes in intracellular catecholamines in rat central nervous system. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR75-40, 1975.

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PROSTAGLANDIN E_1-MORPHINE EFFECTS ON ADENYL CYCLASE ACTIVITY IN A RAT BRAIN SLICE SYSTEM

Principal Investigators: *J. B. Katz and G. N. Catravas*

Prostaglandin E_1 stimulated adenyl cyclase activity was studied in a rat brain slice incubation system to determine if stimulation could be prevented or reversed by morphine.[1] Brain slices were prepared from naive, acutely morphine intoxicated, and morphine tolerant (dependent) and withdrawing rats using a McIlwain tissue slicer set at a 0.30-mm interval. All rats were Sprague-Dawley males (200-250 g). Acute dosages were administered at 40 mg/kg, while tolerant and withdrawing animals were prepared with twice daily injections of 40 mg/kg of morphine sulfate until tolerant to the analgesic properties of the drug. Withdrawal was precipitated with naloxone challenge (0.40 mg/kg), and rats euthanatized and brains removed and sliced 30 minutes after naloxone administration at which time animals exhibited classic signs of morphine withdrawal.

Morphine addition to brain slices stimulated with prostaglandin E_1 did not prevent or reverse stimulation of 3',5'-cyclic adenosine monophosphate accumulation in either naive, acutely morphine intoxicated or tolerant and withdrawing rats. Table 20 reveals that morphine over a wide range fails to significantly alter the approximately twofold stimulation of cyclic adenosine monophosphate accumulation produced by prostaglandin E_1 application to brain slices from rats in all three groups. The data do not support the hypothesis that the prostaglandin E_1 stimulated adenyl cyclase system provides a neurochemical model system which may be used to detect and perhaps quantify insults to the central nervous system induced by a variety of toxic influences including ionizing and nonionizing (microwave) radiation.

Table 20. Lack of Morphine Reversibility of Prostaglandin E_1 (PGE_1) Stimulation of Net 3',5'-Cyclic Adenosine Monophosphate (cAMP) Formation in Brain Slices from Naive, Acutely Morphine-Treated and Tolerant and Withdrawing Rats. Net cAMP formation expressed as counts/min of [^{3}H]cAMP extracted from incubated brain slices per mg of brain slice protein. Values are means ± S. E. Number of rats in each group shown in parentheses.

Treatment group	PGE_1 concentration	Morphine concentration	Net cAMP formation
Naive (4)	0	0	3480 ± 425
	2.9×10^{-6} M	0	6640 ± 350
	2.9×10^{-6} M	2.6×10^{-4} M	6625 ± 345*
Acutely morphine-treated (6)	0	0	3470 ± 280
	0	2.6×10^{-4} M	3595 ± 335
	2.9×10^{-6} M	0	7230 ± 415
	2.9×10^{-6} M	2.6×10^{-4} M	7040 ± 425*
	2.9×10^{-6} M	6.7×10^{-5} M	7215 ± 110*
	2.9×10^{-6} M	2.6×10^{-6} M	7330 ± 220*
Morphine-tolerant and in naloxone-precipitated withdrawal (4)	0	0	3850 ± 470
	4.8×10^{-6} M	0	6630 ± 580
	4.8×10^{-6} M	1.1×10^{-3} M	5890 ± 925*
	4.8×10^{-6} M	8.0×10^{-4} M	6425 ± 350*
	4.8×10^{-6} M	2.6×10^{-4} M	5950 ± 705*

* Values not significantly different ($P > 0.05$) from corresponding sample in each group with PGE_1, and without morphine

REFERENCE

1. Katz, J. B. and Catravas, G. N. Prostaglandin E_1-morphine effects on adenyl cyclase activity in a rat brain slice system. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR76-20, 1976.

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MORPHINE, KETAMINE, AND HARMALINE INFLUENCES ON RAT CEREBELLUM 3',5'-CYCLIC GUANOSINE MONOPHOSPHATE LEVELS

Principal Investigators: *J. B. Katz and G. N. Catravas*

Harmaline, a tremorogenic drug and a known elevator of cerebellar 3',5'-cyclic guanosine monophosphate levels, produced a 500 percent rise in cyclic guanosine monophosphate, from 5 to 25 pmoles/mg protein, when injected at 30 mg/kg, intraperitoneally. This rise was partially blocked by morphine pretreatment, but even 45 mg/kg of morphine failed to block harmaline-induced tremor or to reduce cyclic guanosine monophosphate levels below twice control values. This harmaline-morphine relationship was identical in both acutely morphine injected and chronically morphine treated (tolerant) rats. Ketamine, a dissociative analgesic drug, reduced or blocked harmaline elevation of cerebellar cyclic guanosine monophosphate levels: 20 mg/kg of ketamine-reduced cyclic guanosine monophosphate levels to 30 percent of control values, even in harmaline-treated rats. Isoniazid, a known epileptogenic drug when administered in excess of clinical levels (300 mg/kg intraperitoneally), elevated cerebellar cyclic guanosine monophosphate levels to 700 percent of control levels. Phosphodiesterase activities in soluble fractions of cerebellar homogenates were equal in control and morphine, harmaline, or isoniazid-treated rats. Particulate cerebellar guanylate cyclase activities were equal in control and morphine treated rats; however, guanylate cyclase activity was increased approximately 30 percent in rats receiving harmaline or isoniazid (Figure 34). An understanding of physiologic and pharmacologic control of central nervous system cyclic nucleotide metabolism will permit greater insight into molecular effects of exposure to ionizing and nonionizing radiation. The important and sensitive nature of cyclic nucleotide metabolism in central nervous system function furnishes a model system for the study of a variety of neurotoxic agents and mechanics of their action. Pharmacologic manipulation of enzymes in this model system may offer the opportunity of modifying some neurochemical effects of ionizing and nonionizing radiation exposure.

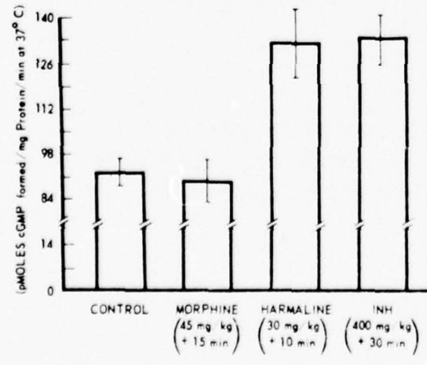

Figure 34.
Particulate cerebellar guanylate cyclase activity in cerebellar homogenates from morphine, harmaline, and isoniazid (INH) treated rats

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CEREBELLAR 3',5'-CYCLIC GUANOSINE MONOPHOSPHATE LEVELS REDUCED BY NARCOTIC AND HYPNOTIC DRUGS: CORRELATION WITH DRUG-INDUCED ATAXIA

Principal Investigators: *J. B. Katz and G. N. Catravas*

Maintenance of normal coordination and precision motor activity requires an intact Purkinje cell system in the cerebellum. Cerebellar cyclic guanosine monophosphate has been shown to reside in, or be functionally associated with, the Purkinje cells. Acute administration of morphine (45 mg/kg, intraperitoneally) or pentobarbital produces a prompt, dramatic reduction in the cyclic guanosine monophosphate pool of the cerebellum of the rat. The reduction is both dose and time dependent. The induction of ataxia by these drugs correlates positively with their ability to depress cerebellar cyclic guanosine monophosphate levels and by inference to interfere with functional activity of cellular cerebellar elements (Figure 35). Animals made tolerant to morphine

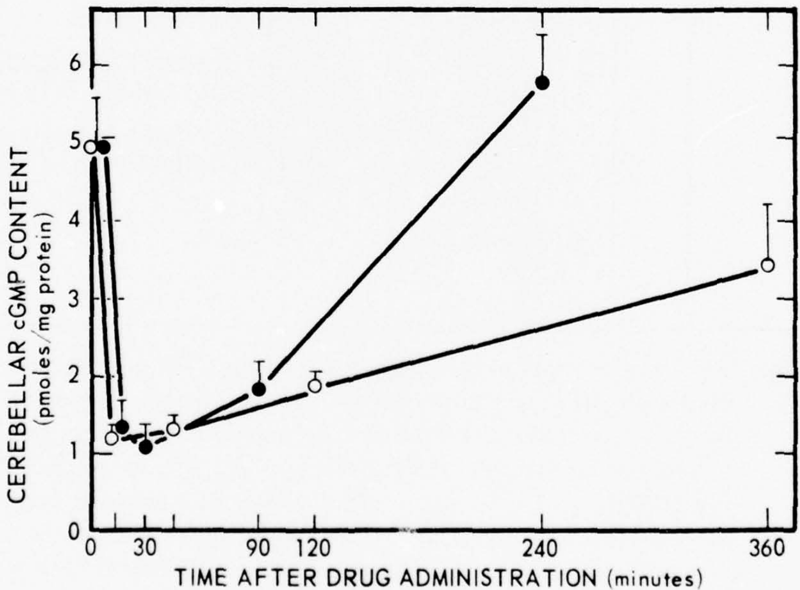

Figure 35. Time course studies: time-dependent effects of acute doses of morphine (45 mg/kg) or pentobarbital (25 mg/kg) on cerebellar cGMP content. All injections intraperitoneally. For morphine study, n = 7, means ± S.E. For pentobarbital study, n = 5, means ± S.E. o: morphine time course; ●: pentobarbital time course.

(i.e., animals refractory to morphine's analgesic properties) through chronic administration of the drug show neither cyclic guanosine monophosphate reduction nor ataxia, even in the presence of a morphine challenge or challenge with the narcotic antagonist naloxone (Figure 36). These studies provide insight into mechanisms and biochemical events underlying normal and ataxic motor behavior, and are thus of value in studying effects of ionizing and nonionizing radiation upon central nervous system function.[1] Disruption of motor coordination and activity during early transient incapacitation following high doses of ionizing radiation may proceed through several mechanisms, one of which may involve perturbations in cerebellar cyclic nucleotide metabolism.

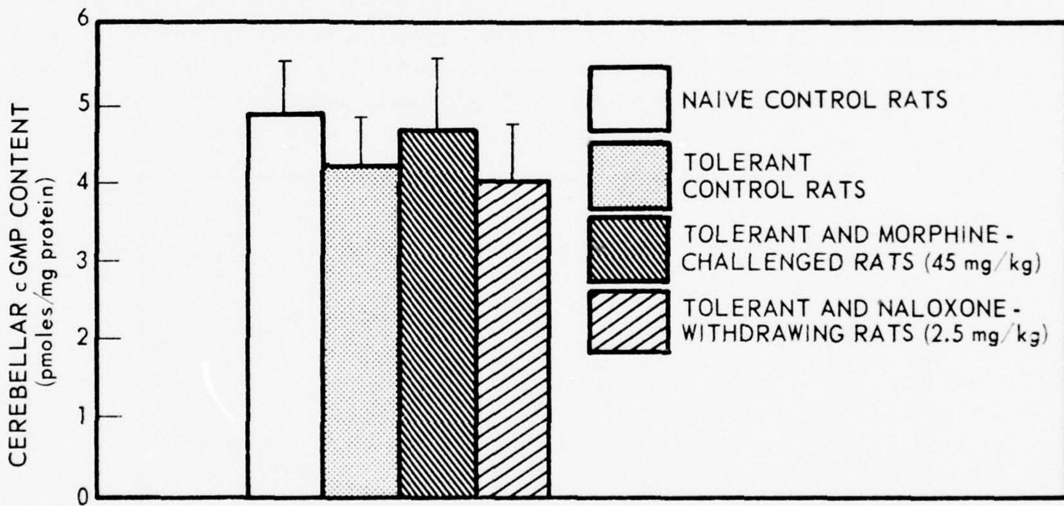

Figure 36. Effect of morphine tolerance, and morphine or naloxone challenge in morphine-tolerant rats on cerebellar cGMP levels. Animals were made tolerant by twice daily injections of morphine sulfate (60 mg/kg, intraperitoneally) for 10 days. A tolerant control group was euthanatized by focused microwave irradiation just prior to final scheduled morphine dose. Tolerant, morphine-challenged rats were injected with 45 mg/kg morphine, intraperitoneally, at this time, and tolerant, naloxone-withdrawing rats were injected with 2.5 mg/kg naloxone, intraperitoneally, at the same time. Both groups were euthanatized 15 minutes later, at which time naloxone-withdrawing rats exhibited the typical abstinence syndrome of diarrhea, shaking, and hypersensitivity to handling. n = 7, means ± S.E. for each group.

REFERENCE

1. Katz, J. B. and Catravas, G. N. Cerebellar cGMP levels reduced by narcotic and hypnotic drugs: correlation with drug-induced ataxia. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR76-30, 1976.

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MONOAMINE OXIDASE ACTIVITY IN NERVOUS AND PERIPHERAL TISSUES OF APLYSIA

Principal Investigators: *J. L. Ribas, G. N. Catravas, J. Takenaga and D. O. Carpenter*

Termination of action of biogenic amines in biological systems is mediated either by reuptake mechanisms or by metabolic degradation. In the nervous system of Aplysia, several laboratories have failed to detect monoamine oxidase in spite of evidence that several biogenic amines function as neurotransmitters in this preparation. We have reinvestigated this problem using a variety of substrates and report the presence of monoamine oxidase in Aplysia. Aplysia californica, weighing approximately 300 g, were dissected; and the kidney, liver, salivary gland, gill, heart, intestine and ganglia were homogenized in cold isotonic KCl or 0.25 M sucrose. Monoamine oxidase activity was measured according to the method of Wurtman and Axelrod[2] using [^{14}C]-tryptamine as substrate. Highest monoamine oxidase activity was found in the liver; appreciable amounts also were found in pooled ganglia, intestine, gill and kidney but salivary gland and heart had none (Table 21). When [^{14}C]-tryptamine was used as substrate according to the method of Robinson et al.[1], the amount of monoamine oxidase activity detected in the nonnervous tissues was at least as great as when tryptamine was used as a substrate. In the pooled ganglia, however, the monoamine oxidase activity measured with tyramine was only 25 percent of that determined using tryptamine. These results indicate that monoamine oxidase is present in the nervous tissue of Aplysia, thus suggesting that deamination may be an important pathway for the termination of action of at least some of the biogenic amines. There may, however, be differences in the monoamine oxidase substrate specificity in different organs.

Table 21. Monoamine Oxidase Activity in Nervous and Peripheral Tissues of Aplysia

TISSUE	SUBSTRATE*	
	TRYPTAMINE	TYRAMINE
Ganglia	1.453 ± 0.186† (3)	3.65 ± 0.08 (2)
Liver	2.474 ± 0.023 (5)	30.22 ± 0.269 (4)
Kidney	0.767 ± 0.027 (4)	19.86 (1)
Gill	0.214 ± 0.214 (2)	16.63 (1)
Intestine	0.382 (1)	
Salivary Gland	N.D.	N.D. (2)
Heart	N.D.	N.D. (1)

* Results are expressed as nmoles of substrate converted per mg of protein per unit of time.

† Values are mean ± S.E.

N.D. None detected

() Number of experiments in duplicate

This study has provided basic data for future experiments on the effects of low-level ionizing radiation on enzyme levels of Aplysia nervous tissue, a relatively simple model compared to mammals. The ultimate goal of this project is to understand the effects of radiation on enzymes and neurotransmitter metabolism of single neurons and to correlate this with similar published experiments in mammals from our department.

REFERENCES

1. Robinson, D. S., Lovenberg, W., Keiser, H. et al. Effects of drugs on human blood platelet and plasma amine oxidase activity in vitro and in vivo. Biochem. Pharmacol. 17:109-119, 1968.

2. Wurtman, R. J. and Axelrod, J. A sensitive and specific assay for the estimation of monoamine oxidase. Biochem. Pharmacol. 12:1439-1441, 1963.

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BIOCHEMICAL CHANGES IN THE BRAIN OF RATS EXPOSED TO MICROWAVES OF LOW POWER DENSITY

Principal Investigators: *G. N. Catravas, J. B. Katz, J. Takenaga and J. R. Abbott*

Experiments were conducted to determine the effects of low power density microwave radiation on macromolecular constituents of the mammalian brain. Sprague-Dawley male rats weighing 200-250 g were used in this series of experiments. Since the plastic cages commonly employed to confine animals during exposure (Plexiglas, polycarbonate, etc.) reflect a considerable portion of the microwave beam and produce extensive field perturbations, a microwave-transparent material, Styrofoam, was used to construct the cages. Coating of the inner walls of the cages with a solution of quinine effectively prevented the rats from chewing through their walls during exposure.

Groups of rats were exposed to microwave radiation (continuous wave) of 2450 MHz frequency and 10 mW/cm^2 power density (8 hours per day for 8 days between 8 a.m. and 4 p.m.) in an anechoic chamber (U. S. Army Microwave Facility, Forest Glen, Silver Spring, Maryland). Control rats, effectively protected from the microwave beam, were kept in the same exposure chamber under the same environmental conditions as the experimental animals. No food or water was given to the animals during exposure, but they had free access to both between exposures.

At the end of the last day's irradiation, several experimental and control rats were euthanatized by decapitation, half being euthanatized immediately after exposure and the remainder 8 hours later. The brains were quickly excised, placed on ice, and the regions to be analyzed were rapidly dissected out and frozen in liquid nitrogen until time of assay. The remaining rats, used to measure levels of neurotransmitters, were euthanatized by a focused high intensity microwave beam. The microwave-induced changes in the prostaglandin E_1 stimulated brain adenyl cyclase and serotonergic systems have been investigated in this series of experiments. Preliminary results indicate an increased sensitivity of brain adenyl cyclase to prostaglandin E_1, with a shift of the dose-response curve to the left. This shift was more pronounced in irradiated animals euthanatized 8 hours postexposure than those euthanatized immediately after exposure. However, the maximal adenyl cyclase activity did not appear to be affected. Marginal changes (increase) in the activity of tryptophan hydroxylase and levels of serotonin in the hypothalamic-thalamic region were also observed. No changes in monoamine oxidase activity were seen. The effects of microwaves on the brain are important considerations for military personnel exposed to radar sites.

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MECHANOSENSORY TRANSDUCTION IN SENSORY AND MOTILE CILIA

Principal Investigator: *M. L. Wiederhold*

It has long been recognized that there are striking anatomical similarities between those cilia classically thought of as serving "sensory" and those serving "motile" functions. It has also been suggested that sensory cilia evolved from motile cilia, and in lower forms there are cases in which motile cilia are utilized in mechanoreceptive organs.

Studies in recent years on the detailed fine structure and biochemistry of motile cilia have far outstripped those on sensory cilia. The current state of knowledge of motile cilia was examined briefly,[1] concentrating on those properties that may be pertinent to the transduction of mechanical stimuli. This review was organized around the hypothesis that mechanosensitivity and motility are complementary aspects of cilia and that the structures responsible for motility may be intimately involved in mechanosensory transduction. Although similar hypotheses have been espoused by several investigators studying invertebrate ciliated mechanoreceptors, with some notable exceptions the possible advantages of at least drawing parallels between the two classes of cilia have largely been abandoned by those working with vertebrate hair cells. This is not to state that such a hypothesis is proven or that it is universally applicable; rather the purpose of the review was to set up a conceptual model with the hope of stimulating research to either support or deny its validity in various mechanoreceptors. In reviewing what data are available or can be inferred concerning mechanosensory transduction in cilia, those aspects of the motile system that clearly are not applicable are noted. In fact, the ciliary structure has been dropped in the evolution of the (adult) mammalian cochlea. This review covers representative examples of a variety of receptors rather than attempting to describe every type of mechanosensitive cilium and every pertinent paper.

Since the ciliated receptor cells in the human inner ear are especially sensitive to ionizing radiation, it is essential to thoroughly understand the function of these cilia in order to understand the damaging effects of radiation.

REFERENCE

1. Wiederhold, M. L. Mechanosensory transduction in "sensory" and "motile" cilia. Annu. Rev. Biophys. Bioeng. 5:39-62, 1976.

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RECTIFICATION IN APLYSIA STATOCYST RECEPTOR CELLS

Principal Investigator: *M. L. Wiederhold*

Membrane slope resistance of Aplysia statocyst receptor cells was measured by passing constant current pulses, using a bridge circuit. In response to downward tilt all cells which responded exhibited depolarization, but this could be accompanied by either decrease, increase, or no measurable change in slope resistance, depending on resting membrane potential.

By altering membrane potential with direct current and measuring slope resistance with constant current pulses, these cells are shown to exhibit both anomalous and delayed rectification (Figure 37). Either hyperpolarization or depolarization from one potential can cause the slope resistance to decrease by as much as a factor of five. The response to standard tilt can be changed from an increase in slope resistance to a decrease, or vice versa, by altering

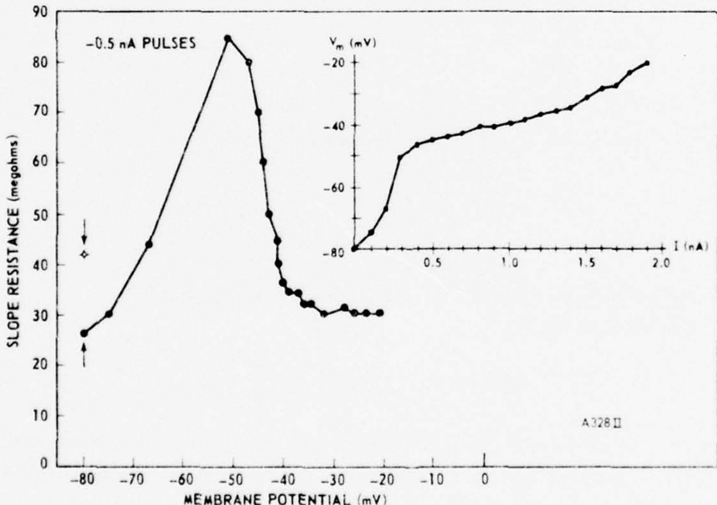

Figure 37. Slope resistance at different membrane potentials. Slope resistance measured with -0.5 nA, 300-msec constant current pulses superimposed on d.c. ranging from 0 to +1.9 nA. Upward-pointing arrow indicates measurement at beginning of run. Open circle with downward pointing arrow is measurement with 0 d.c. at end of run, indicating that the slope resistance increased from 26 to 42 MΩ throughout the passage of current. Inset indicates the average membrane potential (between current pulses) at each value of d.c., measured 20-30 sec after changing d.c.

membrane potential. When membrane potential was held constant during downward tilt, the slope resistance always decreased. Slope resistance, the voltage response to standard tilts and the amplitude of membrane potential fluctuations all vary with average membrane potential in a similar manner.

These findings are incorporated into a circuit model in which anomalous and delayed rectification are represented by voltage-controlled elements. The response to tilt is always modelled as introducing a parallel conductance pathway with a large positive reversal potential. The model demonstrates that slope resistance can be increased by adding a parallel shunt pathway if the latter brings the membrane out of the anomalous rectification region. The model also demonstrates how delayed rectification can greatly alter the reversal potential inferred from measurements at potentials below actual reversal. These subtle characteristics of hair cell-like receptors are only likely to be elucidated through research on model systems such as the Aplysia, yet they are just the sort of mechanism which might be expected to be affected by ionizing radiation. They could thus form a physiological substrate for the selective sensitivity to radiation of cochlear and vestibular hair cells in man.

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RESPONSE OF APLYSIA STATOCYST RECEPTOR CELLS TO PHYSIOLOGIC STIMULATION

Principal Investigators: *M. L. Wiederhold and E. K. Gallin*

The mechanism whereby sound is transformed into nerve impulses in the ear is of critical importance in understanding both the mechanisms of hearing and treatment of diseases of the ear. However, electrical recording from these cells is very difficult. We have studied a simpler receptor preparation that acts through a similar mechanism.

The electrical responses of Aplysia statocyst receptor cells were investigated using intracellular microelectrodes. These ciliated mechanoreceptor cells were stimulated by downward tilting about a horizontal axis. Excitatory tilts produced a depolarizing receptor potential which, if large enough, could generate action potentials.

Large fluctuations in membrane potential were evident during depolarizing receptor potentials and were reduced or sometimes absent when a cell was

tilted upward. Power-density spectra of the noise voltage revealed that most of the energy added by downward tilt is contained in frequency components below 3 Hz. Removing synaptic input to the receptor cell by cutting the statocyst nerve or adding excess Mg^{2+} to the bath did not abolish the increase in fluctuation caused by downward, excitatory tilts.

The depolarizing receptor potential was often associated with a decrease in membrane resistance as measured with constant current pulses, using a bridge circuit. Replacing most of the Na^{+} in the bath with either Tris or Mg^{2+} abolished both potential and resistance changes caused by downward tilt (Figure 38). These results indicate that an increased permeability to Na^{+} underlies the receptor potential.

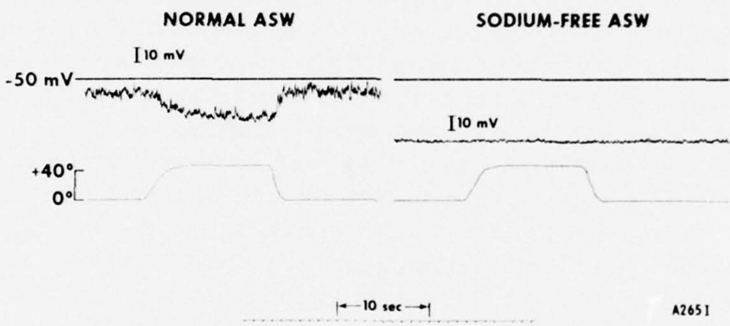

Figure 38. Response to tilting in normal and Tris substituted Na^{+}-deficient seawater. Left-hand side in normal seawater. Upper trace: membrane potential with -50 mV reference line. Lower trace: tilting table position. This cell showed considerable activation in the level table position which was reduced when the table was tilted up to +40° (cell-up position). Right-hand side recorded 25 min after changing to solution in which NaCl was replaced with Tris HCl. Membrane hyperpolarized from -61 mV in normal seawater to -94 mV in level table (0°) position. Average potential at +40° in normal seawater was approximately -79 mV. Potential fluctuations and response to tilting are abolished in low-Na^{+} seawater.

This preparation provides a valuable model of hair cell receptor cells, such as those in the mammalian inner ear. Since the latter are especially sensitive to ionizing radiation, the statocyst provides a system in which the mechanisms of radiation damage can be studied.

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CHRONIC RECORDING OF AUDITORY NERVE POTENTIALS FROM THE CAT

Principal Investigators: *M. L. Wiederhold, AFRRI; and S. A. Martinez, National Naval Medical Center*

Long-term reproducible collection of data is an important technical aspect of long-term studies in the development of a disease state, response to trauma, or therapeutic treatment. A system has been developed which allows repeated recording of auditory-nerve response to transient acoustic stimuli using a closed acoustic system. N_1 and N_2 responses to click (as well as tone- or noise-pips) recorded from the external ear canal near the tympanic membrane resemble those recorded near the round window. The amplitude of the responses recorded from the canal are lower than those from the round window by a factor of five to ten but the shapes of the responses at the two locations are nearly identical. We have developed a system using an inexpensive dynamic ear phone which delivers sound through a closed hollow ear bar inserted into the external ear canal. The tip of the ear bar is made of an insulating plastic, except for a small stainless steel ring at the end which serves as the recording electrode. A calibrated probe microphone incorporated into the ear bar measures sound pressure near the tympanic membrane, allowing better stimulus control than available with free-field systems. Using averaging techniques, measurable responses to clicks over a 90-dB intensity range have been obtained (Figure 39). The peak pressure of the most intense click available is about 130 dB re 0.0002 dyn/cm^2. Response amplitude and latency for fixed stimuli are relatively constant from one recording session to another, separated by weeks or months. A complete intensity series for both condensation and rarefaction clicks can be obtained in less than 1 hour.

For long-term longitudinal studies this system has the advantage of being able to closely monitor stimulus parameters and maintain them constant. A further advantage is that surgical implantation of chronic recording electrodes, with their inherent risk of infection, is avoided. This system can be used to study the effects on hearing of noxious agents such as noise exposure or ionizing radiation.

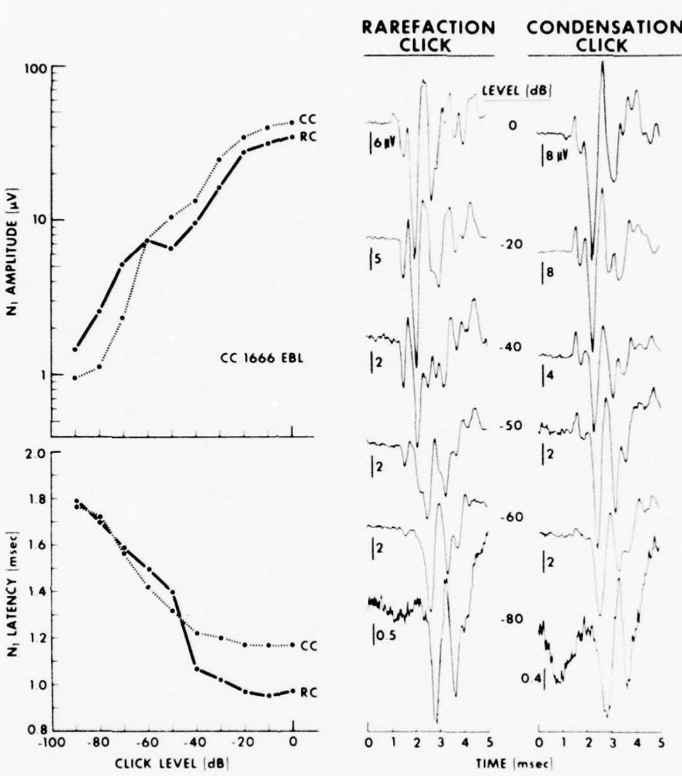

Figure 39. Responses to rarefaction (RC) and condensation (CC) clicks recorded with the earbar electrode using the closed acoustic system. Click level given in dB attenuation from maximum level of 130 dB SPL peak pressure. Left side: amplitudes (upper plots) and latencies (lower plots) of averaged responses to clicks from 0 dB to -90 dB levels. Amplitude measured from base line (average potential in 1 msec preceding presentation of click pulse to earphone) to maximum negative peak of N_1 response. Latency measured from onset of click pulse to earphone to time of maximum negative peak of N_1. Right side: sample averaged responses from which the amplitudes and latencies were measured for both RC's and CC's. Calibration bar to left of each average with μV amplitude of the bar indicated. Each average is of 256 responses for levels above -40 dB and 512 responses for -40 dB and below.

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ELECTROPHYSIOLOGY OF HUMAN MACROPHAGE STIMULATION BY ENDOTOXIN ACTIVATED SERUM

Principal Investigators: *E. K. Gallin, AFRRI; and J. I. Gallin, National Institutes of Health*

It has previously been shown that endotoxin activated serum induces macrophage spreading.[1] To study the initial events of such macrophage stimulation we measured the effects of endotoxin activated serum on the electrophysiological properties of human macrophages cultured for 2-3 weeks. 4 M potassium acetate microelectrodes with resistances of 50-100 megohms were used to record both intracellular potential and resistance changes. The resting potential of cultured macrophages ranged from -10 to -25 mV with an average of -14 mV. Endotoxin activated serum added to the bath caused large hyperpolarizations (20-40 mV) associated with a decrease in membrane resistance. The hyperpolarizations occurred within 2-5 sec and were also observed when endotoxin activated serum was applied to single cells with blunt microelectrodes.

Control serum heated at 56°C for 30 minutes before activation as well as activated serum deficient in C3 were ineffective. C3 deficient serum incubated with C3 did produce membrane hyperpolarizations as seen in Figure 40. Addition of 2 mM Mg-EGTA abolished the hyperpolarizing responses immediately. These hyperpolarizations are similar to the spontaneous hyperpolarizing activations previously reported in both guinea pig and human macrophages.[2] These data suggest that membrane hyperpolarizations related to calcium and potassium fluxes are important in the stimulation of macrophages by complement products generated during endotoxin activation of serum.

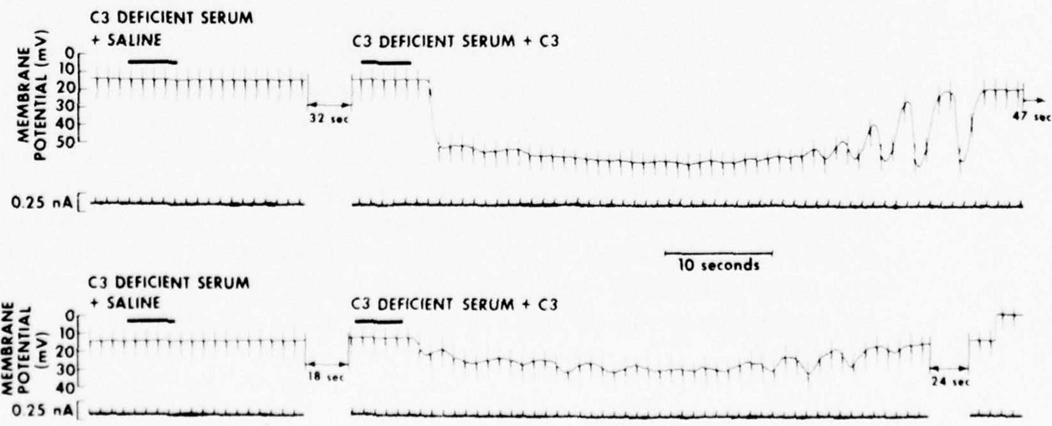

Figure 40. The top tracing is a record of the transmembrane potential of a human macrophage stimulated first with C3 deficient serum incubated with saline for 30 minutes. The C3 deficient serum

The effects of endotoxin released during radiation injury and infection are in large part due to macrophage activation. An understanding of the membrane phenomena associated with macrophage activation is therefore important in the study of radiation injury and other disease states.

REFERENCES

1. Bianco, C., Eden, A. and Cohn, Z. A. Macrophage spreading: a role for complement and coagulation factors. Fed. Proc. 35:335 (Abstract 725), 1976.

2. Gallin, E. K., Wiederhold, M. L., Lipsky, P. E. and Rosenthal, A. S. Spontaneous and induced membrane hyperpolarizations in macrophages. J. Cell. Physiol. 86:653-661, 1975.

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DIFFERENTIAL, INTRACOCHLEAR RECORDING OF THE COCHLEAR MICROPHONIC IN PIGEON

Principal Investigator: *M. G. Pierson*

Using tone bursts as stimuli, the auditory receptor potential, the cochlear microphonic, was characterized as a function of stimulus frequency and intensity

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Figure 40 (continued).
plus saline was applied to the cell by allowing it to diffuse out of the tip of a broken microelectrode (tip diameter 20 μm). Forty seconds later C3 deficient serum incubated with C3 for 30 minutes was added to the cell in the same way resulting in a large hyperpolarization. The tracing below the potential tracing represents the current injected through the microelectrode using the bridge circuit of the amplifier in order to monitor the input resistance of the cell. C3 deficient serum plus saline and C3 deficient serum plus C3 were applied to this cell again and the responses are shown in the bottom half of the figure. The cell responds to a second application of C3 deficient serum plus C3 with a smaller hyperpolarization. At the end of the record the electrode is withdrawn and the transmembrane potential returns to zero.

in the pigeon. A differential electrode technique allowed an isolation of response from a limited portion of the cochlea.

At low input levels, the pigeon's cochlear microphonic was found to exhibit simple linearity as a function of stimulus strength. The low level cochlear microphonic also exhibits fidelity with respect to the middle ear transfer function as a function of frequency. At high input levels the cochlear microphonic becomes nonlinear, exhibiting saturation in response to sufficiently intense signals. At higher input levels, the pigeon's cochlear microphonic shows a prominent nonlinearity centered at the characteristic frequency of the electrodes.

These behaviors are largely consistent with the mammalian cochlear microphonic; however, within the context of avian auditory function, one facet of these results is particularly surprising. Generally it is assumed that cochlear and retrocochlear sensitivity is determined by the middle ear. As expected, the cochlear microphonic measured in this study reflects the avian middle ear properties. According to other studies, however, behavioral and neural frequency response functions of the pigeon are inordinately insensitive at low frequencies. It is probably not coincidental that the apex (the low frequency region) of the avian cochlea consists exclusively of a primitive type of hair cell, the so-called "tall hair cell" whereas the basal avian cochlea through evolution has added a second type of receptor cell, the "short hair cell". It is suggested that those avian receptor cells which transduce low frequency stimuli (tall hair cells) are physiologically less sensitive than short hair cells.

Although there are also two populations of hair cells in the mammalian cochlea, their distribution pattern has precluded their functional separation in the normal animal. As discussed above, the unique distribution pattern of hair cells in the bird's cochlea, in correlation with neural, behavioral and cochlear electrophysiological data, makes such a separation at least tenable in the pigeon. The general implication is that morphological differences in hair cells probably underlie a functional dualism in the mammalian cochlea as well. This latter conclusion is quite important to an understanding of those disorders of hearing in man, in which one population of hair cells is inordinately degenerated. Such disorders include noise-induced hearing loss, drug-induced hearing loss, presbycusis (loss of hearing with advancing age), or the effects of ionizing radiation.

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DOPAMINE MIMICRY AND MODULATION OF L7 GILL MOVEMENTS IN APLYSIA

Principal Investigators: *J. W. Swann, C. N. Sinback and D. O. Carpenter*

Dopamine has been reported in high concentrations in Aplysia gill.[1] Experiments were undertaken to investigate a physiological role for this putative neurotransmitter in the gill. In 10 isolated gill preparations the parietovisceral ganglion was removed and the gill was infused with dopamine. The parietovisceral ganglion was removed to avoid centrally mediated responses to dopamine. In all preparations, dopamine produced contractions of efferent vessel trunklets (the vessels between the distal ends of the pinnules and efferent vessel) and pinnule longitudinal muscles. In eight isolated gill preparations, the threshold concentration of dopamine was 10^{-7} to 10^{-6} M. In the remaining preparations, higher concentrations up to 10^{-4} M were required. In semi-intact preparations of gill plus parietovisceral ganglion, increased spiking of L7 produced efferent vessel trunklet and pinnule longitudinal muscle contractions identical to those induced by dopamine. In a third experimental protocol, semi-intact preparations were used in which the parietovisceral ganglion was isolated during infusion of the gill with dopamine. In 15 such preparations, dopamine at 10^{-7} to 10^{-6} M produced identical contractions as in the isolated gill preparations. In all preparations, firing of L7 during infusion of dopamine at threshold concentrations or higher potentiated the responsiveness of the gill to L7, as compared to L7 gill contractions before and after dopamine infusion. These results show that dopamine can modulate the responsiveness of the gill to L7 and is consistent with a possible role for dopamine as the neurotransmitter for L7. The discovery of a dopamine synapse would lead to a detailed neurophysiological examination of the functioning of dopamine as a neurotransmitter. The clinical implications of such knowledge are suggested by the suspected role of dopamine in such neural disorders as parkinsonism.

REFERENCE

1. Carpenter, D., Breese, G., Schanberg, S. and Kopin, I. Serotonin and dopamine: distribution and accumulation in Aplysia nervous and nonnervous tissues. Int. J. Neurosci. 2:49-55, 1971.

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ORGANIZATION OF RECEPTORS FOR NEUROTRANSMITTERS ON APLYSIA NEURONS

Principal Investigators: *J. W. Swann and D. O. Carpenter*

The application of acetylcholine to neurons of Aplysia has shown the existence of three different responses, mediated by conductance increases to Na^+, Cl^- and K^+, respectively. These neurons also have receptors for various other putative neurotransmitters, including serotonin, dopamine, octopamine, phenylethanolamine, γ-aminobutyric acid, glutamic acid, aspartic acid and histamine. With one possible exception there are at least three types of responses to each of these substances resulting from conductance increases to Na^+, Cl^- and K^+, respectively. These facts suggest that specific receptors (neurotransmitter-binding sites) and ionophores for Na^+, Cl^- and K^+ might be building blocks which can be assembled in any combination. If so, there should be common properties of the receptors for one transmitter mediating the three different ionic responses, as well as similarities in the responses to different transmitters when the conductance change is to the same ion.

We have studied the responses to dopamine on Aplysia neurons.[1] Figure 41 shows examples of the three different conductance increase responses to dopamine. Each of these three neurons was studied with iontophoretic electrodes containing noradrenaline, octopamine and phenylethanolamine in addition to dopamine. Figure 41A shows a neuron with a Na^+ conductance increase response to dopamine while the responses in B and C are due to Cl^- and K^+, respectively. Thus, as for acetylcholine, at least three different ionic responses to dopamine were found. Furthermore, for each response the receptors were also sensitive to noradrenaline and adrenaline, but not to octopamine, tyramine or phenylethanolamine. Cross-desensitization experiments show that the active substances are all acting on one receptor. The same ionic response had similar time courses and temperature sensitivities whether elicited by dopamine or acetylcholine.

Our observation that the various phenylethylamines have similar relative effectiveness in activating the three dopamine responses is consistent with the view that the dopamine binding site is identical irrespective of the conductance change elicited. The similarities in time course, temperature sensitivity and ionic selectivity of responses resulting from similar ionic conductances but activation of receptors to different transmitters suggest that these properties are characteristic of common ionophores. An understanding of the organization of receptors is essential to development of treatment through receptor blocking drugs of radiation-induced injury to the nervous system, especially in the case of early transient incapacitation which results from histamine release.

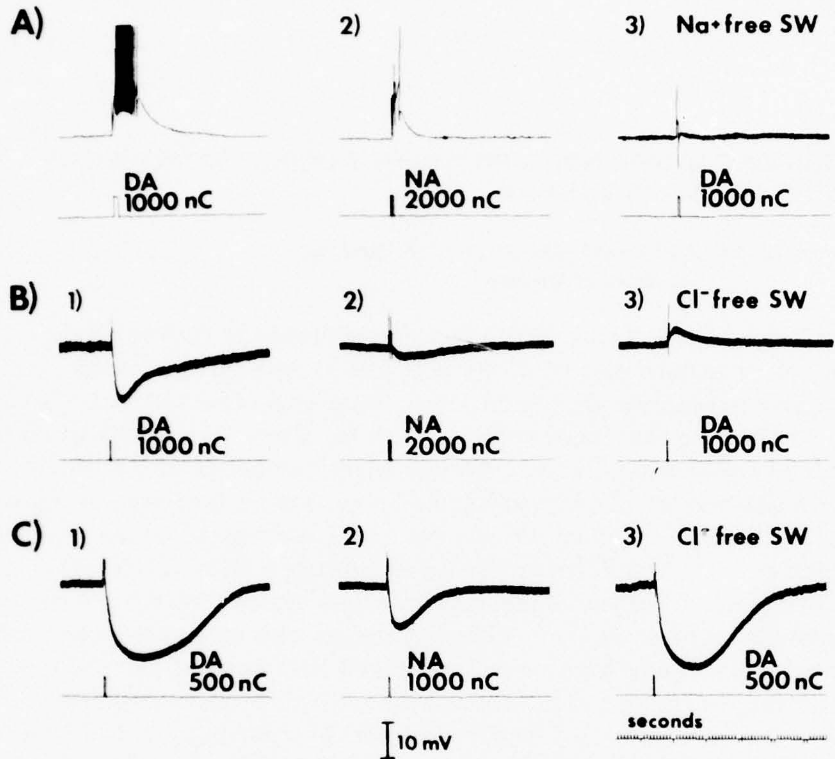

Figure 41. Effects of iontophoretic application of dopamine and noradrenaline on three different unidentified neurons from the abdominal (A and B) and cerebral (C) ganglia. The upper recording in each trace is from the intracellular micropipette, whereas the lower trace shows the duration of the iontophoretic pulse. The total charge passed by iontophoresis is indicated near the pulse. In A3 the ganglion was perfused with seawater where Na^+ was replaced by $Tris^+$, whereas in B3 and C3, all Cl^- was replaced by acetate. The time calibration applies as shown only to A and B. In C the time base is slower by a factor of 2. DA, dopamine; NA, noradrenaline.

REFERENCE

1. Swann, J. W. and Carpenter, D. O. The organization of receptors for neurotransmitters on Aplysia neurons: some generalizations and a hypothesis. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR76-11, 1976.

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DEPLETION OF CARDIAC NOREPINEPHRINE DURING TWO FORMS OF HEMOLYTIC ANEMIA IN THE RAT

Principal Investigators: *J. W. Swann, AFRRI; and J. F. Contrera, University of Maryland*

Knowledge of the status of cardiac norepinephrine during anemia could lead to a better understanding of the role the sympathetic nervous system plays in cardiac function during anemia. Rats were treated with phenylhydrazine. Following the rapid onset of anemia, there was a loss of 60 percent of the stored norepinephrine in the heart within 48 hours of treatment. Associated with the loss of cardiac norepinephrine was an increase in the wet weight of the heart, reaching 40 percent above control 48 hours after treatment (Figure 42).[1] Phenylhydrazine itself probably does not directly mediate this depletion since the vas deferens, brain and spleen have a normal store of norepinephrine at 48 hours. This contention was supported when rats treated with phenylhydrazine were transfused with normal rat red blood cells. This transfusion resulted in nonanemic phenylhydrazine treated rats. The hearts of these rats were not depleted of norepinephrine, but the hearts of the nontransfused phenylhydrazine treated controls were. Anemia was also induced by treating rats with antirat red blood cell serum. The hearts of these rats were also depleted of norepinephrine. These experiments show that during two forms of anemia there is a loss of norepinephrine from the sympathetic neurons innervating the heart. The mechanism underlying this loss of norepinephrine was studied using the experimentally induced immune anemia. Results are consistent with a decrease in the affinity of the cardial sympathetic nerve terminal for norepinephrine combined with an increase in the rate of release of norepinephrine. The physiological implications of a partial loss of norepinephrine from cardial sympathetic nerve terminals remains to be determined. However, an understanding of how anemia alters cardiac function control by the nervous system is critical in development of a total treatment of the anemic patient.

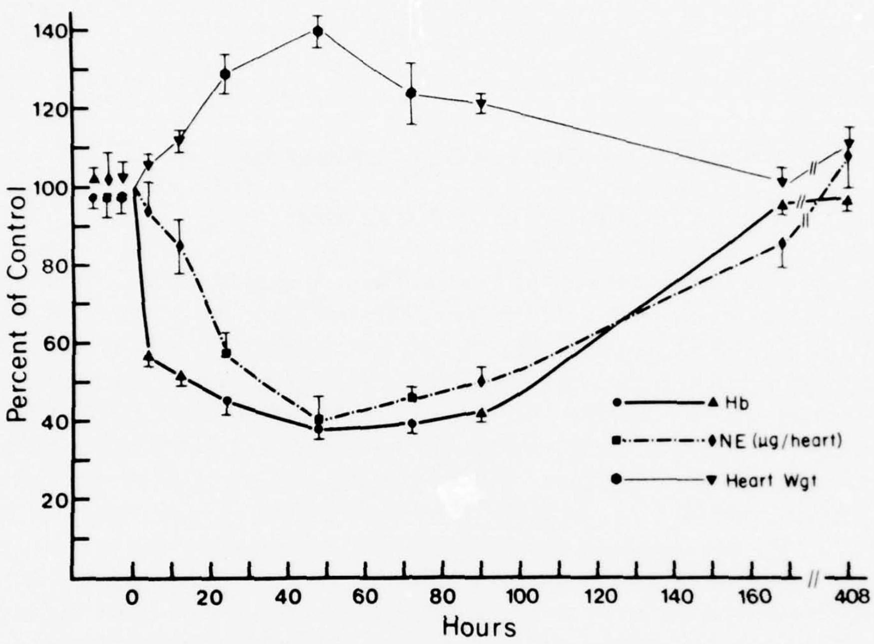

Figure 42. Effect of phenylhydrazine on blood hemoglobin (Hb), cardiac norepinephrine (NE), and heart weight. Data are from two sets of experiments on groups of rats treated with phenylhydrazine (125 mg/kg) at various times before they were euthanatized. Results are expressed as percent of control, mean ± SEM. The 0-hour groups, which are the control groups, were not so treated. A circle, square, or hexagon identifies data for groups of animals from one experiment. For its control Hb = 15.5 ± 0.32 g/100 ml; NE = 0.592 ± 0.03 μg/heart; and wet cardiac weight = 1.164 ± 0.04 g. The other symbols represent data for the second experiment; control group Hb = 16.5 ± 0.03 g/100 ml; NE = 0.562 ± 0.03 μg/heart; and wet weight = 1.054 ± 0.027. N = 5-7 for each point.

REFERENCE

1. Swann, J. W. and Contrera, J. F. Depletion of cardiac norepinephrine during two forms of hemolytic anemia in the rat. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR76-10, 1976.

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THE ELECTRICAL RESISTIVITY OF AQUEOUS CYTOPLASM

Principal Investigators: *K. R. Foster, J. M. Bidinger and D. O. Carpenter*

In order to determine the structure of cellular water in biologic systems, the apparent cytoplasmic resistivity of two different giant cells has been measured using an extension of a previously developed single microelectrode technique.[1] Each cell is penetrated by a metal microelectrode whose complex impedance is measured as a function of frequency between 500 kHz and 5.7 MHz. By plotting the measured impedance data on the complex $\underline{Z}$ plane and extrapolating the data to infinite frequency, the substantial effects of electrode polarization can be overcome. For Aplysia giant neurons and muscle fibers of the giant barnacle, the extrapolated cytoplasmic specific resistivities are 40 and 74 ohm-cm, respectively, at infinite frequency. The barnacle data are in excellent agreement with sarcoplasmic resistivity values derived from the measured cable properties of other marine organisms, and from high frequency conductivity cell measurements in intact barnacle muscle tissue (Figure 43).

The imaginary component of the polarization impedance can be directly measured, both when the electrode is in a known electrolyte solution and when it is in a cell. In the Aplysia neurons, the frequency-dependent part of the electrode impedance is larger when the electrode is in a cell than when it is in an electrolyte solution with the same specific resistivity as the aqueous cytoplasm; however, the phase angle of the frequency-dependent component of the electrode impedance is the same in both cases. The high apparent values of cytoplasmic resistivity found using the single microelectrode technique at lower frequencies probably result from interaction of intracellular membranes with the microelectrode surface, and probably reflect an artifact caused by a reduction of the effective surface area of the electrode, with a corresponding increase in its polarization impedance. It has been suggested that some effects of ionizing radiation might be through mobilization of bound water. These experiments have shown no evidence for bound water, and thus it is unlikely that such a mechanism is significant.

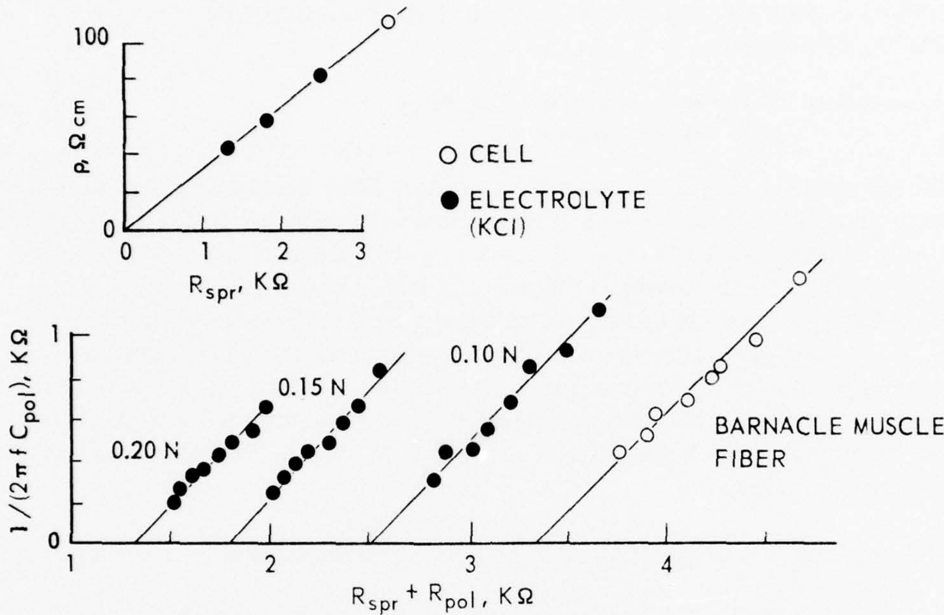

Figure 43. A typical set of experimental data from a single muscle fiber of the barnacle. The complex electrode impedance is shown as a function of frequency, when it is in calibrating solutions of KCl and after it has penetrated the cell. The measurement frequencies range from 0.5 to 5.7 MHz, in equal logarithmic steps. The frequency-dependent part of the measured impedance arises from electrode polarization, both when the electrode is in the electrolyte and (apparently) when it is in the cell. The frequency-independent component of the electrode impedance R_{spr} is proportional to the solution resistivity ρ (inset). This cell has an apparent sarcoplasmic resistivity of 110 ohm-cm, at 20°C.

REFERENCE

1. Foster, K. R., Bidinger, J. M. and Carpenter, D. O. The electrical resistivity of aqueous cytoplasm. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR76-39, 1976.

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THE LOW APPARENT PERMITTIVITY OF ADSORBED WATER IN SYNTHETIC ZEOLITES

Principal Investigators: *K. R. Foster, AFRRI; and H. A. Resing, Naval Research Laboratory*

Recent nuclear magnetic resonance studies have shown that the interstitial water in synthetic zeolites is in many respects similar to the normal fluid. We have measured the dielectric permittivity of hydrated zeolites NaX and NaY (Linde) over the frequency range 1-150 MHz, and estimate that the apparent dielectric constant of this interstitial water is less than 14 (NaX) or 21 (NaY). Apparently, this is an extreme example of the well-known ability of ions to depress the dielectric constant of water. In effect, we have extended previous studies on the dielectric permittivity of concentrated electrolyte solutions to the very high concentrations (c. 18 M) of mobile Na^+ ions present in the hydrated crystals.

The composition of zeolites NaX and NaY are described by the formulas:

$$Na_{86}\,[\,(Al\,O_2)_{86}\ (Si\,O_2)_{106}\,]\cdot 264\ H_2O \qquad (NaX)$$

$$Na_{56}\,[\,(Al\,O_2)_{56}\ (Si\,O_2)_{136}\,]\cdot 264\ H_2O \qquad (NaY).$$

Their crystal structure consists of an open framework of aluminate and silicate tetrahedra enclosing interstitial voids of approximately 13 A diameter. Negative charges in the lattice resulting from the substitution of Si^{4+} by Al^{3+} reside on lattice oxygen atoms, and are neutralized by Na^+ counterions which, in the fully hydrated crystal, float about in the interstitial voids.

Samples of zeolites NaX and NaY were hydrated over saturated aqueous solutions of NaCl and packed by hand into the cup-shaped neutral terminal of a dielectric cell. The cell was designed to permit the sample to be removed without disturbing the powder. Capacitance measurements were performed with a Booton R-X meter and a Wayne-Kerr model 601 impedance bridge, and corrected for the low residual inductance in the system. Samples were dehydrated by heating to 400°C under a vacuum of 10^{-2} mm of Hg. The water content of the hydrated samples, as measured by weight loss during dehydration, agreed well with values calculated from the formulas given above.

Figure 44 shows the measured permittivity (ϵ') of the hydrated and dehydrated samples. Because of the lower powder density employed in this study (0.77 versus 1.19 g/cm^3), the observed permittivities of NaX below 3 MHz are smaller by a factor of about 1.8 than those measured by another investigator (R. A. Schoonheydt, personal communication). The increase in permittivity at low frequencies is due to sample heterogeneity (the Maxwell-Wagner

effect) and to the formation of electric dipoles by association between the mobile cations and the fixed anions in the lattice. From the nuclear magnetic resonance data, the water dipolar absorption in NaX occurs at substantially higher frequencies than these other effects, at least at room temperature. This is evidently not the case with NaY.

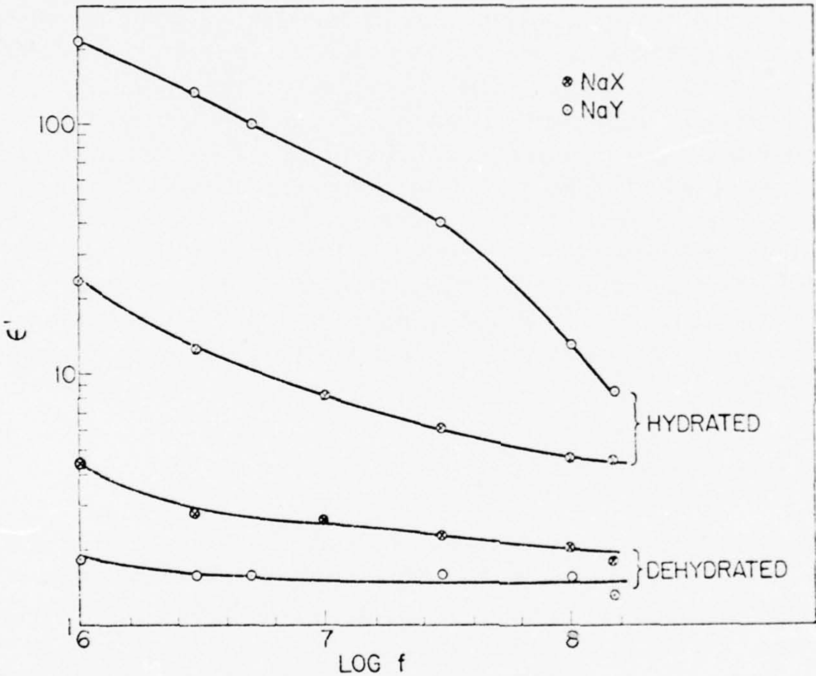

Figure 44. The observed permittivities (ϵ') of zeolites NaX and NaY at 298°K

The water dipolar absorption is surprisingly weak. The maximum possible change in permittivity resulting from this presumed dispersion is the difference between the permittivities of the hydrated and dehydrated samples, measured at 100 or 150 MHz. From these data, it is possible to estimate the maximum apparent permittivity of the interstitial water. We assume that the specific polarization, p, of the sample is additive:

$$p = v_{water}\, p_{water} + v_{lattice}\, p_{lattice}$$

where v_{water} or $v_{lattice}$ is the volume fraction of water or of dry zeolite in the powder. We also assume that the specific polarization is related to the permittivity by the Kirkwood expression:

$$p = (\epsilon' - 1)(2\epsilon' + 1) / 9\epsilon' .$$

Although this formula is essentially empirical, it has been successfully used in other studies of adsorbed molecules; and it predicts a bulk permittivity of 6.3 for dehydrated zeolite NaX, in good agreement with other estimates.[1]

We find the maximum apparent permittivity of the interstitial water in NaX to be about 14 at 298°K and (using the data from Jansen[1]) about 25 at 220°K. Assuming that the Na^+ cations are more strongly hydrated than either the lattice O^- charges (in the zeolite) or the simple anions (in bulk solution), our results can be compared to previous measurements of the permittivity of concentrated NaCl aqueous solutions. While extensive association between the lattice of O^- ions and the counterions undoubtedly is present in the fully hydrated crystal, we still expect most of the interstitial water molecules to be located in the primary hydration spheres of one of these two ion species. Our results show that these water dipoles are "immobilized" (i.e., aligned by local fields within the crystal), yet have rotational and translational "mobilities", estimated from the nuclear magnetic resonance data, similar to molecules in the bulk phase. An understanding of the structure of water in both biologic and model systems is essential to an understanding of how it is manipulated by various forms of electromagnetic radiation.

REFERENCE

1. Jansen, F. J. Electrical properties of synthetic zeolites X and Y with varying silicon to aluminum ratio. Thesis, Kathalieke Universiteit Leuven, Belgium, 1972; Jansen, F. J. and Schoonheydt, R. A. ACS Adv. Chem. Series 121:96-105, 1973.

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EFFECT OF DIMETHYLSULFOXIDE ON THE DIELECTRIC PROPERTIES OF CANINE KIDNEY TISSUE

Principal Investigators: *K. R. Foster, AFRRI; R. T. Bell III, R. Whittington and B. Denysyk, Naval Medical Research Institute*

One major difficulty with attempts to prolong the viability of solid organs by freezing is the need to thaw the tissue rapidly and uniformly after storage. An attractive approach, using a commercial microwave oven operating at 2450 or 915 MHz, has had only limited success, partially because of the nonuniform heating pattern produced by the microwave field.

To ascertain the probable effect of a commonly used cryoprotective agent, dimethylsulfoxide, on the electromagnetic heating process, we measured the conductivity and permittivity of canine kidney tissue over the temperature range -20°C to +20°C, and over the frequency range 3 to 900 MHz.

Kidneys, weighing 60 to 80 g, were removed from adult male mongrel dogs by laparotomy incision. Anesthesia was induced with sodium pentobarbital and later maintained with a mixture of halothane, nitrous oxide and oxygen. The surgical procedures were done in connection with an immunological study on renal rejection, requiring autotransplantation of one kidney and removal of the contralateral organ. The removed kidneys were perfused either with canine plasma on a Belzer perfusion apparatus or with gravity-infused physiological saline, and then stored at -10°C in a standard freezer until experimentally used. Samples of the dimethylsulfoxide-perfused tissue were analyzed by tritium labeled distribution studies to verify that the dimethylsulfoxide had uniformly penetrated the tissue. Samples of approximately 1 cm^3 volume were removed from the cortex and medulla, and packed into a stainless steel and Teflon dielectric cell constructed from a length of 50-ohm coaxial air line, as described elsewhere.[1] The sample temperature was controlled to within 1°C by delivering cold nitrogen gas through a coaxial jacket surrounding the cell. Measurements over the frequency range of 3-100 MHz were performed using a Booton R-X meter and a Wayne-Kerr model 801 admittance bridge.

For our purposes, the most useful parameter is the specific conductivity, σ, of the tissue. Figure 45A shows the conductivity at 10 MHz of dimethylsulfoxide-perfused and saline-perfused organs. Each point represents the average of six measurements on three dimethylsulfoxide-perfused organs, or of four measurements on two saline-perfused kidneys. The standard error of the mean is approximately 25 percent except at -20°C, when it increases to nearly 100 percent because of the lower values of σ. At -20°C, dimethylsulfoxide increases the conductivity of the tissue by nearly fivefold; at 20°C, it decreases the conductivity by 40 percent. These effects are observed in dimethylsulfoxide-saline solutions as well (Figure 45B). The tissue conductivity at temperatures below -10°C was constant over the frequency range of 3 to 500 MHz, and that of the thawed tissue increased twofold (saline-perfused) to fourfold (dimethylsulfoxide-perfused), from 3 to 900 MHz. At 900 MHz, the conductivities of the dimethylsulfoxide-perfused and saline-perfused organs are roughly the same. Dimethylsulfoxide significantly reduced the change in the dielectric properties of the tissues as it was thawed.

Unfortunately, we cannot realistically calculate the dielectric heating of a partially thawed organ irradiated by a microwave oven. However, detailed calculations, assuming concentric tissue spheres whose dielectric properties are characteristic of frozen and thawed tissue irradiated with plane electromagnetic waves, show that the greatest nonuniformity in the dissipated power occurs at

the interface between the two sections. This nonuniformity results from the mathematical boundary condition that the tangential component of the electric field, E_t, be continuous across the interface between the thawed and frozen sections. The power deposited by this field component is proportional to σE_t^2. To improve the uniformity of the heating, it therefore appears most important to reduce the difference in the conductivities between the "frozen" and the "thawed" tissue. Since the conductivity of the "thawed" tissue rises more quickly with frequency than that of the "frozen" tissue above 100 MHz, our data suggest that more uniform heating might be obtained using frequencies lower than 915 or 2450 MHz.

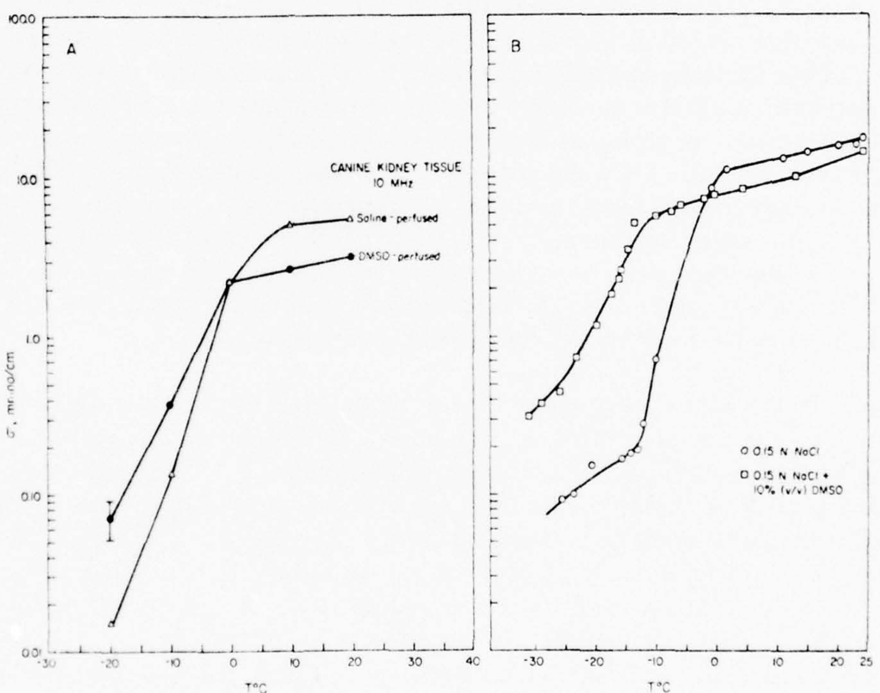

Figure 45. A. The specific conductivity (σ) of saline- and DMSO-perfused canine kidney tissue as a function of temperature. The conductivities of cortical and medullary tissues were the same to within data reproducibility and are included together. The S. E. M. for the data at -20°C is indicated; the remaining data at higher temperatures have somewhat smaller relative uncertainties. B. The specific conductivity of 0.15 N NaCl and 0.15 N NaCl-10 percent dimethylsulfoxide (DMSO) mixtures (v/v) as a function of temperature. The similarity between A and B shows that the DMSO-induced increase in the conductivity of frozen tissue results from a colligative property of the DMSO-electrolyte solution.

Our results show a significant effect of the cryoprotectant dimethylsulfoxide on the conductivity of canine kidney tissue. Presumably, this will substantially improve the microwave heating pattern in frozen tissues. This effect, in addition to pharmacological considerations, should be a factor in the choice of a cryoprotective agent for solid organs which are to be thawed by radiofrequency or microwave irradiation.

REFERENCE

1. O'Konski, C. T. and Edwards, A. Cell for dielectric measurements in the high radio frequency region. Rev. Sci. Instr. 39:1456-1458, 1968.

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PROPERTIES OF WATER IN REVERSED MICELLES. A NUCLEAR MAGNETIC RESONANCE STUDY

Principal Investigators: *M. J. McCreery, AFRRI; H. J. C. Yeh, National Institutes of Health; and D. O. Carpenter, AFRRI*

The microscopic environment of ions and water at the membrane water interface is of great importance in understanding membrane phenomena. While it is generally accepted that the charged surface of the membrane imparts some order to water and counterions adjacent to the interface, there is little agreement over not only the rigidity of this order, but also the distance that it extends into the aqueous media. A study was undertaken to investigate the properties of interfacial water via high resolution nuclear magnetic resonance, utilizing a simple model system. Reversed micelles formed by the surfactant Aerosol-OT in a nonpolar solvent can trap pools of water whose size may be altered by varying the [water] to [surfactant] ratio R. Linewidths at half-height and chemical shift of the water signal were measured from spectra recorded on a Varian HA-100 nuclear magnetic resonance spectrometer equipped with a temperature controller (Figure 46). Calculations of the spin-spin relaxation time T_2, obtained from linewidth data (Figure 47), suggest that the rotational mobility of the water within these trapped pools is similar to that of "bulk" water until the pools are approximately 25 Å in diameter. Micelles of smaller diameter exhibit a marked decrease in water mobility. The water in at least this model system, therefore, does not seem to be structured until it is within 10 to 20 Å of the negatively charged interface.

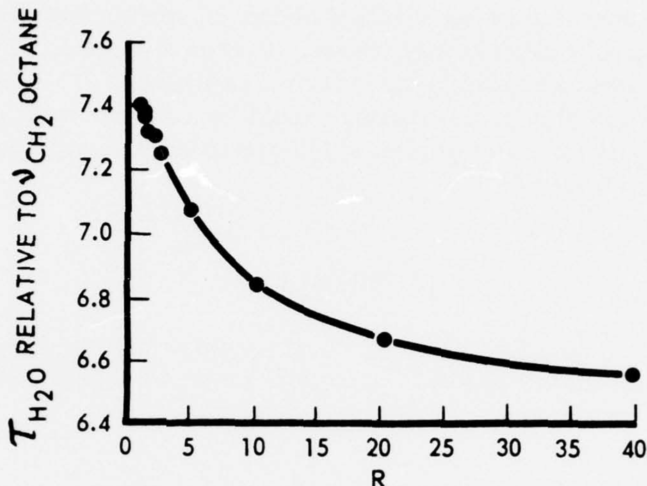

Figure 46. Chemical shift (τ) of the H_2O protons as a function of $[H_2O]/[\text{surfactant}]$ ratio (R) as measured on a Varian HA-100 nuclear magnetic resonance (NMR) spectrometer at $30^o \pm 1^oC$. The $[H_2O]$ was held constant at 1 M while the [surfactant] was altered to vary R. τ_{H_2O} is expressed relative to the chemical shift of the methylene protons on octane.

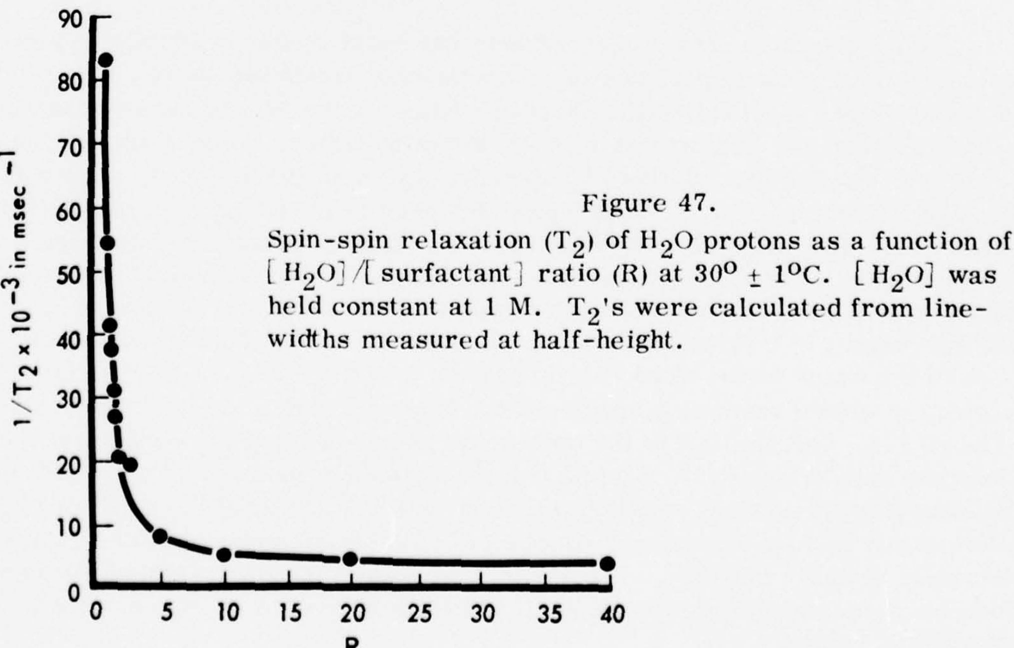

Figure 47.

Spin-spin relaxation (T_2) of H_2O protons as a function of $[H_2O]/[\text{surfactant}]$ ratio (R) at $30^o \pm 1^oC$. $[H_2O]$ was held constant at 1 M. T_2's were calculated from line-widths measured at half-height.

Further experimentation using the nuclear magnetic resonance method of Creekmore and Reilley[1] has yielded an estimation of the hydration number for sodium ions in the water phase of these aggregates. Each of these ions is thus hydrated by an average of 1.0 water molecule at $R = 5.0$ to 0.6 water molecule at $R = 0.8$, considerably lower than the hydration number of 4.5 for sodium ion in "bulk" solution. These results imply "ion-pairing" between sodium ions and the negatively charged sulphonate at the water-surfactant interface. Since ion-pairing is a form of ion binding, this model system is one which can provide valuable information for ion-water interactions and alterations of their normal interactions by radiation.

REFERENCE

1. Creekmore, R. W. and Reilley, C. M. Nuclear magnetic resonance determination of hydration numbers of electrolytes in concentrated aqueous solutions. J. Phys. Chem. 73:1563-1568, 1969.

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EFFECTS OF ESERINE AND NEOSTIGMINE ON THE INTERACTION OF α-BUNGAROTOXIN WITH APLYSIA ACETYLCHOLINE RECEPTORS

Principal Investigators: *D. O. Carpenter, AFRRI; L. A. Greene, National Institutes of Health; W. G. Shain, Jr., AFRRI; and Z. Vogel, National Institutes of Health*

In an effort to determine how several clinically useful drugs interact with acetylcholine receptors, we have studied their interaction with α-bungarotoxin binding sites.[1] Binding of ^{125}I α-bungarotoxin to acetylcholine receptors of a ganglionic homogenate of the marine mollusc Aplysia is blocked by the anticholinesterases eserine ($I_{50} = 4 \times 10^{-6}$ M) and neostigmine ($I_{50} = 2 \times 10^{-4}$ M). The classical acetylcholine antagonist d-tubocurarine blocks with an $I_{50} = 2 \times 10^{-4}$ M. Eserine ($I_{50} = 5.4 \times 10^{-6}$ M) and neostigmine ($I_{50} = > 10^{-3}$ M) also block toxin binding to a solubilized receptor preparation. Unlike their relative potency in blocking toxin binding, neostigmine is a more potent inhibitor of Aplysia acetylcholinesterase ($I_{50} = 1.4 \times 10^{-8}$ M) than is eserine ($I_{50} = 2.5 \times 10^{-7}$ M). α-Bungarotoxin does not affect esterase activity or interfere with the ability of eserine to block the esterase. The response to acetylcholine recorded through intracellular microelectrodes is blocked by α-bungarotoxin.

Neither eserine nor neostigmine blocks the acetylcholine response but rather prolongs and increases it as expected from their effects on the esterase (Figure 48). Eserine (10^{-4} M) blocks the α-bungarotoxin inhibition of the physiologic acetylcholine response. These results indicate that eserine and neostigmine block the binding of α-bungarotoxin by interacting with a site which is different from both the esterase and the cholinergic sites of the acetylcholine receptor. These experiments provide further information on the mechanisms of action of specific components of snake venoms. Furthermore, these investigations increase our knowledge of the normal and pathologic bases of movement and coordination in higher animals and man, and how these are affected by various drugs, since all movement in vertebrates is mediated through acetylcholine receptors similar to those described here.

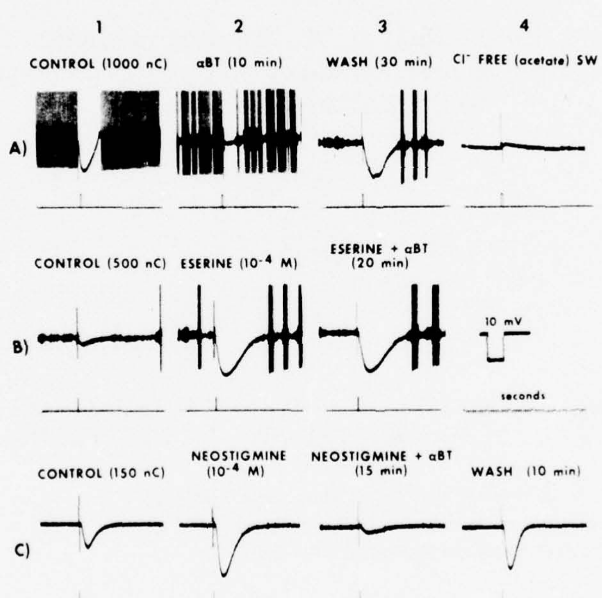

Figure 48. Interactions of α-bungarotoxin (αBT) with eserine and neostigmine on the response to iontophoretic acetylcholine, recorded from an unidentified neuron in the lower left quadrant of the abdominal ganglion of Aplysia. The intracellular recording of electrical activity is shown in the upper trace. The neuron was initially spontaneously active but became silent with time. The second trace indicates the duration of the iontophoretic pulse. The total charge passed is different for records A, B and C and the amount is indicated in column 1. The preparation was perfused with artificial seawater containing 100 mM added $MgSO_4$ to block spontaneous synaptic transmission, and drugs were added as indicated. All records were taken at 22°C. Record A-2 shows the reduction of the response to 1000 nC acetylcholine observed

REFERENCE

1. Carpenter, D. O., Greene, L. A., Shain, W. G., Jr. and Vogel, Z. Effects of eserine and neostigmine on the interaction of α-bungarotoxin with Aplysia acetylcholine receptors. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR76-16, 1976.

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EFFECT OF CURARE ON RESPONSES TO DIFFERENT PUTATIVE NEUROTRANSMITTERS IN APLYSIA

Principal Investigators: *D. O. Carpenter, J. W. Swann and P. J. Yarowsky*

Aplysia neurons have specific receptors for acetylcholine, dopamine, octopamine, phenylethanolamine, histamine, gamma-aminobutyric acid, glutamic acid and aspartic acid. We have studied the effects of curare on responses resulting from iontophoretic application of these putative neurotransmitters onto Aplysia neurons.[1] Each of these substances may, on different

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Figure 48 (continued).
after perfusion for 10 min of high Mg^{++} seawater containing 2 mg/ml bovine serum albumin and 3.6×10^{-6} M α-bungarotoxin. A-3 shows the effect of the same amount of acetylcholine given after 30 min of washing with high Mg^{++} seawater. Record A-4 shows the response 10 min after beginning perfusion with seawater in which all Cl^- was replaced by acetate. B-1 is a smaller control response before addition of eserine, while B-2 shows the effect of the same amount of acetylcholine 15 min after addition of 10^{-4} M eserine. α-Bungarotoxin (3.6×10^{-6} M) did not affect this response even after 20 min. After the eserine effect was at least partially reversed by washing, another control was taken (C-1) and neostigmine (10^{-4} M) was added (C-2). The facilitated response in neostigmine was depressed by 3.6 $\times 10^{-6}$ M α-bungarotoxin (C-3). After washing (C-4) the blockade was reversible.

specific neurons, elicit at least three types of response, caused by a fast depolarizing Na^+, a fast hyperpolarizing Cl^- or a slow hyperpolarizing K^+ conductance increase. All responses resulting from either Na^+ or Cl^- conductance increases, irrespective of which putative transmitter activated the response, were sensitive to curare. Most were totally blocked by $\leq 10^{-4}$ M curare (Figure 49). Gamma-aminobutyric acid responses were less sensitive and were often only depressed by 10^{-3} M curare. K^+ conductance responses,

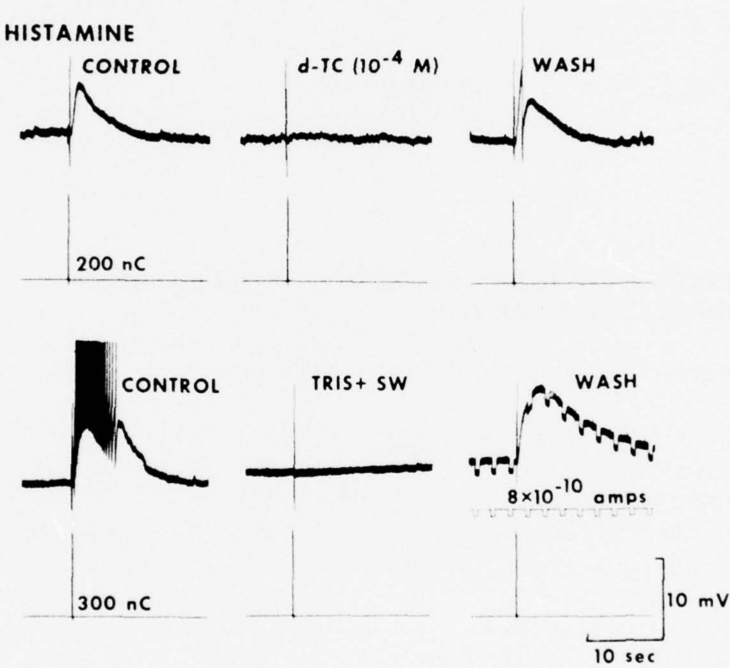

Figure 49. Curare blockade of a depolarizing histamine response. Each upper trace shows recording from an unidentified neuron in the lower right quadrant of the cerebral ganglion (-38 mV) while the lower trace indicates the pulse of 200 nC (upper records) or 300 nC (lower records) histamine. The lower right records also show the constant current pulses (8×10^{-10} A) used to monitor membrane resistance. Curare (d-TC) was applied for 5 min at 10^{-4} M, and the response recovery was taken following a 15 min wash with seawater. The larger response (below) was totally blocked after a 15-min perfusion with $Tris^+$ (Na^+ free) seawater. After 5 min of wash with normal seawater, the histamine response has recovered without return of control spike generation, and under these circumstances constant current pulses fail to demonstrate a conductance change during the response. The membrane did not show rectification over the potential spread of the response.

irrespective of the transmitter, were not curare sensitive. These results are consistent with a model of receptor organization in which one neurotransmitter receptor may be associated with any of at least three ionophores, mediating conductance increase responses to Na^+, Cl^- and K^+, respectively. In Aplysia nervous tissue, curare appears not to be a specific antagonist for the nicotinic acetylcholine receptor but rather to be a specific blocking agent for a class of receptor-activated Na^+ and Cl^- responses. In addition, the presence of three different kinds of ionic response, including both depolarizing and hyperpolarizing responses, suggests that any neurotransmitter, at least in Aplysia, may be either excitatory or inhibitory. This conclusion has significance in understanding a variety of human diseases in which synthesis or metabolism of one or more transmitters is abnormal, such as Parkinson's disease, various choreas and athetoses and possibly some mental illnesses such as schizophrenia. The elucidation of the mechanisms whereby histamine affects neurons provides a basis for understanding how histamine, released from mast cells by ionizing radiation, might activate receptors on muscle or cerebral blood vessels to cause early transient incapacitation following exposures to very high doses of ionizing radiation.

REFERENCE

1. Carpenter, D. O., Swann, J. W. and Yarowsky, P. J. Effect of curare on responses to different putative neurotransmitters in Aplysia. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR76-27, 1976.

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LOCALIZATION OF AND SPECIFIC RECEPTORS FOR PHENYLETHANOLAMINE IN APLYSIA

Principal Investigators: *J. M. Saavedra, National Institute of Mental Health;*
J. L. Ribas, J. W. Swann and D. O. Carpenter, AFRRI

We have attempted to document a role for phenylethanolamine as a neurotransmitter. Phenylethanolamine was detected in the nervous system of Aplysia. Phenylethanolamine was assayed by an enzymatic-isotopic technique.[1] It was found in all ganglia with the greatest amount in the buccal (4.6 ± 4 ng/mg protein) and the lowest in the pedal (1.4 ± 0.1 ng/mg protein).

Of all of the regions of the ganglia, the neuropile showed the greatest concentration, approximately five times that of other regions. The amine was also present in both connective (4.6 ± 0.9 ng/mg protein) and posterior parapodial (2.8 ± 0.8 ng/mg protein) nerves. It could not be detected, however, in any of the major identified neurons.

Specific receptors for phenylethanolamine were found in the nervous system of Aplysia. These receptors were not responsive to iontophoretic application of structurally related putative neurotransmitters (phenylethylamine, dopamine, norepinephrine, octopamine and histamine) from five-barreled electrodes. Such receptors are rare, and most frequently found in buccal or cerebral ganglia (Figure 50). Three types of responses were found, due to Na^+, Cl^- or K^+ conductance increases, respectively. The Cl^- conductance response was most frequent. These results, demonstrating presence of and specific receptors for phenylethanolamine, suggest that this amine may be considered to be a putative neurotransmitter in the nervous system of Aplysia. Since phenylethanolamine is also present in the mammalian central nervous system, these results are consistent with the possibility that phenylethanolamine may be a neurotransmitter there as well. If phenylethanolamine is a neurotransmitter in simple nervous systems, it may also be in man. Furthermore, it may be involved in human disease states or in response to various injuries to the nervous system.

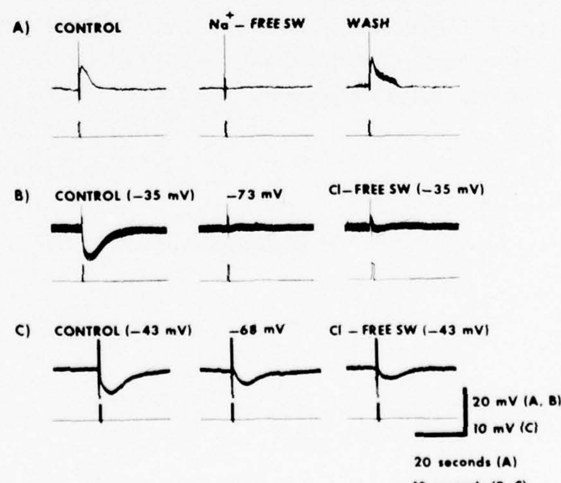

Figure 50. Responses of Aplysia neurons to phenylethanolamine. A, B, and C (upper trace) are intracellular recordings from unidentified neurons in the buccal and cerebral ganglia respectively. The iontophoretic current application for a five-barreled electrode is indicated in the lower trace and was 500, 500 and 1000 nC. The neuron in A was unresponsive to 1000 nC of dopamine and octopamine but had a depolarizing response to acetylcholine. The

REFERENCE

1. Saavedra, J. M. Enzymatic-isotopic method for octopamine at the picogram level. Anal. Biochem. 59:628-633, 1974.

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A COMPARISON OF IONOPHORES ACTIVATED BY ACETYLCHOLINE AND GAMMA-AMINOBUTYRIC ACID IN APLYSIA NEURONS

Principal Investigators: *P. J. Yarowsky and D. O. Carpenter*

Identified neurons in Aplysia possess three different ionic responses to acetylcholine and to gamma-aminobutyric acid. Both substances have receptors on the soma as well as in the neuropile. Swann and Carpenter[1] compared the acetylcholine and dopamine responses and found similarities in the responses due to movement of the same ions on activation of different receptors. They suggested that a single receptor may be associated with any of at least three ionophores which mediate permeability changes to Na^+, Cl^- and K^+. We have found that gamma-aminobutyric acid and acetylcholine responses have similar times to peak for different ionic conductance responses: for Na^+ conductances, acetylcholine = 1.8 ± 0.2 sec, gamma-aminobutyric acid = 2.0 ± 0.1 sec; for Cl^- conductances, acetylcholine = 2.4 ± 0.2 sec, gamma-aminobutyric acid = 2.4 ± 0.4 sec; for K^+ conductances, acetylcholine

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Figure 50 (continued).
middle record was taken 4 min after beginning perfusion with seawater in which $Tris^+$ replaced all Na^+. The right record was taken 10 min after wash was begun. The neuron in B was insensitive to dopamine, norepinephrine, octopamine and histamine, while that in C had a very small hyperpolarizing (K^+) response to dopamine but was unresponsive to octopamine, norepinephrine and acetylcholine. In B and C the neurons were penetrated with two independent intracellular electrodes for recording and current passage. The middle records show the responses after membrane potential was hyperpolarized past the equilibrium potential for Cl^- (about -60 mV) and show that the response in B reverses while that in C does not. The Cl^- response in B is also abolished by replacement of Cl^- in the seawater with acetate (right record in B).

= 12.2 ± 1.7 sec, gamma-aminobutyric acid = 13.1 ± 1.5 sec. The properties of the ionic selectivity of the Cl^- ionophore were studied on cell R2, which has Cl^- responses to both acetylcholine and gamma-aminobutyric acid. Cells were impaled with two independent microelectrodes. Acetylcholine and gamma-aminobutyric acid responses were elicited by iontophoresis. The inversion potential was measured in seawater and after partial substitution of the external Cl^- by impermeant anions (SO_4 and acetate). The resulting shift in the inversion potential for both substances was similar and corresponded to changes in E_{Cl} (Figure 51). However, the gamma-aminobutyric acid and acetylcholine receptors on R_2 do not cross desensitize and are therefore distinct. Furthermore, α-bungarotoxin and strychnine block the acetylcholine but not the gamma-aminobutyric acid response. These results indicate that the receptors for acetylcholine and gamma-aminobutyric acid are distinct but the similarity of the ionic conductances to the two substances is consistent with the view that the ionophores associated with the receptors are identical. These observations on receptor organization are significant in that they are probably applicable to a wide variety of neurotransmitter substances, and are therefore important in understanding human nervous and muscular disease states, as well as the development of therapeutic drugs.

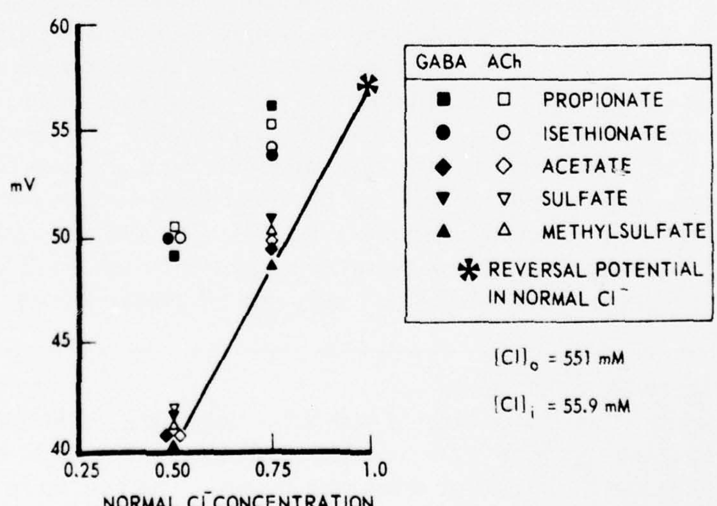

Figure 51. Graph of inversion potentials for ACh and GABA responses in R_2 following various anion substitutes for Cl^-. Reversal potential is indicated by * in normal Cl^- (-58 mV). Following replacement of Cl^- by anions so that 50 or 70 percent of Cl^- remained, the reversal potentials for ACh and GABA shifted in the same direction. Substitutions with acetate, sulfate, or methylsulfate followed the Nernst equation for reduction of external Cl^-, while propionate and isethionate substitutions did not follow this equation.

REFERENCE

1. Swann, J. W. and Carpenter, D. O. Organisation of receptors for neurotransmitters on Aplysia neurones. Nature 258:751-754, 1975.

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ASPARTATE: DISTINCT RECEPTORS ON APLYSIA NEURONS

Principal Investigators: *P. J. Yarowsky and D. O. Carpenter*

While L-glutamate has been regarded as a putative excitatory neurotransmitter in both vertebrates and invertebrates, L-aspartate has been reported only to act like glutamate. However, recent experiments in lobster muscle and the mammalian central nervous system have suggested that aspartate may have a distinct role as a neurotransmitter. Both aspartate and glutamate are involved in a number of cellular processes such as metabolism, protein formation, and intracellular osmotic and ionic regulation. Aspartate has been found to be a major anion in excitatory, inhibitory, and sensory nerve tissue in the lobster and crab.

We have studied the nervous system of the marine mollusc Aplysia and have recorded from single neurons during application of aspartate, glutamate and acetylcholine in an effort to determine if specific receptors were present.

Responses to aspartate were recorded in cells from all ganglia studied, although not all cells responded to either aspartate or glutamate. Separate and distinct responses to aspartate were obtained in only a small percentage of the neurons. These were occasionally found in the abdominal ganglia, but were more frequent in the buccal ganglia.

As shown in Figure 52, there was no relation between aspartate receptors and those for acetylcholine or glutamate. For all three substances, three types of response were found due to Na^+, Cl^- and K^+ conductance increases, respectively.

Specific aspartate receptors have not previously been found in either vertebrate or invertebrate preparations. Aspartate is asymmetrically concentrated in the Aplysia nervous system.

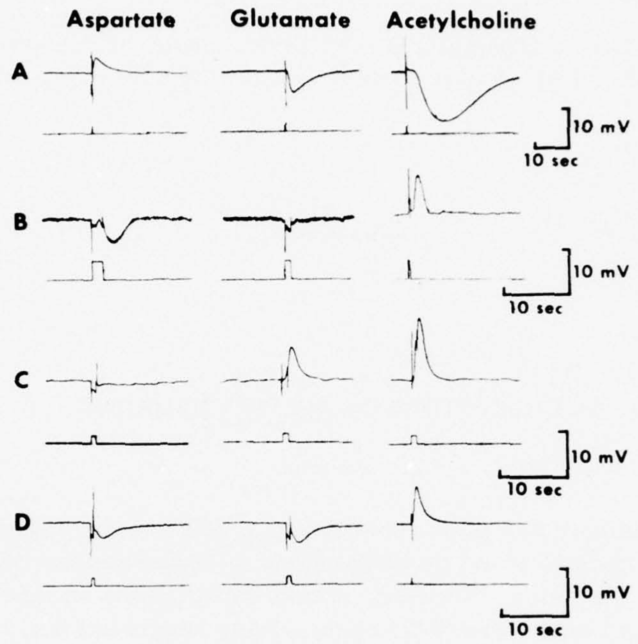

Figure 52. Effects of iontophoretic application of L-aspartate, L-glutamate, and acetylcholine (ACh) on four different neurons from the abdominal (A and D) and buccal (B and C) ganglia. The upper recording in each trace is from the intracellular micropipette, while the lower trace shows the duration of the iontophoretic pulse. The cell in (A) had a resting membrane potential (RMP) of -50 mV, and iontophoretic currents were (in nanocoulombs): aspartate, 50; glutamate, 50; and ACh, 50. In (B), the cell had a RMP of -48 mV and currents were (in nanocoulombs): aspartate, 400; glutamate, 400; and ACh, 100. No response was seen with bath application of glutamate (10^{-4} M). In (C), the cell had an RMP of -45 mV and currents were (in nanocoulombs): aspartate, 500; glutamate, 200; and ACh, 200. No response was seen with a bath application of aspartate (10^{-4} M). In (D), the RMP of the cell was -52 mV and currents were (in nanocoulombs): aspartate, 400; glutamate, 400; and ACh, 200.

In Aplysia, the receptors activated by iontophoretic application of serotonin and acetylcholine appear to be identical to those existing at serotonergic and cholinergic synapses, respectively. Our results suggest that aspartate may also function as a neurotransmitter, since there are specific receptors for aspartate which can selectively change the membrane permeability of some neurons

to Na^+ or Cl^- (or both). Since these experiments strongly suggest that aspartate is a neurotransmitter, it must be considered when searching for effects on the nervous system by injurious agents such as drugs and radiation.

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BIOCHEMICAL PROPERTIES OF A β-BUNGAROTOXIN

Principal Investigators: *M. A. Donlon, G. S. Tobias, W. G. Shain, Jr. and G. N. Catravas*

This report describes a potent tool for studying calcium effected release that has been implicated in radiation- and drug-induced pathology.

The β-bungarotoxin most extensively studied has a molecular weight of 21,800 and is a major component of Bungarus multicinctus venom. We have investigated the biochemical properties of a minor component of the venom having a molecular weight of 11,000. The molecular weight was determined by three independent methods: sodium dodecyl sulfate polyacrylamide gel electrophoresis, analytical sedimentation velocity and amino acid analysis. The molecule was labeled with ^{125}I and the binding characteristics of the monoiodinated toxin were studied. Binding to synaptosomes and mitochondria was rapid, saturable and could be inhibited by preincubat[illegible] with unlabeled toxin. Binding was also inhibited by Ca^{++} in the incubation mixture. This β-bungarotoxin also shows Ca^{++}-dependent phospholipase A activity. These results suggest that β-bungarotoxin may act through two steps: first a specific binding (not Ca^{++}-requiring) and then a phospholipase A activity (Ca^{++}-requiring). Classically, phospholipase A is remarkably heat stable at pH below 5.9 but is inactivated by boiling at pH above 7. Phospholipase A activity of β-bungarotoxin is destroyed by heat treatment (100°C, 5 min) at pH 8.6 but is unaffected at pH 5.4. In addition, the lethality of β-bungarotoxin is abolished after high pH heat treatment (Table 22). These data suggest that the phospholipase activity is associated with lethality. However, bee venom phospholipase A has no lethality, suggesting that both presynaptic specificity of binding and associated phospholipase A activity are necessary for β-bungarotoxin toxicity. Finally, we have demonstrated a Ca^{++}-dependent inhibition of adenosine diphosphate-dependent oxygen consumption in crude mitochondrial preparations, when β-bungarotoxin's phospholipase A activity is inactivated by heat treatment at high pH. This suggests that this inhibition

may be unrelated to β-bungarotoxin toxicity. These observations suggest that β-bungarotoxin may be useful in studying altered release mechanisms occurring during stress and radiation injury.

Table 22. Summary Table of the Properties of a Presynaptic Neurotoxin, β-Bungarotoxin. The toxicity is dependent on the presence of phospholipase A (PLA) activity which is retained by boiling at low pH and destroyed at pH's above 7. The PLA activity requires Ca^{++} and ADP-dependent O_2 uptake can be inhibited in mitochondrial preparations without PLA activity in the presence of Ca^{++}.

PROPERTY	UNTREATED	BOILED	
		LOW pH	HIGH pH
TOXICITY	+	+	−
PLA ACTIVITY a) c̄ Ca^{++}	+	+	−
b) s̄ Ca^{++}	−	−	−
ADP-DEPENDENT O_2 UPTAKE a) c̄ Ca^{++}	+	+	+
b) s̄ Ca^{++}	+	+	−

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PURIFICATION AND BIOCHEMICAL CHARACTERIZATION OF A β-BUNGAROTOXIN

Principal Investigators: *G. S. Tobias, M. A. Donlon, W. G. Shain, Jr. and G. N. Catravas*

Specific proteins in snake venoms may be very valuable tools for the study of the nervous system. A chromatographic procedure has been described for the separation of the neurotoxic proteins in Bungarus multicinctus venom.[3] The most positively charged toxic protein isolated was examined for purity, molecular weight, amino acid composition and toxicity. Nine protein peaks were isolated from the crude venom by column chromatography on CM-Sephadex

C-25. Peak IX (β-IX), the most electropositive protein peak, ran as a single band on pH 4.3 and sodium dodecyl sulfate polyacrylamide gel electrophoresis. The molecular weight of β-IX was estimated to be 10,000 daltons by analytical sedimentation analysis. This value was consistent with the electrophoretic mobility of β-IX in sodium dodecyl sulfate polyacrylamide gels (Figure 53). The amino acid composition of β-IX was comparable to that of the major β-bungarotoxin peak (β-VII) previously reported,[1,2] suggesting that β-IX may be a subunit of the major toxin. β-IX was toxic to mice when injected intravenously. Animals which received lethal doses exhibited hyperexcitability followed by ataxia, convulsions and death. The minimum lethal dose was 0.12 μg/g body weight. β-Bungarotoxin acts presynaptically at the neuromuscular junction to inhibit transmitter release. β-IX exhibited a Ca^{++}-dependent phospholipase activity comparable to that of β-VII in a pH stat assay in which egg yolk lecithin served as the substrate. The enzyme activity was lost after β-IX was boiled for 5 minutes at pH 8.6. β-IX inhibited the muscle twitch evoked by nerve stimulation in an isolated nerve-muscle preparation but did not inhibit that evoked by bath-applied acetylcholine. Thus, β-IX, as β-VII, acts presynaptically to inhibit transmitter release. The investigation of the toxin and its action provides a model system for studying the effects of other forms of stress on neuronal activity.

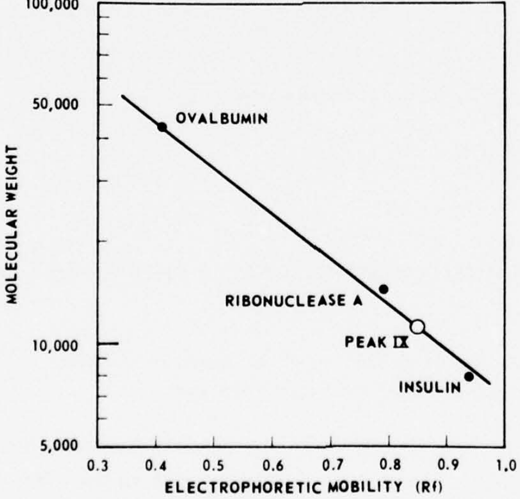

Figure 53. Comparison of molecular weight and electrophoretic mobility of known proteins and β-bungarotoxin (β-IX) in sodium dodecyl sulfate polyacrylamide gels. 20-30 μg of ovalbumin, insulin ribonuclease, and β-IX were run separately and in various combinations on sodium dodecyl sulfate polyacrylamide disc gels. The molecular weight of β-IX was estimated to be 10,000 daltons and this was confirmed by analytical sedimentation analysis and amino acid analysis.

The elucidation of the mechanism of action of β-IX can lead to an understanding of presynaptic events involved in the release of neurotransmitters. These Ca^{++} ion dependent events may be related to functional incapacitation due to radiation injury seen in early transient incapacitation following large doses of radiation.

REFERENCES

1. Kelly, R. B. and Brown, F. R. Biochemical and physiological properties of a purified snake venom neurotoxin which acts presynaptically. J. Neurobiol. 5:135-150, 1974.

2. Lee, C. Y., Chung, S. L., Kau, S. T. and Luh, S.-H. Chromatographic separation of the venom of Bungarus multicinctus and characterization of its components. J. Chromatogr. 72:71-82, 1972.

3. Tobias, G. S., Donlon, M. A., Shain, W. G., Jr. and Catravas, G. N. Purification and biochemical characterization of a β-bungarotoxin. Bethesda, Maryland, Armed Forces Radiobiology Research Institute Scientific Report SR76-18, 1976.

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ANALYSIS OF BINDING AND PHOSPHOLIPASE A PROPERTIES OF β-BUNGAROTOXIN

Principal Investigators: *G. S. Tobias, M. A. Donlon, W. G. Shain, Jr., AFRRI; and B. Marinetti, University of Rochester*

β-Bungarotoxin acts presynaptically to inhibit neuromuscular transmission. We have previously shown that β-bungarotoxin has the following two properties: (1) ^{125}I β-bungarotoxin binds to rat brain synaptosomes, and the binding is inhibited by Ca^{++}; and (2) the toxin has Ca^{++}-dependent phospholipase A activity. We have proposed that β-bungarotoxin exerts its effect on synaptic transmission through a sequence of events: first binding and then phospholipase A activity. We have been investigating several additional aspects of β-bungarotoxin's binding and phospholipase A properties. We have found that cations inhibit ^{125}I β-bungarotoxin binding to synaptosomes in the following order of inhibitory potency: $Ca^{++} > Ba^{++} > Sr^{++} >> Mg^{++} > K^{+} > Na^{+}$.

Binding is also decreased following pretreatment of synaptosomal membranes with trypsin or chymotrypsin. Pretreatment with neuramidinase, concanavalin A, or phospholipases C or D does not alter binding. However, phospholipase A activity from Vipera russelli venom is inhibitory while bee venom phospholipase A is not. Sr^{++} is 30 percent of that with Ca^{++}. Incubation of synaptosomal membranes (3 mg protein) with β-bungarotoxin (20 μg) for 15 min at 37°C results in a partial hydrolysis of phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine to their respective lyso-compounds and fatty acids. Using ^{14}C-glycerol labeled synaptosomes, rapid hydrolysis of phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine occurred during the first 10-15 min of incubation with the extent of hydrolysis being PE >PC >PS (Figure 54). The hydrolysis due to endogenous phospholipase activity and that contributed by β-bungarotoxin were significantly inhibited when Sr^{++} was substituted for Ca^{++} in the incubation medium. These results

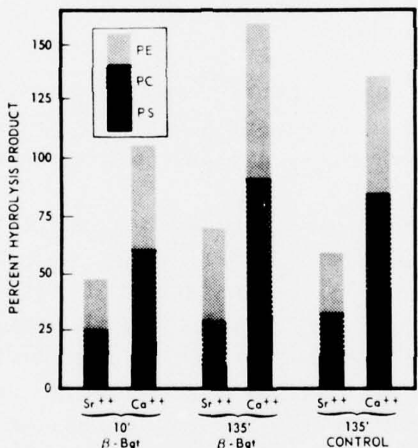

Figure 54. The effect of Ca^{++} and Sr^{++} on the hydrolysis of synaptosomal phospholipids by β-bungarotoxin. Rat brain synaptosomes, whose phospholipids had been prelabeled with ^{14}C by prior intraventricular injection of ^{14}C-glycerol, were incubated for 10 or 135 min in Krebs Ringer solution with 0.6 mM $CaCl_2$ for $SrCl_2$ with or without 20 μg β-bungarotoxin. Following incubation the phospholipids, phosphatidylethanolamine (PE), phosphatidylcholine (PC), and phosphatidylserine (PS), and their respective lyso-compounds were extracted, separated by two-dimensional thin-layer chromatography and assayed by liquid scintillation counter. The results are expressed as percent hydrolysis of specific lysophospholipid divided by the sum of specific phospholipid plus lysophospholipid times one hundred.

suggest that β-bungarotoxin binds to a proteinaceous site and rapidly hydrolyzes membrane phospholipids. An understanding of the biochemical events involved in transmitter release from the presynaptic terminal is an essential first step in the future clarification of radiation damage to the process of nerve transmission.

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β-BUNGAROTOXIN: RELATIONSHIP OF PHOSPHOLIPASE ACTIVITY TO TOXICITY

Principal Investigators *G. S. Tobias, M. A. Donlon, W. G. Shain, Jr. and G. N. Catravas*

Calcium dependent release of neuroeffector substances may be responsible for radiation-induced early transient incapacitation. We have been developing β-bungarotoxin to study the control of calcium release.

β-Bungarotoxin has been shown to inhibit presynaptic transmitter release.[1] Wagner et al.[2] and Wernicke et al.[3] have suggested that the neurophysiologic effect is mediated by changes in mitochondrial metabolism. Application of the toxin on a neuromuscular preparation causes an initial increased rate of acetylcholine release from the presynaptic terminal followed by a complete inhibition of release. We have reported that a different bungarotoxin (peak IX) labeled with ^{125}I binds rapidly and saturably to both rat brain synaptosomal and mitochondrial fractions, and that binding is inhibited by Ca^{++}. It thus appears that the toxin binding site is similar on both brain fractions. To resolve the interaction of bungarotoxins with this binding site on mitochondria, phospholipase A activity and oxygen consumption were assayed similar to Wernicke et al.[3] Minced rat cerebral cortex was incubated at 37°C in the presence and absence of toxin which was previously treated by boiling at high and low pH. Crude mitochondrial pellets were prepared from these minces, and oxygen consumption was measured using an oxygen electrode (Figure 55). Both the major bungarotoxin peak (VII) and peak IX exhibit Ca^{++}-dependent phospholipase A activity. The phospholipase A activity and the toxicity are abolished by boiling for 3 min at pH 8.6. Both peaks inhibit adenosine diphosphate-dependent oxygen consumption. However, peak IX does not require Ca^{++} for this effect. These results suggest that (1) peak IX differs from peak VII; i.e., peak IX does not require Ca^{++} for binding or for adenosine diphosphate-dependent mitochondrial oxygen consumption; (2) toxicity

is related to phospholipase A activity; and (3) the mode of action of peak IX is through two steps: first a specific binding (not Ca^{++} requiring) and then a phospholipase A activity (Ca^{++} requiring). The relationship between the mitochondrial inhibition of adenosine diphosphate-dependent oxygen consumption and the neuromuscular blockade remains to be determined.

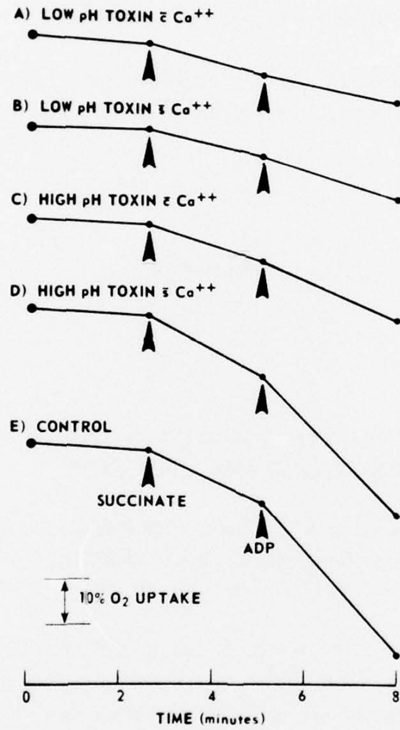

Figure 55. Succinate-dependent oxygen uptake by brain mitochondria. Tracings of oxygen consumption are shown for the various toxin and Ca^{++} additions. The arrows mark the succinate at 14 mM or ADP at 1.75 mM.

This study has described a second mode of function of a potential tool for studying radiation-induced calcium-requiring phenomena. However, the enzymatic activity of the toxin can be destroyed, preserving its effects on calcium flux. Thus the inactivated toxin may be a potent tool in understanding the mechanism(s) of histamine release during early transient incapacitation.

REFERENCES

1. Chang, C. C., Chen, T. F. and Lee C. Y. Studies of the presynaptic effect of β-bungarotoxin on neuromuscular transmission. J. Pharmacol. Exp. Ther. 184:339-345, 1973.

2. Wagner, G. M., Mart, P. E. and Kelly, R. B. Beta-bungarotoxin inhibition of calcium accumulation by rat brain mitochondria. Biochem. Biophys. Res. Commun. 58:475-481, 1974.

3. Wernicke, J. F., Vanker, A. D. and Howard, B. D. The mechanism of action of β-bungarotoxin. J. Neurochem. 25:483-496, 1975.

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β-BUNGAROTOXIN: THE RELATIONSHIP OF PHOSPHOLIPASE A ACTIVITY TO NEUROMUSCULAR TRANSMISSION

Principal Investigators: *D. R. Livengood, M. A. Donlon, AFRRI; R. S. Manalis, University of Cincinnati College of Medicine; G. S. Tobias and W. G. Shain, Jr., AFRRI*

At least two different species of β-bungarotoxin have been isolated and studied.[1-3] We are proposing that the toxin may function by first binding to a proteinaceous moiety of the presynaptic membrane and then by phospholipase A enzymatic activity. Subthreshold end-plate potentials were recorded in a frog neuromuscular preparation in 0.36 mM Ca^{++}, 2.9 mM Mg^{++} saline. End-plate potential amplitude and membrane potential of muscle fibers were analyzed during treatment with purified β-bungarotoxin or toxin treated by boiling at pH 8.6 to destroy phospholipase A activity. Purified enzymatically active β-bungarotoxin (0.2-2.0 μg/ml) causes a rapid decay in 5-10 min in end-plate potential amplitude. This frequently was followed by a rebound of the end-plate potential to levels in excess of control. This is in turn followed by a second decrease to zero (100-150 min). The toxin blocks the end-plate potential but not iontophoretically applied acetylcholine in a curarized preparation. Enzymatically inactivated β-bungarotoxin (0.05-2.0 mg/ml) also causes a rapid decrease in amplitude of the end-plate potentials. No rebound of end-plate potential activity has been seen with inactivated toxin. Loss of end-plate potential amplitude with inactivated β-bungarotoxin appears to be

dose dependent. We suggest that a molecule of β-bungarotoxin has two functional components: (1) a phospholipase A active site and (2) a site that binds to the presynaptic membrane, probably at the Ca^{++} binding site and thereby blocks transmission. The effect of β-bungarotoxin on frog sartorius muscle twitch response is shown in Figure 56.

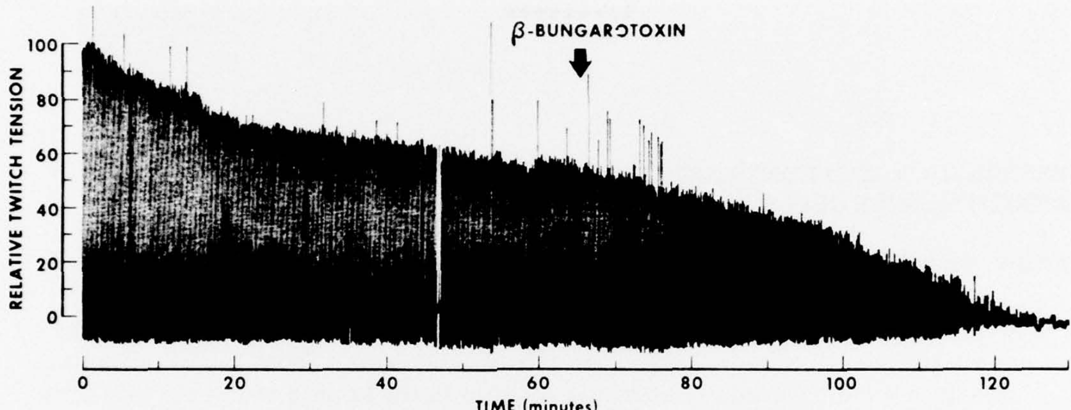

Figure 56. Effect of β-bungarotoxin on the frog sartorius muscle twitch response. The frog sciatic nerve was stimulated at 10 min^{-1} and the isometric twitch tension of the sartorius muscle was monitored with a strain gauge. After the preparation had maintained a steady-state twitch response for 30 min (at t = 60 min), β-bungarotoxin was added to the bathing Ringer's solution to a concentration of 1 μg/ml.

The mechanism of radiation damage induced incapacitation of animals may be related to transient alteration of neurosecretory processes. This study examines the electrophysiological aspects of neuromuscular blockade using a presynaptic toxin to probe the biophysical events occurring at this radiation sensitive site.

REFERENCES

1. Donlon, M. A., Tobias, G. S., Shain, W. G. and Catravas, G. N. Binding properties of a pre-synaptic bungarotoxin to rat brain homogenates. In: Neuroscience Abstracts, 5th Annual Meeting of the Society for Neuroscience, New York City, November 2-6, 1975, Vol. 1, Abstract 119. Bethesda, Maryland, Society for Neuroscience, 1975.

2. Kelly, R. B. and Brown, F. R. Biochemical and physiological properties of a purified snake venom neurotoxin which acts presynaptically. J. Neurobiol. 5:135-150, 1974.

3. Wernicke, J. F., Oberjat, T. and Howard, B. D. β-neurotoxin reduces neurotransmitter storage in brain synapses. J. Neurochem. 22:781-788, 1974.

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DISSOCIATION AND CONTINUOUS CELL CULTURE OF HUMAN SMOOTH MUSCLE CELLS

Principal Investigators: *E. S. Chock and W. G. Shain, Jr.*

Many studies require a reproducible preparation of smooth muscle cells, but such a preparation is difficult or impossible to obtain from intact animals. We have attempted to obtain continuous lines of cells in culture. Cell cultures of smooth muscle have been established from human oviduct by enzymatic dissociation. Repeated treatments of minced tissue in Ca^{2+}, Mg^{2+} free Hanks' saline containing collagenase (20 units/ml) and elastase (10 units/ml) resulted in single cell suspensions. Cells from the later dissociation treatments were most homogeneous with respect to smooth muscle morphology. Cells have been maintained in continuous culture for at least 1 year and have a doubling time of 5.5 days. By light microscopy the muscle cells were observed to have a prominent nucleus and to be flat and of irregular shape. The cell shape appears to be determined by the displacement of prominent bundles of filaments. Cultures were fixed for thin section and surface replica electron microscopy in phosphate-buffered glutaraldehyde and osmium tetroxide. Filament bundles containing numerous dense bodies coursed through the cell soma and large cytoplasmic extensions terminating on the inner surface of the extracellular membrane. Small vesicles were frequently associated with points of filament termination. The replicas suggest that the surface membrane is smooth except in those areas where caveolae are arranged in longitudinal rows. Replicate cell cultures were analyzed for stimulation of intracellular concentrations of cyclic adenosine monophosphate after 15-min incubations with dopamine, noradrenaline, isoproterenol, carbamylcholine (all 10^{-4} M), and prostaglandin E_1 (1.67 μg/ml). Only prostaglandin E_1 treatment showed significant changes in cyclic adenosine monophosphate concentrations, stimulating basal levels (35 pmoles/mg protein) to 647 pmoles/mg protein. A method for the rapid dissociation and establishment of continuous cell cultures exhibiting a smooth muscle phenotype has been developed.

The establishment of these cell lines allows the possibility of adapting the procedure to compare sensitivity of various primate smooth muscles to direct

effects of radiation and to sensitivity to histamine, which is thought to be the causative agent in early transient incapacitation. This technique, because it allows direct comparison of human and nonhuman cell lines, will allow a direct extrapolation of radiosensitivity data to man.

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ELECTROPHYSIOLOGICAL PROPERTIES OF SMOOTH MUSCLE IN DISSOCIATED CELL CULTURE

Principal Investigators: *C. N. Sinback and W. G. Shain, Jr.*

In order to compare radiation effects on a number of different species, including man, homogeneous smooth cultures have been developed. Human oviduct smooth muscle cells grown for as long as 1-1/2 years in vitro express electrophysiological properties of smooth muscle in vivo. Single isolated cells and cells in contact with neighboring cells were impaled with one to two microelectrodes. The mean resting potential was 42 ± 9 mV (n = 87). Input resistances were calculated from electrotonic potentials due to current injected via microelectrodes. In 10 isolated single cells the input resistance was 54 ± 28 MΩ. Using photographs to calculate cell surface area, the specific resistance was 41 ± 8 $k\Omega\text{-cm}^2$. The time constant of the electrotonic potential was 96 ± 31 msec. These values agree with predictions of single cell properties. The input resistance of cells in contact with neighboring cells was 18 ± 14 MΩ (n = 72). Current passed into either of two connected cells always elicited electrotonic potentials in both cells (n = 10). Thus, current spread across cell contacts accounted for lower input resistance of connected cells. In a subpopulation of cells, active depolarizing potentials were elicited by depolarization to a threshold potential or by release from hyperpolarization. Active response amplitude and duration at half-amplitude were 10 mV and 200 msec, respectively. Although spontaneous active responses were not seen, pacemaker potentials were elicited by 10 mM $BaCl_2$. Muscle excitability depended on calcium since 2 mM EGTA or 10 mM $CoCl_2$ abolished active responses. This report describes the normal electrophysiology of human smooth muscle cells in culture. This information will serve as base-line information for the study of neuroeffectors important in early transient incapacitation, e.g., histamine, and direct effects of radiation on smooth muscle metabolism.

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PROSTAGLANDIN E_1 STIMULATION OF CYCLIC ADENOSINE MONOPHOSPHATE LEVELS: INHIBITION BY PUTATIVE NEUROTRANSMITTERS IN A NEURONAL SOMATIC CELL HYBRID

Principal Investigators: *J. C. Blosser, P. R. Myers and W. G. Shain, Jr.*

Prostaglandins and cyclic adenosine monophosphate have been reported to control or modulate a number of neurotransmitter effects in the central nervous system. We have used a model cell culture system to study these effects. A somatic cell hybrid (TCX17), a subclone of the embryonic mouse sympathetic ganglion cell X neuroblastoma (N18TG2) cell line NX31, was tested for the ability of neurotransmitter substances to antagonize prostaglandin E_1 stimulation of cyclic adenosine monophosphate levels. In the presence of phosphodiesterase inhibitor Ro20-1724 (10^{-4} M), cells incubated for 3 min in the presence of 10^{-8} M prostaglandin E_1 exhibited a tenfold increase in cyclic adenosine monophosphate levels. The prostaglandin E_1 stimulation as well as basal levels of cyclic adenosine monophosphate could be inhibited by coincubation with either 10^{-5} M carbachol, 10^{-5} M norepinephrine, or 10^{-5} M dopamine (Table 23). In contrast, both serotonin and morphine were ineffective in altering either basal or prostaglandin E_1-stimulated increases in cyclic adenosine monophosphate at 10^{-5} M, the latter despite the presence of opiate receptors in this cell line.[1] The carbachol inhibition could be reversed by preincubation with 10^{-8} M of either atropine or scopolamine but not by 10^{-6} M of α-bungarotoxin or d-tubocurarine, suggesting the presence of muscarinic receptors. Phentolamine and phenoxybenzamine but not dichloroisoproterenol could reverse norepinephrine inhibition.

Table 23. Effect of Neurotransmitters on PGE_1 Stimulation of Cyclic Adenosine Monophosphate (cAMP) Levels in the TCX11 Hybrid Clone

Additions	cAMP (pmoles/mg protein)
Basal	78.8 ± 8.4
PGE_1 10^{-8} M	253 ± 32
PGE_1 + Dopamine 10^{-5} M	122 ± 10
PGE_1 + Norepinephrine 10^{-5} M	113 ± 23
PGE_1 + Carbachol 10^{-5} M	133 ± 34
PGE_1 + Serotonin 10^{-5} M	313 ± 22

Drugs were incubated with cells as described. Results are expressed as ± S.D. for three separate plates of cells.

In addition, isoproterenol (10^{-5} M) could not mimic the norepinephrine inhibition, consistent with an adrenergic receptor. Chlorpromazine, Stelazine and bulbocapnine (all 10^{-6} M) blocked the dopamine inhibition suggesting the presence of a dopamine receptor. Electrophysiologically, dopamine elicits a depolarizing response in TCX17.[2] Thus the possibility exists that a neurotransmitter which elicits a conductance change can also modulate prostaglandin E_1 alterations in cyclic adenosine monophosphate levels in this cell line.

This report continues the description of a model system for neuron-drug interactions. The report is of significant importance in describing the separation of opiates and cyclic nucleotides, and demonstrates the value of such cell lines as models for studying drug interactions in a simple control system.

REFERENCES

1. Blosser, J. C., Abbott, J. R. and Shain, W. Sympathetic ganglion x neuroblastoma somatic cell hybrids with opiate receptor activity. Fed. Proc. 34(3):713 (Abstract 2797), 1975.

2. Myers, P. R., Livengood, D. and Shain, W. Pharmacological specificity and the effects of d-tubocurarine on a depolarizing dopamine response. In: Neuroscience Abstracts, 5th Annual Meeting of the Society for Neuroscience, New York City, November 2-6, 1975, Vol. 1, p. 641. Bethesda, Maryland, Society for Neuroscience, 1975.

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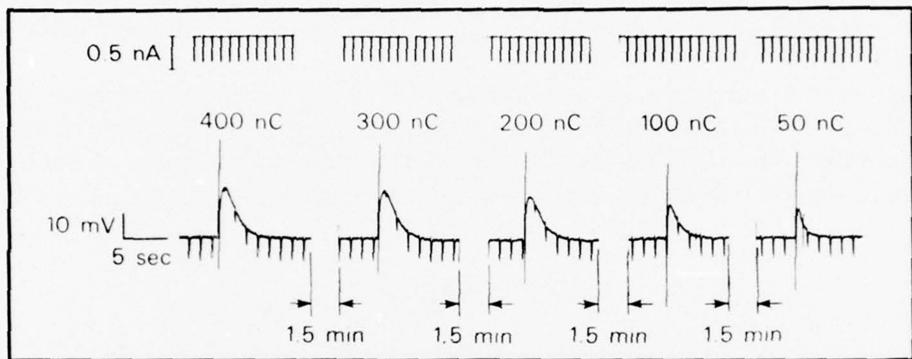
EVIDENCE FOR A DOPAMINE RECEPTOR ANTIBODY

Principal Investigators: *P. R. Myers, M. A. Donlon, K. F. McCarthy, M. J. McCreery, D. R. Livengood and W. G. Shain, Jr.*

Subtle changes in cell surfaces caused by irradiation can be monitored using antisera produced against normal cell membranes. We have used a specific cell culture line to provide such a tool.

Antiserum to the somatic cell hybrid TCX11 was produced to obtain an antibody to the dopamine receptor found on these cells. TCX11 is a subclone

of NX31 (a neuroblastoma X embryonic mouse sympathetic ganglion cell hybrid). Intracellular recordings from TCX11 show a depolarizing, conductance increase response upon iontophoretic application of dopamine (Figure 57). The response is specific in that dopamine antagonists block the response, agonists mimic dopamine, and cholinergic antagonists are without effect.



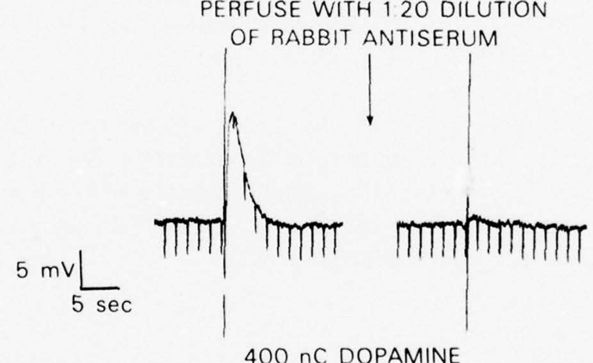


Figure 57. Upper panel. Depolarizing, conductance increase response elicited by dopamine in TCX11. Upper trace shows current pulses passed across membrane to test membrane resistance. Lower trace indicates dose dependency of the response amplitude to decreasing iontophoretic charge (representing decreasing quantitites of dopamine). Lower panel. Blockade of the dopamine response by decomplemented antiserum. Blockade was reversible upon perfusion with medium free of antiserum. Control serum did not antagonize the response.

Cells were grown in dibutyryl cyclic adenosine monophosphate to induce differentiation, harvested, washed, concentrated and injected into rabbit footpads (10-14 x 10^6 cells). After a period of repeated small injections, serum was collected and decomplemented. Bath applications of antiserum showed a dose-dependent inhibition of the dopamine response while control serum was

without effect. An immunoglobulin peak which inhibited the response was obtained after Sephadex G200 gel filtration or DEAE chromatography. To ascertain antibody specificity, dopamine receptor binding in rat caudate was examined by the method of Creese et al.[2] The antiserum inhibited dopamine binding while control serum had no effect. Apomorphine inhibited dopamine binding 50-60 percent while chlorpromazine inhibited 20-30 percent relative to controls. The antiserum at a dilution of 1:50 would inhibit binding 40-50 percent. Similarly, the antiserum at 1:40 dilution inhibited the dopamine-sensitive adenyl cyclase described by Clement-Cormier et al.[1] Preliminary experiments show selective blockade by the antiserum of dopamine responses in Aplysia, but not acetylcholine responses. Collectively, these results suggest the presence of an antibody which effects dopamine receptor activity.

Radiation effects on plasma membranes have been studied in a general way by observing sulfhydryl modifications of surface proteins. However, knowledge of specific protein modifications due to radiation injury is limited due to a lack of substances which uniquely interact with only one protein. The antibody used in this study provides a unique tool for dissection of radiation effects on a single functional protein within the cell membrane.

REFERENCES

1. Clement-Cormier, Y. C., Parrish, R. G., Petzold, G. L., Kebabian, J. and Greengard, P. Characterization of a dopamine-sensitive adenylate cyclase in the rat caudate nucleus. J. Neurochem. 25:143-149, 1975.

2. Creese, I., Burt, D. R. and Snyder, S. H. Dopamine receptor binding: differentiation of agonist and antagonist states with 3H-dopamine and 3H-haloperidol. Life Sci. 17:993-1001, 1975.

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TRANSPORT OF GAMMA-AMINOBUTYRIC ACID BY GLIAL CELLS IN CULTURE

Principal Investigators: *D. L. Martin, D. A. Brown and W. G. Shain, Jr.*

Gamma-aminobutyric acid is usually considered to be a neurotransmitter, but may have other roles in other cells. In this study, gamma-aminobutyric

acid transport was studied in cultured glial cells obtained by dissociation of fetal or neonatal rat superior cervical ganglia and in a morphologically similar rat glioma cell line obtained from a spinal tumor (1056A cells) in order to confirm observations on glial transport previously made with intact superior cervical ganglia. The rate of uptake in Hepes buffered Hanks' balanced salt solution was nearly constant for over 2 hours at 37°C for all gamma-aminobutyric acid concentrations studied. Kinetic analysis of uptake in 1056A cells revealed two components with apparent Michaelis constants of approximately 0.1 μM and 150 μM and a third component with an apparent K_m greater than 600 μM. Uptake of 0.025 μM gamma-aminobutyric acid by 1056A cells was about 46 percent of control when NaCl was replaced with either choline chloride or sucrose whereas uptake of 10 μM and 1 mM gamma-aminobutyric acid was not strongly sodium dependent. Ganglion glia also possessed a high affinity uptake system ($K_m \simeq 0.1$ μM). Uptake of 0.025 μM gamma-aminobutyric acid by both ganglion glia and 1056A cells was much more strongly inhibited by β-alanine than by L-2,4-diaminobutyric acid (Figure 58). Representative α-amino acids (alanine, glycine, histamine and leucine) were found to be extremely poor inhibitors of this uptake. The inhibitory potency of β-alanine relative to diaminobutyric acid was increased at 150 μM gamma-aminobutyric acid. At this gamma-aminobutyric acid concentration, the IC50 for β-alanine was less than 75 μM but 1.5 mM diaminobutyric acid inhibited by only 22 percent. The present results on substrate specificity and sodium dependency of the high affinity uptake system are similar to those obtained previously with intact superior cervical ganglia. Since gamma-aminobutyric acid is known to

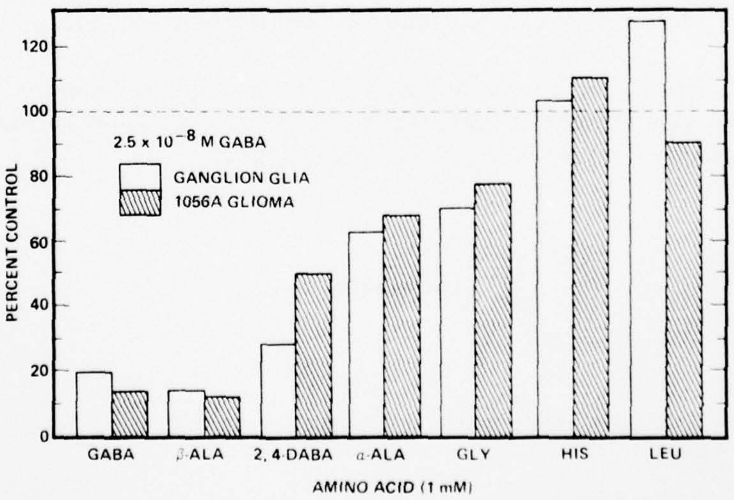

Figure 58. Effects of amino acids on the uptake of ^{3}H gamma-aminobutyric acid by superior cervical ganglion glia and 1056A glioma cells in culture

be the principal inhibitory neurotransmitter in the nervous system, and is involved in such human diseases as epilepsy and some movement disorders, this gamma-aminobutyric acid transport system may have a direct involvement in human disease states.

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