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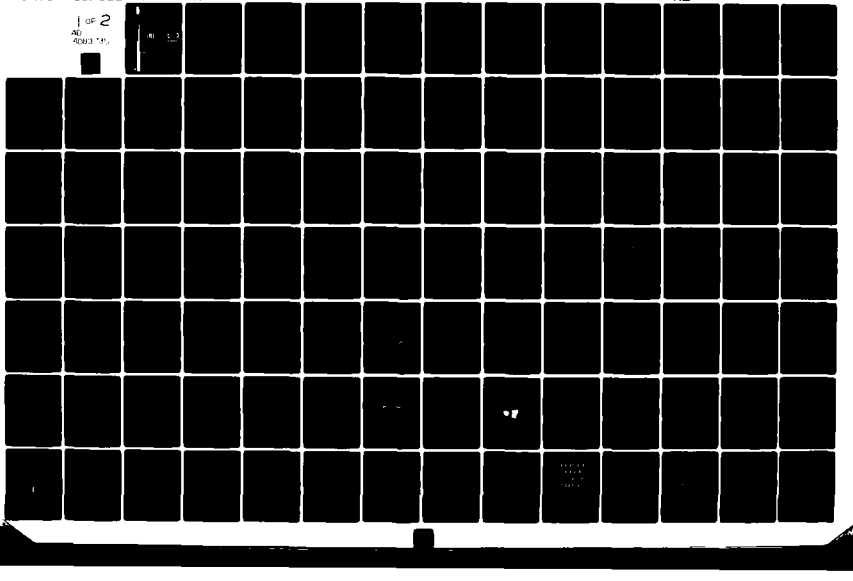

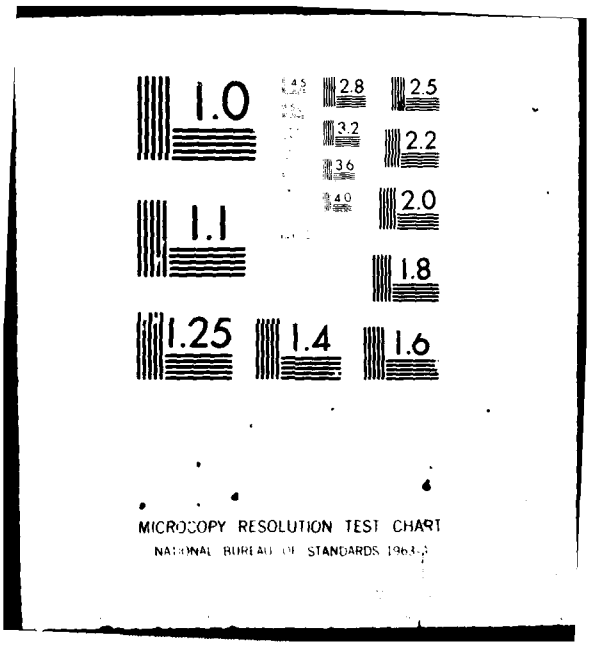
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ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE

LEVEL II

ANNUAL RESEARCH REPORT

1 JULY 1976 – 30 SEPTEMBER 1977

Defense Nuclear Agency
Bethesda, Maryland 20014

Research was conducted according to the principles enunciated in the "Guide for the Care and Use of Laboratory Animals," prepared by the Institute of Laboratory Animals Resources, National Research Council.

Studies involving human patients were performed in conformity with the "recommendations guiding doctors in clinical research" as stated in the Declaration of Helsinki of the World Health Medical Association (1964).

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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 1976 to 30 September 1977.

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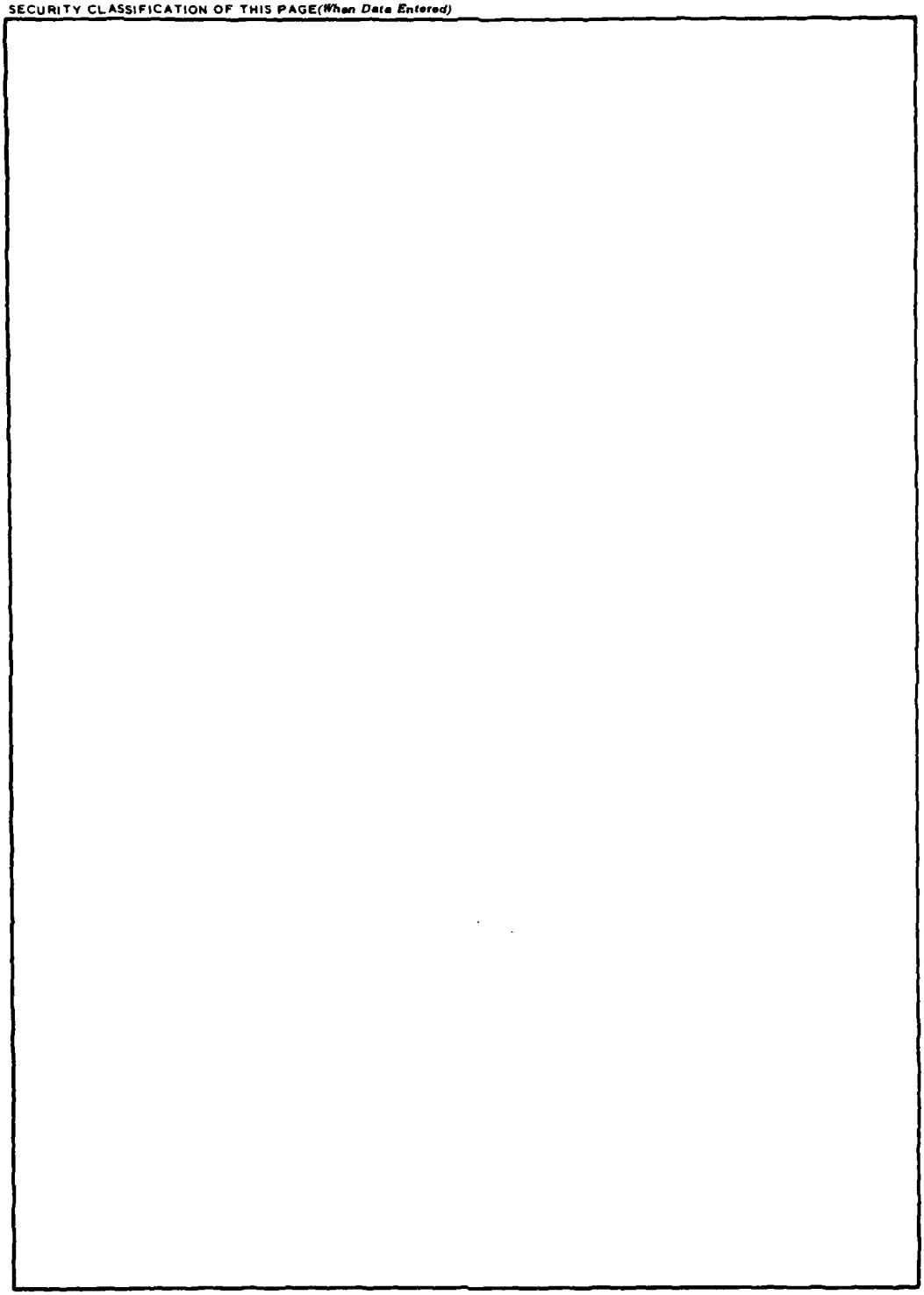

CONTENTS

BEHAVIORAL SCIENCES DEPARTMENT

The Behavioral Sciences Department performs research to determine the acute effects of radiation, chemicals, and drugs on the behavior, performance, psychoneurological integrity, and physiology of experimental animals for extrapolation of these data to man. Research projects in support of this effort are conducted in the three Divisions of the department: the Experimental Psychology Division, Physiological Psychology Division, and Experimental Neurology Division. Collaborative efforts are also under way with other departments of the Institute as well as with the Naval Medical Research Institute, the National Institutes of Health, and several universities in the nearby area.

Primary research efforts of the Department have been through the use of animal models in order to assess the capability of man to function in a tactical nuclear weapons radiation environment, to perform complex mental tasks and/or physical tasks, to determine the degree of degradation of performance as a function of task complexity and radiation exposure, to determine the bioelectric and biochemical changes that occur postirradiation in the central nervous system, and to develop data on possible methods to prevent or modify performance decrements caused by high-dose radiation exposure.

Behavioral toxicology studies have also been developed and evaluated to detect changes in the electroneurophysiological and performance capability in primates and rodents. These unique tests are providing information for establishing maximum permissible occupational levels for industrial and military environments, and they have been developed into models for use in radiation-injury studies involving subtle changes in behavior.

Results of the Department's research programs are forwarded to the military services and appropriate agencies by informal reports, incorporation into committee and working-group reports, discussion, and correspondence. They are also made available to the scientific community through publications and oral presentations at scientific meetings.

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BEHAVIORAL INCAPACITATION AS A FUNCTION OF A PULSED, WHOLE-BODY, MIXED-FISSION-SPECTRUM, HIGH-NEUTRON RADIATION DOSE

Principal Investigator: R. W. Young
Technical Assistance: J. R. Harrison, P. Mannon, and C. A. Boward

In order for military planners to produce meaningful combat-casualty criteria, it is necessary to know the relationship between the degree of effect on performance and the radiation dose for a full range of doses in a given type of radiation field. This study attempts to provide that information by defining the dose-response function for behavioral incapacitation in the monkey for a mixed-fission-spectrum, high-neutron radiation field (neutron/gamma ratio of 3.0).

Fifty male rhesus *(Macaca mulatta)* monkeys, trained to perform a paired, shock-avoidance, visual discrimination task, were irradiated with single whole-body doses of pulsed radiations from the AFRRI TRIGA reactor. The radiation field was moderated with 2" of lead in order to produce an incident radiation field having a neutron-to-gamma ratio of 3.0. Ten subjects were irradiated at each of five dose levels. The mean midline tissue doses obtained were 1606, 2037, 2767, 3600, and 4566 rads, respectively, for the five groups.

Considering 1 min or more of not performing the task as the criterion for behavioral incapacitation, the data indicate that 20% of the animals were incapacitated at 1060 rads, 40% were incapacitated at 2037 rads, 50% at both 2767 and 3600 rads, and 80% at 4566 rads. These data were treated using the Finney method of probit analysis in order to produce a dose-response function and 95% confidence limits for behavioral incapacitation as a function of dose. The results of that analysis are presented in Figure 1. This analysis indicates that the median effective dose for producing behavioral incapacitation is 2838 rads for this radiation field.

In addition to performance, the occurrence of emesis and the survival time after irradiation were recorded for each subject. Twenty-four of the 50 monkeys vomited after exposure, with the incidence of vomiting decreasing as dose increased. Of the 24 animals that vomited, only 3 exhibited behavioral incapacitation. Mean survival times were 93 hours at 1606 rads, 54 hours at 2037 rads, 17 hours at 2767 rads, 14 hours at 3600 rads, and 13 hours at 4566 rads.

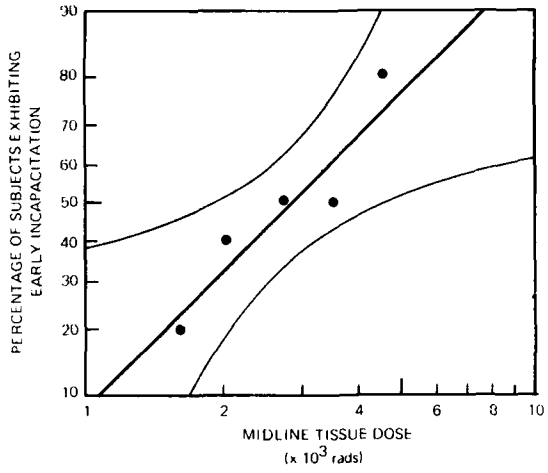

Figure 1. Percent early behavioral incapacitation as a function of dose ($n/\gamma = 3$)

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INCIDENCE OF RADIATION-INDUCED INCAPACITATION AND DEGREE OF PERFORMANCE DEGRADATION AS A FUNCTION OF TASK

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

Physical conditioning and type of behavioral task have both been identified as possible significant variables in determining the effect of ionizing radiations on postirradiation performance, incapacitation, and survival time. Previous work at this laboratory has left unclear the relationship of these variables. During the first study of these variables, unfettered monkeys (irradiated with 4600 rads) performing a physical activity task

had longer postexposure survival times than chair-restrained monkeys performing a cognitive task. This finding was consistent regardless of whether the chair-restrained monkeys had or had not been physically conditioned before irradiation, thus discounting physical conditioning as the explanation for the difference. Despite this postirradiation, task-related difference in survival time, no significant differences in performance or incapacitation were observed. This lack of difference in postirradiation performance as a function of task is inconsistent with previous findings in which clear differences in physical and cognitive performances were observed in monkeys irradiated with 2000 rads in a different radiation field. Taken together, these findings suggest that a dose of 4600 rads may be of sufficient magnitude to incapacitate subjects for any meaningful behavior, physical or cognitive, and thus mask any differences that may exist for the effects of radiations on these types of performance. The study reported here was conducted to test this hypothesis by comparing physical and cognitive behavior after a lower radiation dose.

Twenty monkeys were trained to either one of two behavioral tasks and then irradiated with a pulsed, whole-body dose of 2050 rads (midline tissue dose) of ionizing radiations (n/γ = 3.0). Ten animals were chaired and trained to a simultaneous visual discrimination task. The other ten subjects were trained to operate a physical activity wheel as a nonmotorized treadmill.

The postirradiation data are summarized in Table 1. At 2050 rads, physical performance was much more affected than was performance of the visual discrimination task. Both the number of incapacitations and the extent of the radiation effect on the physical activity task was much greater than the effects on visual discrimination performance. Despite this greater behavioral effect on the physical activity subjects, the survival time for these animals was significantly longer ($p = 0.05$, Mann-Whitney U-test) than that observed for the chaired monkeys.

To date, four findings summarize the observations of these comparison studies. First, there appears to be a point beyond which the magnitude of the radiation dose overwhelms any differences in postirradiation task performance that may exist. Second, there are whole-body radiation doses at which the performance of a physical activity task is much more significantly affected than cognitive performance. Third, either physical activity, after irradiation, increases survival time or restraint after irradiation foreshortens survival. Fourth, the mechanisms underlying behavioral changes after irradiation are fundamentally different from those that produce death.

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Table 1. Radiation-Induced Performance Incapacitation, Degradation, and Survival Time as a Function of Task After Exposure to Midline Tissue Dose of 2050 Rads

Task	Percent of Group Incapacitated (n = 10)	Percent of Test Period Incapacitated	Percent of Base-line Performance (Mean) When Not Incapacitated	Time of Onset (Mean) When Not Incapacitated (in Minutes)	Duration (Mean) of Incapacitation (in Minutes)	Survival Time (Mean) (in Hours)
Physical Activity	90	21	77	22	25	110
Visual Dis-crimination	40	1	99	7	16	54

THE ACCELEROD: A METHODOLOGICALLY MORE SENSITIVE TECHNIQUE FOR ASSESSING RODENT INTEGRATED MOTOR FUNCTION

Principal Investigators: V. Bogo and R. W. Young, *AFRRI,*
T. Hill, *Naval Medical Research Institute*
Technical Assistance: G. G. Kessell, W. A. Hunt, and T. K. Dalton

Use of an accelerod (a gradually accelerating rotarod) to evaluate neuromuscular intoxicants in rats suggests that toxicity can be demonstrated at lower dose concentrations than previously reported with the conventional rotarod. The rotarod is a test of motor coordination in which a trained subject maintains its balance by walking on a suspended rod that is revolving in one direction at a constant speed for a specified time (1). Subtle decrements in performance cannot be assessed with the pass/fail scoring criterion of this procedure, and measurement of improved performance is possible only by testing fatigue limits. The accelerod was designed to eliminate these methodological limitations by measuring either impaired or improved ability as a continuous variable. This is achieved by a trained subject's maintaining its balance on a rotating rod that gradually accelerates from 1 mph until the subject is no longer able to maintain balance.

A series of experiments has been undertaken to compare the sensitivity of the rotarod and accelerod in detecting signs of motor intoxication. Two toxic substances have been tested: acrylamide (a progressive, peripheral neurotoxin) and ethanol (a short-term depressant of the central nervous system). The results from the acrylamide study presented in Figure 1 illustrate that significant performance-related toxicity first occurred 2 days after initial dosing in the 50-mg/kg group and 8 days after initial dosing in the 25-mg/kg group. This compares to 6 and 17 days, respectively, for the first evidence of toxicity reported with use of the rotarod (1).

In the acute study of ethanol intoxication, the onset of motor incoordination was about the same for both devices; however, rats tested on the accelerod showed significant loss of motor competence longer than animals tested on the rotarod. In light of the increased sensitivity of this technique for detecting toxically induced motor impairment, it is currently being evaluated with a central nervous system stimulant, d-amphetamine, to see if it is equally sensitive in quantifying motor facilitation.

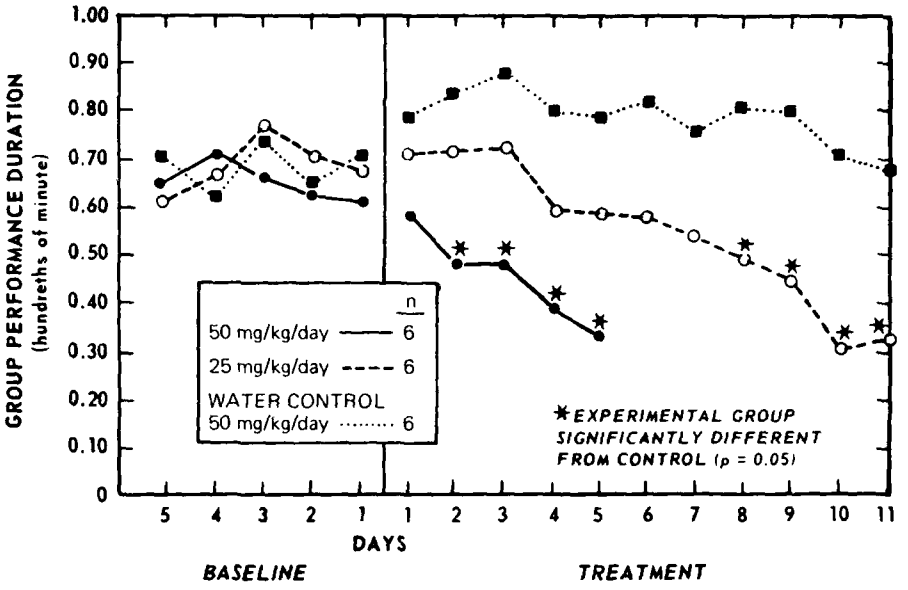

Figure 1. Accelerod performance as a function of two levels of daily acrylamide dosing

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EFFECTS OF PETROLEUM-DERIVED DIESEL FUEL MARINE ON THE BEHAVIOR OF RATS

Principal Investigators: V. Bogo and R. W. Young, *AFRRI*
Collaborator: T. Hill, *Naval Medical Research Institute*
Technical Assistance: G. G. Kessell

In order to assure future supplies of diesel fuel marine (DFM), the U.S. Navy is actively assessing alternatives to petroleum-derived DFM. As part of a current project to demonstrate the feasibility of extracting and refining DFM from shale, the Navy is evaluating the relative toxicity of shale- and petroleum-derived DFM. Since no baseline data exist for petroleum DFM, the first phase of the evaluation is devoted to obtaining this toxicity information.

The initial studies were lethality investigations that sought to set upper limits for petroleum DFM toxicity on orally dosed rats. These studies concentrated on survival, necropsy, and histopathological findings, as well as observations on general condition, spontaneous behavior, home-cage activity, food intake, water consumption, and weight. In these range-finding studies, the animals were dosed at 0.2-log intervals, with no dose exceeding 5% of an animal's body weight. The subjects were given either single doses of 3, 5, 12, 19, 30, or 48 ml/kg or up to five daily doses of 4, 6, 10, 16, 26, or 42 ml/kg/day, and were followed for 14 days. All animals that received a total dose of 30 ml/kg or more at a rate of at least 15 ml/kg/day were severely affected (e.g., depressed weight, reduced food and water intake, extensive hair oiliness and matting). Seventy percent of these animals died within 3 to 6 days after exposure. Necropsy and histopathology indicated substantial lung and intestinal involvement. At the lower cumulative doses of 12-30 ml/kg in which the dose rate was at least 10 ml/kg/day, erythema, lesions, and wrinkled skin as well as alopecia were observed, but there were no deaths. At the lowest dose rate of 4 or 6 ml/kg/day, all effects decreased in a dose-dependent manner. As reflected in all measures, the oral ingestion of a single or multiple dose of DFM produced adverse effects, especially at the higher levels. It appears that a cumulative oral dose of 45 ml/kg is an approximate threshold dose for lethality in the rat. However, since 45 ml/kg is approximately 5% of the body weight of the animals, this suggests that petroleum-derived DFM is not grossly toxic.

Because of depression of home-cage activity, observations of ataxia, and sensitivity to handling, a more comprehensive follow-on study was initiated in which specific assessment of motor function and sensory sensitivity was added to the previous battery of measurements. In that study, animals dosed with 3 or 5 ml/kg of DFM could not be distinguished from untreated and water controls on a complex motor coordination test and a test of sensory sensitivity. However, there was an overall decrease in general activity for up to 1 week after dosing, suggesting an overall depression rather than a specific neurotoxic effect. Since all studies to date have demonstrated lung involvement, and inhalation is the primary route of occupational exposure, future emphasis will be placed on subchronic inhalation studies of behavioral effects.

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CHEMICAL NEUROTOXICITY IN NEONATAL SWINE

Principal Investigators: R. M. Cartledge and R. W. Young
Technical Assistance: W. F. Fry, C. A. Boward, and E. A. Ketterer

It has been suggested that chemical neurotoxins, such as hexachlorophene and acrylamide monomer, may affect the neonatal nervous system more severely than the adult nervous system. Hexachlorophene is an antibacterial agent that has been shown to produce vacuolar encephalopathy of the brain stem reticular activation system. Acrylamide monomer, a widely used vinyl monomer in the production of high molecular polymers, has been shown to produce a potent neurotoxin that causes axonal degeneration. Since hexachlorophene and acrylamide are chemicals to which neonates can be exposed, the purpose of this study is to evaluate their effects on neonatal swine.

Three litters of miniature swine were used. In each litter of six pigs, three pigs were treated with a chemical and three served as water-dosed controls. The first litter was given 6 mg/kg/day of hexachlorophene. The second and third litters were given acrylamide at 10 mg/kg/day and 15 mg/kg/day, respectively. The chemicals were administered orally once daily for 8 weeks, beginning when the pigs were 3 days old. The weight, cage activity, and sensitivity to shock of all animals were measured daily. Visual-evoked responses were collected twice weekly from the hexachlorophene group and the 10-mg/kg acrylamide group, once dosing began. These responses were averaged after 16 stimulations (once every 10 sec) with a Grass PS22 photostimulator at a maximum flash intensity. Somatosensory-evoked responses were collected every other weekday from the 15-mg/kg acrylamide group. These responses were averaged after 64 stimulations (once every 2 sec) with an Ortec dual-channel stimulator.

There were no significant differences in the weight, cage activity, or shock sensitivity between the control animals and the animals in either chemical group. The visual-evoked responses of the 10-mg/kg acrylamide group were also not significantly different. However, initial analysis indicates that there were significant differences in the visual-evoked responses for the hexachlorophene group and in the somatosensory-evoked responses for the 15-mg/kg acrylamide group when compared to the control group. This suggests that evoked responses may be a more sensitive indicator of neurotoxicity than the nonelectrophysiological measures examined in this study.

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ACUTE AND CHRONIC PROPYLENE GLYCOL DINITRATE EXPOSURE IN THE MONKEY

Principal Investigators: J. L. Mattson, *U.S. Air Force School of Aerospace Medicine*
R. W. Young and C. G. Franz, *AFRRI*
C. R. Curran, *U.S. Air Force Air Training Command*
M. J. Cowan, Jr., *Naval Medical Research Institute*

Rhesus monkeys *(Macaca mulatta)* were exposed to propylene glycol 1,2-dinitrate (PGDN) vapors on either an acute (4-hour) or chronic (125-day) schedule (1). During acute exposures, PGDN concentrations ranged from a low of 2 parts per million (ppm) to a high of 33 ppm. Free-operant avoidance behavior and visual-evoked responses were monitored during the exposure. Free-operant avoidance was not affected at any dose level. However, the late positive (100-150 ms) wave of the visual-evoked response increased 20% at 2 ppm and decreased 25% at concentrations up to 33 ppm. Other monkeys were exposed to successively increasing concentrations of PGDN vapors at 0.3, 0.8, 1.6, and 4.2 ppm for 23 hours per day and a total of 125 days. Consecutive exposure levels lasted 35, 56, 20, and 14 days, respectively. Daily performance testing included alternating sessions of discrete-trial cued-avoidance and free-operant avoidance separated by 3-min rest periods. None of the four PGDN concentrations had a discernible effect on either type of avoidance performance, and there were no measurable changes in overall behavior. Necropsy and histopathological examinations were negative.

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1. Mattson, J. L., Young, R. W., Curran, C. R., Franz, C. G., Cowan, M. J., Jr., and Jenkins, L. J. Acute and chronic propylene glycol dinitrate exposure in the monkey. Arch Environ Health, in press.

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EVIDENCE FOR A DISSOCIATION BETWEEN ACUTE HYPOTENSION AND PERFORMANCE DECREMENT AFTER A LARGE DOSE OF IONIZING RADIATION

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

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Within a few minutes after exposure to a high dose of whole-body ionizing radiation, animals often exhibit a transient period of hypotension which may accompany a decrement in performance (1). The spontaneously hypertensive rat (SHR) exhibits baseline blood pressures 40-80 mm Hg (systolic pressure, indirect method) above that of normotensive controls. This study investigates the possibility that the SHR might be provided some protection against a radiogenic incapacitation.

Male SHR's (300-350 g) were exposed to 10,000 rads of high-energy electrons, immediately after which blood pressure was measured for one-half hour. Normotensive control rats (WKY strain) received the same treatment. SHR's showed a large drop in blood pressure ($p = 0.05$, Wilcoxon matched-pairs, signed-ranks test) postirradiation although their pressures stayed above those of the nonirradiated normotensive controls. Surprisingly, after irradiation, control-group blood pressure did not differ significantly ($p > 0.05$, Wilcoxon) from a baseline taken before exposure.

In a second experiment, performance of SHR and normotensive controls was evaluated on an active avoidance task after exposure to a dose of radiation identical to that used in the initial study. Both groups showed a significant drop in ability to avoid foot shock in the 30 min following exposure ($p = 0.031$, sign test). No difference was noted between the two groups.

Although spontaneously hypertensive rats maintain a postirradiation blood pressure exceeding that of the nonirradiated controls, this group still exhibits a performance decrement. On the other hand, rats in the normotensive control group, which showed no consistent drop in blood pressure, also exhibited a decrement in ability to perform after exposure. These preliminary findings indicate a dissociation between acute decreases in blood pressure and the behavioral incapacitation observed after large doses of ionizing radiation.

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1. Bruner, A., Bogo, V., and Henderson, E. A. Dose rate effects of ^{60}Co irradiation on performance and physiology in monkeys. Topical Report DNA-3660T, Defense Nuclear Agency, Washington, D.C., 1975.

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GENOTYPE-DEPENDENT CHANGES IN BEHAVIOR AND CEREBRAL GLUCOSE UTILIZATION IN RESPONSE TO MORPHINE IN TWO SPECIES OF MICE

Principal Investigators: H. Teitelbaum, D. E. McClain, and G. N. Catravas

In contrast to mice of the DBA/2J strain, which became somnolent after administration of morphine, the C57BL/6J mouse strain responds to morphine with locomotor hyperactivity and excitement (1). To determine the neuronal basis for this genetic difference in response to opiates, carbon-14 deoxyglucose mapping of the brain is being used to determine which regions of the brain are differentially affected by morphine in the two strains of mice.

The brain regions that show higher glucose utilization in the C57BL/6J strain are the anterior thalamus, the mammillary nuclei of the posterior hypothalamus, and the anterior amygdaloid region. The subthalamic region binds a large amount of carbon-14 deoxyglucose also. When amphetamine is given, both strains of mice show locomotor hyperactivity and changes in glucose utilization similar to the changes seen with morphine in the C57BL/6J strain.

In addition to its use in determining the site of action of drugs in the central nervous system, the carbon-14 deoxyglucose mapping procedure is being used to relate changes in motivated behavior after radiation exposure to localized changes in cerebral glucose utilization.

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1. McClain, D. E., Catravas, G. N., and Teitelbaum, H. Age and genotype dependent changes in morphine induced locomotor activity and brain histamine levels. Neurosci Abstr 3: 297, 1977.

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PERSISTENCE OF LATERAL HYPOTHALAMUS-MEDIATED BEHAVIORS AFTER A SUPRALETHAL DOSE OF IONIZING RADIATION

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

In rats a sufficiently large dose of ionizing radiation produces an early transient incapacitation (ETI) characterized by symptoms resembling those observed after bilateral lateral hypothalamic lesions; i.e., akinesia and decrements in various motivated behaviors. Since high-frequency, bipolar electrical stimulation of the lateral hypothalamus produces vigorous locomotor activity in the rat, we chose to challenge radiation-induced akinesia with this stimulation. Substantia nigra stimulation also produces a locomotor response (more stereotypic in nature) and thus provides a means of comparing the effects of lateral hypothalamic stimulation to those produced by activation of another subcortical structure.

Behaviors were quantified by use of a Lafayette instrument activity platform which measures minute vibrations. Baseline activity was recorded in the habituated animal during five 15-sec stimulations. On a different day subjects received 10,000 rads of high-energy electrons immediately followed by electrical brain stimulations administered in a program identical to that during the baseline.

The probability of producing locomotion through lateral hypothalamic stimulation was identical before and after irradiation. However, substantia nigra stimulation failed to produce the significant increase in activity postirradiation that it had before exposure.

Because radiation-produced ETI also affects motivated behaviors, a second experiment investigated the persistence of lateral hypothalamic self-stimulation after 10,000 rads of high-energy electrons. After exposure, bar pressing for lateral hypothalamic stimulation suffered only a slight drop when compared to two other subcortical areas that support similar behaviors (see Figure 1). There is a statistically significant difference between the radiogenic changes produced in lateral hypothalamic self-stimulation and those produced in similar substantia nigra and septal mediated behaviors ($p < 0.01$, Kruskall-Wallis one-way analysis of variance).

These results suggest that stimulation of the lateral hypothalamus can produce relief from radiation-produced akinesia. In addition, the lateral hypothalamus may be less sensitive to ionizing radiation than are the other subcortical structures sampled. If it is the case that specific brain areas are differentially altered by ionizing radiation, this information may be a potentially useful tool in determining the neurological basis for the early transient incapacitation syndrome.

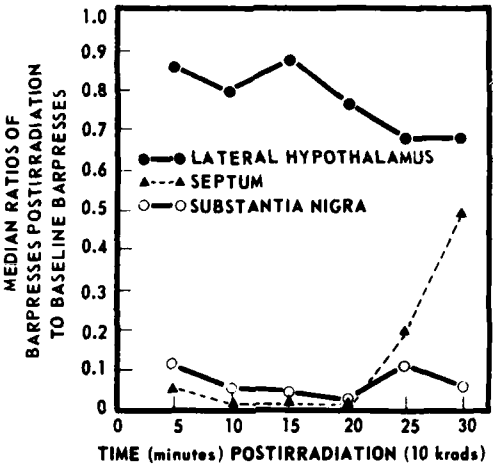

Figure 1. Vigorous self-stimulation of the lateral hypothalamus persists after radiation exposure even though similar behaviors mediated by other subcortical structures are strongly attenuated.

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BRAIN CYCLIC NUCLEOTIDE LEVELS AFTER ACUTE AND CHRONIC ADMINISTRATION OF ETHANOL

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

Present evidence suggests that cyclic nucleotides play a role in synaptic transmission. Because of reports that acute and chronic administration of ethanol induces alterations in transmitter function, a study was undertaken to determine the regional brain levels of adenosine-3',5'-cyclic monophosphate (cAMP) and guanosine-3',5'-cyclic monophosphate (cGMP) after different conditions of ethanol treatment. Male Sprague-Dawley rats were either treated with a single dose of ethanol (6 g/kg, per os) or rendered ethanol-dependent by the method of Majchrowicz (1). At appropriate times the animals were sacrificed by focused microwave fixation. The brains were excised and dissected into the following parts: cerebellum, brain stem, thalamus, hippocampus, caudate nucleus, and cerebral cortex. Acute ethanol treatment reduced cGMP levels

after 2 hours by at least 50% in most areas of the brain. After 1 hour, cerebellar cGMP was depleted by 95%. cGMP levels gradually returned to control values as blood ethanol was eliminated. In ethanol-dependent rats still intoxicated, cGMP was depressed in all areas, but during the withdrawal syndrome it had returned to control levels. No alterations were observed in cAMP levels under any of the experimental conditions. The data suggest that changes in brain cGMP may be involved in some of the actions of ethanol.

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ALTERATIONS IN STRIATAL DOPAMINE RELEASE AFTER ACUTE AND CHRONIC ADMINISTRATION OF ETHANOL

Principal Investigators: W. A. Hunt and J. H. Darden

The effect of ethanol treatment on dopamine turnover has been controversial. A single dose of ethanol has been reported to increase, decrease, or not affect dopamine turnover, depending on the method of measuring turnover, the size of the dose, and the time after administration. Similar results have been reported during the withdrawal period in ethanol-treated animals. These variations in results suggest that the dopaminergic system may not be in a steady state, a condition that must be met to render valid any turnover studies. In an attempt to assess dopaminergic activity by a different experimental approach, the present investigation explores the measurement of dopamine release *in vitro* in the caudate nucleus of the rat as a function of acute and chronic administration of ethanol.

Male Sprague-Dawley rats were treated with a single dose of ethanol or rendered ethanol-dependent by the method of Majchrowicz (1). This entailed the oral administration of ethanol at 9-15 g/kg/day in three to five divided doses for 4 days. Dopamine release was measured as the rate of efflux of tritiated dopamine from slices of caudate nucleus after stimulation with high potassium ion (K^+).

A single dose of ethanol accelerated K^+-stimulated dopamine release in a dose-dependent manner until blood ethanol concentrations reached about 300 mg/dl.

Above this amount, dopamine release progressively declined until K^+ was incapable of stimulating release. When animals were rendered ethanol-dependent, dopamine release was elevated while they were still intoxicated. However, 6-8 hours later, when a fully developed withdrawal syndrome had developed, dopamine release was inhibited.

The results observed after acute and chronic treatment with ethanol do not appear to be due to a direct effect since ethanol added to the incubation medium in physiologically compatible concentrations had no effect on dopamine release and since no ethanol was present in striata of treated animals at the time the high K^+ concentrations were added. Since the alterations in dopamine release are observed only in treated animals, the possible role of acetaldehyde, a metabolite of ethanol, was assessed. When acetaldehyde was added to the incubation medium at the same time as K^+, dopamine release was enhanced in a concentration-dependent manner. However, the concentrations of acetaldehyde that produced a significant effect are in excess of those found in blood and brain after a dose of ethanol that would stimulate dopamine release.

The data suggest that acute and chronic administration of ethanol have a biphasic effect on striatal dopamine release and that this effect is induced by indirect means. Also, some of the signs of the ethanol withdrawal syndrome may be related to a functional dopamine deficiency.

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FUNCTIONAL PROPERTIES OF POSTERIOR PARIETAL CORTEX OF THE MONKEY. I. SENSORY RESPONSES

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

On the basis of clinical observations and experimental studies, the posterior parietal cortex has been thought to associate information from the visual and somatosensory modalities with behavior such as limb movements and eye movements. By recording from several hundred movement-related single neurons in areas 5 and 7 of three awake,

trained monkeys, we have found that such cells respond to passive visual and/or somatosensory stimulation. In addition, the responses of some of these cells can be modulated by certain behavioral conditions (to be discussed).

Passive visual stimuli were presented while the monkey fixated a spot of light on a tangent screen; the animal was not required to make any movement toward the visual stimuli. Posterior parietal cells have very large receptive fields, and these frequently include a whole visual quadrant. On occasion, the fields include a whole hemifield and can be bilateral. These visual fields can include the fovea. Some cells have tonic responses to stimuli whereas other respond phasically. The activity of these cells during eye movement tasks is determined by these visual properties; i.e., tonic cells discharge during fixations and tracking eye movements, and phasic cells discharge with saccadic eye movements. Parietal cells are not sensitive to the orientation of stationary stimuli. Most parietal cells respond very well to large stimuli and less well to small spots of light. The visual responses of these cells can be modulated by the intensity of the stimulus. Many cells respond equally well to all directions of stimulus movement, although a subset of neurons are directionally selective.

Passive somatosensory stimuli were delivered while the animal sat quietly in its chair and could not see its limbs. Somatic receptive fields of posterior parietal neurons are similar to the visual receptive fields in size; they are quite large. Somatic receptive fields frequently include a whole limb, either the forelimb or hindlimb. Some cells respond to passive somatosensory stimulation of the ipsilateral or contralateral limb, and others respond best to simultaneous bilateral stimulation. Although many posterior parietal neurons are excited by somatic stimulation, some can have their spontaneous activity inhibited.

We believe that our data support the association hypothesis for the function of posterior parietal cortex, and we suggest that this area, in conjunction with other parts of the visual and somatosensory systems, helps to determine a sensory environment in which movement might be appropriate.

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FUNCTIONAL PROPERTIES OF POSTERIOR PARIETAL CORTEX OF THE MONKEY. II. BEHAVIORAL ENHANCEMENT OF VISUAL RESPONSES

Principal Investigators: M. E. Goldberg and D. L. Robinson, *AFRRI*
G. B. Stanton, *Howard University*

Neurons in areas 5 and 7 of the rhesus monkey have been described to discharge in association with eye and hand movements. We have shown that such neurons have visual and/or somatosensory responses that can be demonstrated in the absence of any active response on the part of the monkey toward the stimulus used to excite the neuron. When the animal uses the stimulus as the target for an eye or hand movement, the discharge of some neurons in the posterior parietal cortex can be enhanced.

Three rhesus monkeys were trained to fixate a spot of light and then to make one of three responses: to continue to fixate the spot and ignore any other visual stimuli that might appear on the screen, to reach out and touch a panel if it is illuminated, or to make a saccadic eye movement to a small peripheral target. In this paradigm the same visual stimulus could take on three different meanings to the monkey: it could be a target for a hand movement, a target for an eye movement, or an irrelevant and negligible stimulus. Extracellular single-unit recordings were done while the animals were performing their tasks. All behavioral control and on-line data analysis was done using a PDP 11-10 computer.

The visual responses of nearly half the visually responsive neurons in areas 5 and 7 were brisker and more regular when the animal was going to make a saccadic eye movement to fixate or reach out to touch the target. This enhancement response was not entirely dependent on the presence of the movement because if, in a series of trials, the monkey made an occasional erroneous response, the enhancement was still present. The response, however, was absolutely dependent on the presence of the stimulus. If the monkey made the proper movement when the proper stimulus was absent, the neuron did not discharge in relation to the movement.

The enhancement is spatially specific. When the animal sees two stimuli (one in the receptive field of the neuron and the other outside of the receptive field), the neuron has an enhanced discharge only when the animal makes a saccadic eye movement to fixate the stimulus in the receptive field. There is no enhancement when the animal makes saccades to a stimulus outside of the receptive field.

These data indicate that the posterior parietal cortex acts as an association area for sensory and behavioral data. Those association processes may provide a physiological

basis for the psychological process of attention. These data do not support the hypothesis that posterior parietal neurons command movement except to delineate an environment in which movement may be appropriate.

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CORTICOCORTICAL AND CORTICOTHALAMIC PROJECTIONS TO AREA 7 OF MONKEY CEREBRAL CORTEX

Principal Investigators: G. B. Stanton and W. L. R. Cruce, *Howard University*
M. E. Goldberg and D. L. Robinson, *AFRRI*

Thalamocortical and corticocortical connections to area 7 of the parietal cortex were studied as part of an ongoing investigation of visually evoked responses in this area in alert, trained monkeys. Multiple injections of 0.5 μl of horseradish peroxidase (30%) were made unilaterally in area 7 of two *Macaca mulatta* monkeys. After 48 hours the monkeys were anesthetized and perfused through the heart with a saline wash followed by a buffered fixative solution of 0.5% paraformaldehyde, 2.5% glutaraldehyde, and 1.5% sucrose (pH 7.4). Brains were blocked and stored in cold 30% sucrose until sectioned (50 μ thick). Sections were developed in diaminobenzidine and hydrogen peroxide and then counterstained with cresyl violet. The distribution of peroxidase-labeled cells was plotted with an XY plotter driven by potentiometers attached to the microscope stage.

Labeled neurons were found to be distributed in a band radiating dorsolaterally and rostrally from a region of greatest concentration in the dorsocentral part of the medial pulvinar. Labeled cells were found in pulvinar oralis and in the lateral posterior nucleus, ventral to the lateral dorsal nucleus. Isolated labeled neurons were found in the pars postrema of the ventral lateral nucleus, in the central lateral nucleus, and in paralaminar portions of the mediodorsal nucleus. Many labeled neurons were found in the basal nucleus of Meynert. In the cortex, labeled cells appeared in the cingulate gyrus, in the inferior bank of the arcuate sulcus, and in area 5.

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VISUAL SUBSTRATE OF SACCADIC EYE MOVEMENTS: A MODEL FOR THE SPATIAL CODING OF BEHAVIOR

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

Visually guided eye movements require that the oculomotor system change eye position on the basis of information transmitted to it by the visual system. The visual system, in turn, must analyze a prospective target in terms of three questions: what is the stimulus, where is it, and is it behaviorally significant enough to become the target for the next saccade? We have studied how the brain answers those questions using the techniques of single-unit recording from awake, trained monkeys.

Hubel and Wiesel (1) showed that neurons in striate cortex have receptive fields with very precise stimulus requirements in terms of shape, orientation, direction of motion, size, and (to some extent) color. Baizer, Robinson, and Dow (2) showed that neurons in area 18 maintain this degree of stimulus specificity and that different neurons exercise specificity in different parameters. These neurons could easily be important in the analysis of what or where an object is.

Neurons in the superficial grey and optic layers of the superior colliculus have, in distinction, large receptive fields without stimulus specificity in terms of shape, orientation, color, or (usually) direction of movement. Although an ensemble of collicular neurons could conceivably locate an object in space, they could not perform a very adequate qualitative analysis of the object. Many of these neurons do show evidence of processing behavioral information relevant to the stimulus. Goldberg and Wurtz (3) showed that when an animal is going to use the stimulus in the receptive field of a neuron as the target for an eye movement, the response to that stimulus is enhanced. The enhanced component of the response is presumably a result of the behavioral significance of the stimulus. This enhancement is spatially specific; when the animal is going to fixate a stimulus outside the receptive field of the neuron, the neuron's response to the stimulus in the receptive field is not enhanced. Therefore, although these neurons could not carry much information about what an object is, they do carry information about the stimulus' importance to the animal. Wurtz and Mohler (4) showed for striate neurons and Robinson, Baizer, and Dow (5) showed for prestriate neurons that a neuron in these areas may show slight enhancement before an eye movement but that the enhancement is not dependent on whether or not the eye movement is to the receptive field of the neuron.

When an animal moves its eyes, many stimuli are swept across the retina. Since a moving stimulus is a very salient event for many visual neurons, eye movement-induced stimulus movement may act as a falsely salient stimulus unless there is a mechanism to differentiate between self-induced stimulus movement and real stimulus movement. Wurtz (6) showed that striate cortical neurons respond, whether or not the movement is stimulus-induced or eye movement-induced. In contrast, Robinson and Wurtz (7) showed that some neurons in the superior colliculus can make this differentiation.

Almost inevitably, neurons that make this differentiation show specific enhancement when the stimulus in their receptive field is going to be used as the target for an eye movement; neurons that do not differentiate rarely show enhancement. Thus, for the superior colliculus, the spatial coding of behavioral significance seems to be paired with a filtering mechanism to eliminate falsely salient stimuli, and the filtering mechanism is not present in those collicular and striate cortical neurons that do not code for behavioral information.

Two cortical areas do show spatial coding of behavioral information. These areas are the frontal visual cortex (area 8) and the posterior parietal cortex (areas 5 and 7). Neurons in frontal cortex have large receptive fields that are relatively independent of stimulus parameters, and they show pre-eye movement enhancement that is spatially specific. Neurons in the posterior parietal cortex that seem to discharge in association with eye or hand movement can be shown to have visual and somatosensory responses in the absence of behavior. The receptive fields of these neurons are large, and the neurons are not fastidious about stimulus parameters. We have yet to demonstrate even an inhibitory surround for any visual neuron in areas 5 and 7. However, the response of these neurons is frequently enhanced when the stimulus is the target for behavior, and the enhancement is spatially specific.

In summary, the visual processing preceding eye movements seems to involve two sorts of neurons: (a) neurons in striate and prestriate cortex that perform detailed qualitative analysis on the stimulus without bearing any information about the behavioral significance of the stimulus, and (b) neurons in the superior colliculus, frontal cortex, and parietal cortex that say a great deal about the behavioral significance of the stimulus but little about its qualitative properties.

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BIOCHEMISTRY DEPARTMENT

The Biochemistry Department was formed in July 1976 to provide centralization and management of biochemical research programs. Its primary objectives are (a) to develop a better understanding of the mechanisms of damage to biological systems as a result of the action of various toxic agents such as radiation and chemical agents, and (b) to provide more effective methods for detecting and evaluating such changes and to relate them to subsequent injury in man. A number of approaches and models are used, and special emphasis is given to the development of biochemical indicators of radiation injury.

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

The Physiological Chemistry Division is primarily concerned with identifying biochemical indicators of disease and injury in biologic fluids. At present, major emphasis is being placed on tissue lipids and on changes in serum glycoproteins and trace metals. Considerable progress has been made in a study of gallium-67 distribution in irradiated animals in collaboration with the Nuclear Sciences Department. Alterations in whole-body retention, tumor uptake, and serum binding of gallium-67 were found to be related to radiation-induced increases of serum iron. Investigations have continued on elevations of serum glycoproteins and protein-bound carbohydrates as indicators of radiation injury, trauma, and other disease states. Collaborative studies with the National Cancer Institute and other medical centers are concerned with identifying and comparing various tests for estimating tumor burden as well as further changes in these markers due to radiotherapy and other treatment. An expanded research effort in the immunological effects of radiation includes a collaborative study on portal irradiation with the Physiological Chemistry Division and Immunological Chemistry Division of the Viral Oncology Branch, NCI. This study is based on the idea that irradiation of portals containing different lymphoreticular tissues should result in various degrees of immunosuppression. Various adjuvants that either stimulate or potentiate the immune response will then be tested on radioprotectors. The effect of radiation and combined therapies on mouse tumor growth is also being studied.

The Molecular Biology Division is concerned with elucidation of the biochemical mechanisms of injury induced by toxic agents such as ionizing and nonionizing radiation, chemical agents, and drugs. Two operationally oriented research projects funded by the U.S. Navy concern bioeffects as a result of exposure to nonionizing radiation. A completed work unit was concerned with the biochemical alterations of lens proteins due to microwave irradiation. Also under investigation are the effects of microwaves on neurotransmitter levels and the enzymes involved in their metabolism in the mammalian brain. The effects of radiation and of chemical agents on the central nervous system of the subhuman primate are also being investigated. A model was developed based on the chronic implantation of a catheter in the fourth ventricle of the monkey connected to a subcutaneously implanted Ommaya reservoir. Since cerebrospinal fluid is a product of neural tissue, it is expected to reflect biochemical changes occurring in

the central nervous system as the result of an insult to the organism. With the cooperation of the Agricultural Research Service (U.S. Department of Agriculture), experiments have been initiated to determine the effect of bovine transfer factor on the immune system of the mouse. Transfer factor has been shown to correct immune deficiencies in experimental animals; the mouse model will therefore be used to determine if the factor has any beneficial effect on the immune system of the irradiated animal. Collaborative studies are also in progress with investigators from the Neurobiology and Behavioral Sciences Departments of AFRRI to determine the relationship between brain histamine levels and postirradiation performance decrement.

The Immunological Chemistry Division's research efforts are concerned with studies on the nature of the interaction of stromal tissue with hemopoietic cells and its importance in postirradiation hemopoietic regeneration. A mutant mouse model extremely sensitive to radiation is being used in these studies. A series of new studies is soon to be initiated aimed at the isolation of hemopoietic and progenitor cells and the measurement of their potential as a modifier of radiation injury. The Fluorescence Activated Cell Sorter will be used in these studies to obtain pure population of hemopoietic and progenitor cells.

The aims of the Biochemistry Department's research efforts are to develop biochemical indicators of damage induced by radiation, chemical agents, and different disease conditions and to elucidate underlying mechanisms of injury and repair. Efforts are directed toward applying developed techniques to diagnosis and treatment of conditions peculiar to the battlefield environment. Research projects conducted in the Department are interrelated and complementary.

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STEROL METABOLISM IN NERVOUS TISSUE

Principal Investigator: J . F. Weiss, *AFRRI*
Collaborators: P. Paoletti, *University of Pavia,* Italy,
R. Fumagalli, *University of Milan,* Italy

The high concentration of cholesterol in nervous tissue and its localization in cell membranes--notably myelin--indicate an important role for this sterol and related compounds in the growth, maturation, and metabolism of the brain. However, very little has been known not only about sterol function but also (until recently) about sterol composition and pathways of biosynthesis and metabolism in the brain. The introduction of drugs inhibiting specific steps of cholesterol biosynthesis in the nervous system has made possible a different approach to solving these complex problems. This involves inducing selective accumulation of precursors which can be separated and identified by techniques such as gas chromatography-mass spectrometry. Although sterol metabolism is slower in mature brain compared to developing and neoplastic brain, sterol synthesis and turnover do occur in mature nervous tissue and can be affected by disease, injury, and drugs. For example, ionizing radiation appears to block brain cholesterol synthesis, and this effect can be modified by radioprotective agents or radiosensitizers.

The following abstract from Paoletti *et al.* (1) provides evidence that brain sterol changes occur in disease states and that cholesterol precursors or catabolites can be used as biochemical indicators of brain disease or injury.

ABSTRACT

Studies on the role of desmosterol as a marker of glioma (and other brain tumor growth) are reviewed. Experimental studies indicate that desmosterol (24-dehydrocholesterol) is related to rapid growth of ıe central nervous system, including neoplasia. Clinical studies undertaken during the past 10 years show that desmosterol can be further increased in the cerebrospinal fluid of patients with brain tumors by a short treatment with triparanol, a drug that blocks the conversion of desmosterol to cholesterol. Results are shown in Figure 1 relating the cerebrospinal fluid cholesterol and desmosterol levels to the histology and site of tumors in patients before surgery as well as examples of the use of sterol measurements as indicators of tumor activity in patients undergoing chemotherapy.

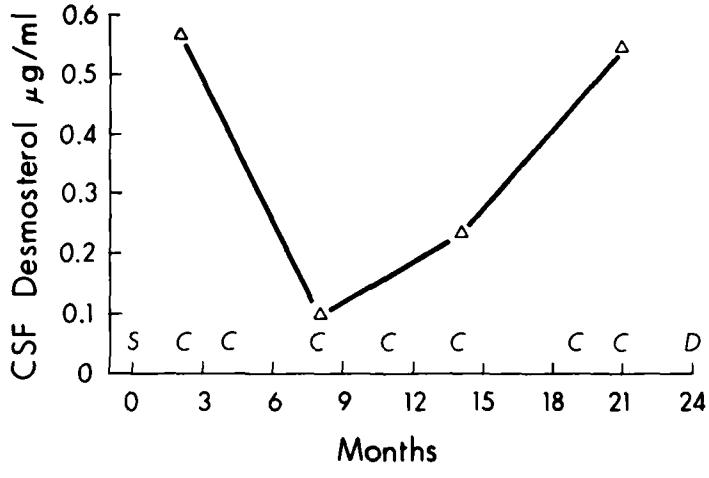

Figure 1. Cerebral spinal fluid desmosterol after triparanol administration (250 mg b.i.d. x 5 days) during the follow-up of a patient receiving treatment for a glioma. S = surgery; C = chemotherapy; D = died.

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EFFECT OF RADIATION ON TRACE METAL DISTRIBUTION

Principal Investigators: W. P. Bradley, P. O. Alderson, and J. F. Weiss, *AFRRI*
Collaborators: W. C. Eckelman, *George Washington University;*
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Technical Assistance: C. J. Morrisey, E. L. Barron, W. W. Wolfe,
J. K. Warrenfeltz and M. E. Flynn

Serum metal concentrations have been observed to change after irradiation. Magnesium is decreased whereas copper, zinc, and iron are increased. Small doses of radiation were reported to affect whole-body retention and serum binding of the radionuclide gallium-67. An investigation into radiation effects on serum metal concentrations was begun at AFRRI with early emphasis on gallium-67.

The mechanism of decreased gallium-67 retention after whole-body irradiation is unknown. To investigate this mechanism and to determine the effects of prior irradiation on gallium-67 tumor uptake, Sprague-Dawley rats bearing a subcutaneous Walker-256 carcinosarcoma were exposed to cobalt-60. Each animal received 10 μCi of gallium-67 citrate intravenously 24 hours after exposure to whole-body doses of 250, 500, 750, or 1000 rads. Control animals received gallium-67 but were not irradiated. Urine was collected for 48 hours after exposure to determine the percent injected dose (ID) excreted. Animals were sacrificed at 48 hours after injection, and the percent ID/g in tumor and other tissues was determined. A blood sample was also obtained to determine the serum iron, unbound iron-binding capacity (UIBC), and transferrin level. Tumor uptake and serum UIBC were decreased in irradiated animals whereas serum iron levels and gallium-67 urinary excretion were increased. There was a significant correlation between the UIBC and the gallium-67 tumor uptake ($r = 0.78$, $n = 49$) (Figure 1). Transferrin levels in the irradiated population were not different from control values. Local irradiation of the tumor without whole-body exposure had no effect on gallium-67 tumor uptake or urinary excretion. Similarly, whole-body irradiation with the tumor shielded did not prevent decreased gallium-67 tumor uptake. Injection of N-acetylphenylhydrazine to increase serum iron by increasing red cell hemolysis duplicated the effect caused by irradiation. The results indicate that the decreased gallium-67 retention and tumor uptake seen after whole-body irradiation are related—at least in part—to saturation of transferrin by increased levels of circulating iron (1, 2).

Future studies will be aimed at determining the effect of irradiation on other serum metal ion concentrations, with emphasis on the mechanism of observed alterations and their potential use as biological dosimeters.

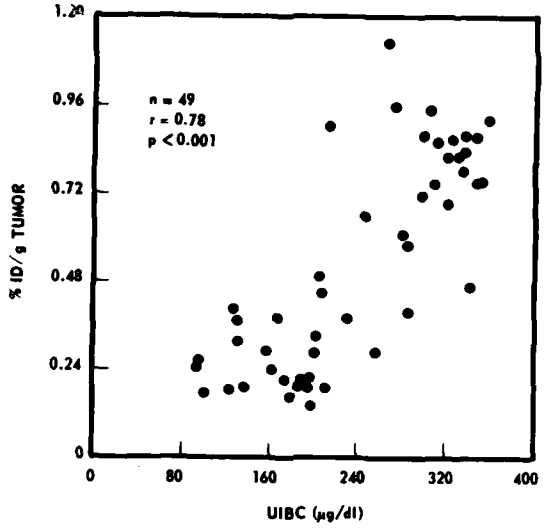

Figure 1. Relationship between unsaturated iron-binding capacity and percent injected dose per gram tumor in control and irradiated rats. Unbound iron-binding capacity (UIBC) was determined at the time of sacrifice, which was 72 hours after irradiation and 48 hours after injection of gallium-67.

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SERUM GLYCOPROTEINS IN DISEASE AND INJURY

Principal Investigators: J. F. Weiss, W. P. Bradley, and A. P. Blasco, *AFRRI*
Collaborators: P. B. Chretien and D. M. Crnic, *National Institutes of Health*
W. J. Fouty, *National Naval Medical Center*
R. A. Morantz, *University of Kansas*
J. S. McDonald, *Georgetown University*
W. G. Shain, *AFRRI*
Technical Assistance: M. J. Ryan, W. W. Wolfe, L. R. Wooldridge, and K. M. Hartley

Changes in the levels of different serum glycoproteins occur as a response to various types of disease and injury. Acute-phase glycoproteins (haptoglobin), other alpha globulins, and the carbohydrate moieties of glycoproteins have been found to increase in irradiated animals. Similar responses occur after trauma and after inflammatory, infectious, and neoplastic diseases in humans. After combined injuries in mice, more pronounced changes develop in the serum protein composition than after only skin wounding or irradiation. The overall aim of the current research effort is to contribute to an understanding of (a) the increased production of serum glycoproteins in response to injury and (b) possible methods of altering their production.

Clinical studies to date concern changes in glycoprotein-associated carbohydrates and specific serum glycoproteins in cancer patients. Studies of this population provide the opportunity to obtain information on the effects of the disease state as well as the added effects of treatment such as surgical trauma, radiotherapy, chemotherapy, and hyperthermia.

Preliminary evidence indicates that decreased levels of α_2-HS-glycoprotein or increased levels of the acute-phase proteins might serve as indicators of impaired immune status. There is evidence from other investigators that these proteins may have functional significance in altering cell-mediated immunity. Determination of immune status is extremely important in various types of trauma, disease, and injury, and future studies will emphasize the immunological implications of serum glycoprotein changes. Recent clinical studies complemented with animal studies are briefly described in the following abstracts.

ABSTRACT 1

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 with levels of 17 normal serum glycoproteins, carcinoembryonic antigen, and with lymphocyte reactivity to phytohemagglutin. 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. Increases in protein-bound carbohydrates in tumor

bearers were not related to increases in carcinoembryonic antigen. Increased levels of the acute-phase proteins occurred in individuals with depressed *in vitro* lymphocyte reactivity to phytohemagglutinin. A significant positive correlation was found between lymphocyte reactivity and levels of α_2-HS-glycoprotein. The results in Table 1 suggest that serum protein-bound carbohydrates or glycoproteins may be of adjunctive value in assessing tumor burden and immune reactivity in cancer patients (1).

Table 1. Correlation of Lymphocyte Reactivity With Serum Proteins

	Correlation Coefficients		
	Tumor-Bearing, Cured, and Normal Subjects (n = 53)	Tumor-Bearing and Cured Subjects (n = 46)	Tumor-Bearing Subjects (n = 23)
α_1-Antitrypsin	-0.24	-0.30*	-0.29
Haptoglobin	-0.29*	-0.28	-0.23
α_1-Acid Glycoprotein	-0.22	-0.23	-0.15
α_2-Macroglobulin	+0.26	+0.25	+0.37
Albumin	+0.32*	+0.29	+0.34
α_2-HS-Glycoprotein	+0.37†	+0.47†	+0.60†

* $p < 0.05$

† $p < 0.01$

Twelve other proteins and CEA showed no significant correlation with lymphocyte reactivity.

ABSTRACT 2

Serum glycoprotein levels, lymphocyte reactivity to phytohemagglutinin, and T-cell levels were determined in patients with increasing tumor burden and during radiotherapy. Studied were 109 patients with head and neck squamous carcinomas and 33 normal controls. In the patients, but not normals, α_2HS-glycoprotein levels correlated with lymphocyte reactivity to phytohemagglutinin and T-cell levels ($p < 0.05$). Mean α_2HS-glycoprotein levels and T-cell levels did not show consistent significant differences among normals, patients with primary tumors only, and those with metastases.

However, haptoglobin and C-reactive protein levels showed significant progressive increases among these groups. During radiotherapy of localized tumors, the a_2HS-glycoprotein levels and T-cell levels declined whereas other proteins increased or did not change. The results in Figure 1 show that in this study these serum glycoproteins provided insight into immune reactivity, tumor burden, and effect of radiotherapy comparable to that obtained with lymphocyte reactivity to phytohemagglutinin and T-cell levels. Because of the technical and practical advantages of the serum assays, the equivalence of these glycoprotein levels with lymphocyte reactivity to phytohemagglutinin, T-cell levels, and other parameters of cellular immunity in assessing tumor burden should be further tested in other malignancies and during other therapies (2).

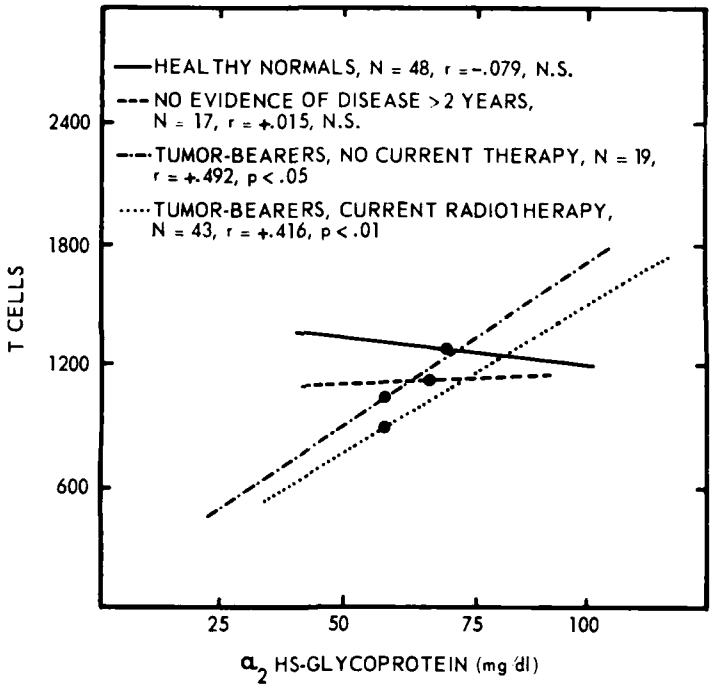

Figure 1. Correlation of T-cell and a_2HS-glycoprotein levels

ABSTRACT 3

Patients with nonneural tumors have elevated levels of serum glycoproteins synthesized by the liver. The increases in a-globulins are related to tumor burden and possibly to the immune status of the patient. This aspect of the host-tumor response has not been considered in detail in patients with brain tumors. In the present study, various glycoprotein assays were performed on serum from patients with primary intracranial tumors of varying histology. Compared to normal, significant increases ($p < 0.01$) in

serum protein-bound carbohydrates (fucose, sialic acid, neutral hexoses), determined by colorimetric assays, were found in a group of preoperative patients (n = 25) with gliomas. The increases were similar to those seen in patients with localized nonneural tumors. Significant increases also were found in the acute-phase proteins, α_1-acid glycoprotein, α_1-antitrypsin, haptoglobin, and C-reactive protein, determined by radial immunodiffusion in a group of patients (n = 10) with gliomas (Figure 2). No significant increases were found in the immunoglobulins. Levels of protein-bound carbohydrates and the acute-phase proteins of the α-globulin fraction of serum are highly correlated, and both measurements probably reflect the same host response to tumor presence. Glycoprotein synthesis or utilization appears to differ in various rat brain tumor model systems. While rats with primary nitrosourea-induced tumors of the central nervous system (n = 25) had increased levels of protein-bound fucose, there was a depression of fucose, related to tumor growth, in rats bearing subcutaneous tumors derived from the primary tumors (3).

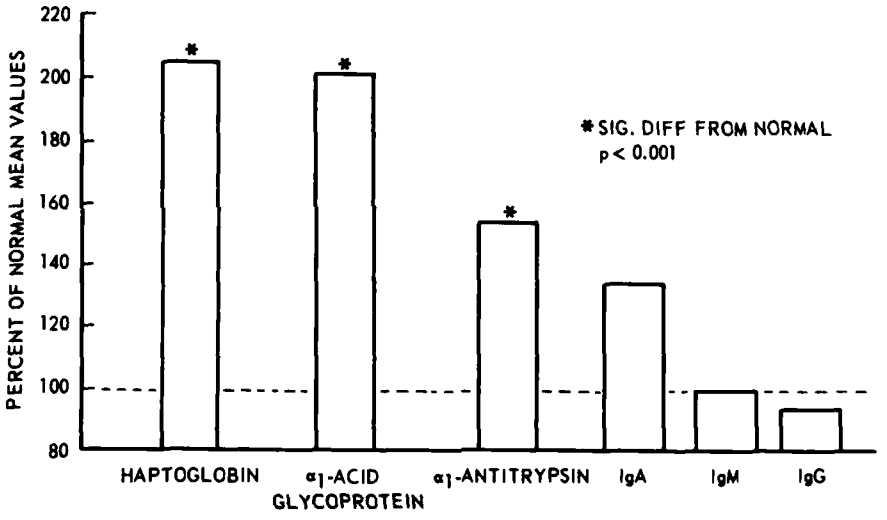

Figure 2. Serum acute-phase proteins and immunoglobulins in patients with gliomas

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MORPHINE REDUCES CEREBELLAR GUANOSINE 3',5'-CYCLIC MONOPHOSPHATE CONTENT AND ELEVATES CEREBROSPINAL FLUID GUANOSINE 3',5'-CYCLIC MONOPHOSPHATE CONTENT IN RHESUS MONKEY

Principal Investigators: J. B. Katz, G. N. Catravas, and S. J. Wright, Jr.

Biochemical indications that guanosine 3',5'-cyclic monophosphate (cGMP) may play an important role in cerebellar function include its high concentration in cerebellum and a high-affinity cGMP-binding protein in rat cerebellum (1). Morphine pentobarbital (2) and ethanol (3) administered systematically have been shown to depress cGMP levels in rat cerebellum. Experiments in our laboratory failed to reveal a depression of guanylate cyclase or stimulation of phosphodiesterase activities in cerebella removed from acutely morphine-treated rats. Since cerebellar cGMP levels could perhaps change as a consequence of cGMP exit from cerebellar tissue into surrounding cerebrospinal fluid, we decided to determine if cerebrospinal fluid cGMP levels would rise after morphine administration.

Five rhesus monkeys were chronically implanted with silastic catheters placed in the fourth ventricle and connected to compressible polyethylene Ommaya reservoirs placed subcutaneously over the occiput. Cerebrospinal fluid could be aseptically aspirated from the reservoir in the awake animal.

Before administration of a cataleptic dose of morphine (20 mg/kg, intramuscularly), duplicate baseline cerebrospinal fluid samples were taken 15 min apart after repeated reservoir pumping to insure good mixing. Mixing was repeated before each sampling in the time course experiment. As shown in Figure 1, a statistically significant increase in cerebrospinal fluid cGMP concentration is apparent within 15 min after opiate administration and persists at a significantly elevated level for several hours.

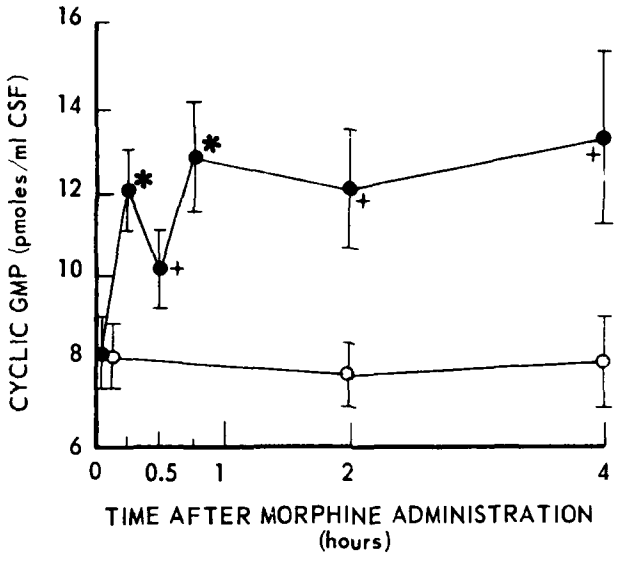

Figure 1. Time course measurement of cGMP in cerebrospinal fluid (CSF) following morphine administration to rhesus monkeys. CSF samples (0.5 ml) were removed from both control monkeys (n = 5, o—o) or morphine-intoxicated monkeys (n = 5, ●—●, 20 mg/kg morphine sulfate, intramuscularly) and frozen immediately in liquid nitrogen. Values are mean ± S.E. *$p < 0.02$, compared to control value. †$p < 0.05$, compared to control value.

In a second experiment, a group of monkeys was anesthetized with an initial loading dose of 75 mg ketamine hydrochloride and maintained and immobilized with ketamine hydrochloride (7 mg/kg/hour, intravenously), 60% nitrous oxide, and pancuronium bromide (0.04 mg/kg/hour, intravenously). Subsequent hemicraniectomy permitted frontal, parietal, occipital cerebrocortical, and cerebellar biopsies. Following anesthesia and hemicraniectomy, morphine was administered (20 mg/kg, intramuscularly) and 45 min later, biopsy specimens were taken. Specimens were immediately plunged into liquid nitrogen and subsequently assayed for cGMP content. The control group for this second experiment was treated identically to the first except that morphine was not administered. Table 1 demonstrates that cGMP levels in all cerebrocortical regions were unaffected by the opiate. Only cerebellar cGMP levels changed significantly, showing a 35% decrease relative to anesthetized controls. Although the controlling factors of brain tissue and cerebrospinal fluid cGMP levels are poorly understood, it is possible that, under certain conditions, a reciprocal relationship may exist between cGMP levels in certain brain regions and in cerebrospinal fluid.

Table 1. Effect of Morphine on Cerebrocortical and Cerebellar cGMP Content in Anesthetized Monkeys

Group	Morphine Dose I.M., mg/kg	cGMP Content (p moles/mg protein) Cerebellum	Cerebral Cortex Frontal	Cerebral Cortex Parietal	Cerebral Cortex Occipital
Control	0	0.72 ± 0.06	0.37 ± 0.06	0.39 ± 0.04	0.41 ± 0.04
Morphine-treated	20	0.47 ± 0.03*	0.39 ± 0.10	0.36 ± 0.08	0.43 ± 0.14

* $p < 0.02$, compared to control value

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CEREBROSPINAL FLUID CYCLIC AMP LEVELS IN RHESUS MONKEYS: DAILY FLUCTUATIONS

Principal Investigators: J. B. Katz, C. Valases, G. N. Catravas, and S. J. Wright, Jr.

Cyclic adenosine monophosphate (cAMP) has been suggested as a second messenger in central nervous system synaptic events by analogy with its role in other tissues and by results of *in vitro* and *in vivo* experiments with nervous tissues (1,2). Increases in cerebrospinal fluid cAMP levels have resulted from *in vitro* and *in vivo* administration of putative adrenergic transmitters, and other experimental and clinical conditions

[including epilepsy, transient cerebral ischemia (migraine), and hydrocephalus] have correlated to alteration of cerebrospinal fluid cAMP levels. Yet in contrast to these efforts to relate complex pathophysicological states to cerebrospinal fluid cAMP levels, little is known about the reasons for such elementary findings as the wide normal range of cerebrospinal fluid cAMP values. Therefore, we decided to examine neurologically intact rhesus monkeys to determine the presence or absence of intrinsic daily fluctuations in cerebrospinal fluid cAMP levels.

Rhesus monkeys (4.5-6.0 kg) were chronically implanted with Ommaya cerebrospinal fluid reservoirs connected to silastic catheters placed in the fourth ventricle. This method permits sterile aspiration of 0.5-1.0 ml of cerebrospinal fluid from the awake animal on a chronic basis. To insure equilibration of cerebrospinal fluid between the reservoir and the subarachnoid space, reservoirs were compressed and allowed to fill several times before sampling. In the first experiments, monkeys were maintained on a 12-hours-light/12-hours-dark (12L/12D) lighting schedule. Cerebrospinal fluid samples (0.5 ml) were taken every 3 hours over a 72-hour period. In later experiments, animals were adapted to continuous, dim light (less than 0.25 footcandles) for 5 days before the 72-hour sampling period commenced. Aspirated samples were immediately frozen in liquid nitrogen, stored at -80°C, and later assayed radioimmunologically for cAMP content.

Part *a* of Figure 1 contains three records of monkey cerebrospinal fluid cAMP content as a function of time during the 72-hour period, using animals exposed to a 12L/12D-light schedule. Fluctuations in cAMP levels appear to be evident in all subjects. Part *b* of Figure 1 demonstrates that when the animals were maintained in continuous, dim light for 5 days before the 72-hour dim-light sampling period, a trend is evident toward a lower mean cAMP level in the dim-light condition (17.2 ± 2.0 pmoles cAMP/ml cerebrospinal fluid in 12L/12D animals versus 10.3 ± 0.7 pmoles/ml in dim-light animals). Since the S.D. of the light/dark animals (17.2) is greater than that of the dim-light animals (10.3), one tends to believe that the observed fluctuations of cerebrospinal fluid cAMP levels were not only significant with respect to measurement error, but also were more prominent under light/dark conditions. No statistically significant dark/light differences for the 12-hours-light/12-hours-dark group were found.

It is not clear from our experiments whether the changes we observed in the cerebrospinal fluid cAMP levels reflect changes in brain cAMP, changes in transport of cAMP from brain to cerebrospinal fluid, degradation of cAMP within the intercellular spaces of the brain, or a combination of these factors. The potential influence of environmental lighting on cerebrospinal fluid cAMP concentration remains to be delineated. Knowledge of temporal fluctuations of cerebrospinal fluid cAMP levels demonstrated in our study may be useful in future experiments.

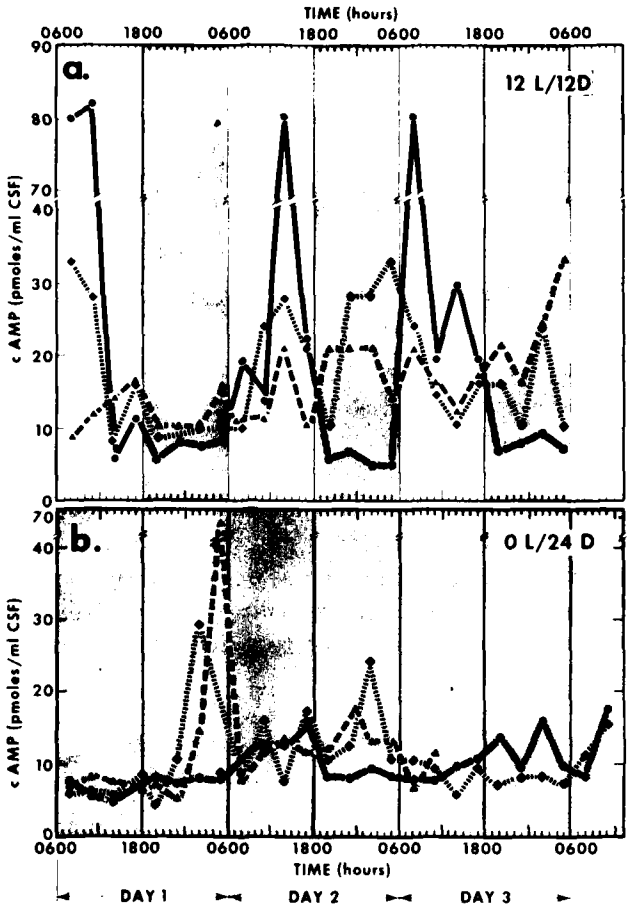

Figure 1. Cerebrospinal fluid cyclic adenosine monophosphate (cAMP) levels in rhesus monkeys over a 72-hour period. Three monkeys were accustomed either to a 12-hours-light/12-hours-dark schedule (12L/12D) as presented in *a* or to a 24-hour dim-light schedule (0.25 footcandles) (OL/24D) as shown in *b*.

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AGE- AND GENOTYPE-DEPENDENT CHANGES IN MORPHINE-INDUCED LOCOMOTOR ACTIVITY AND BRAIN HISTAMINE LEVELS

Principal Investigators: D. E. McClain, G. N. Catravas, and H. Teitelbaum

Previous work (1) has shown an inverse relationship between morphine-induced changes in brain histamine levels and changes in locomotor activity in the same strain of mouse (Commonwealth Serum Laboratories). We have been able to confirm this relationship using the C57BL/6J and DBA/2J strains of inbred mice at about 2 months of age.

Thirty minutes after injection of morphine there is a dose-dependent decrease in histamine levels in mice of the C57BL/6J strain associated with a dose-dependent increase in locomotor activity. In contrast, increased histamine levels and decreased locomotor activity are seen when mice of the DBA/2J strain are given increasing doses of morphine.

There is, however, an age dependence in the animal's brain histamine response to morphine stimulation. Figures 1 and 2 show the relationship of whole-brain histamine levels in 2- and 5-month-old mice of both strains at various times after injection of 20 mg/kg of morphine. In both the C57BL/6J and DBA/2J strains, the 5-month-old mice (Figures 1*B*, 2*B*) show a distinctly altered histamine response compared to the 2-month-old mice (Figures 1*A*, 2*A*). On the other hand, there were no differences in the locomotor response to morphine when comparing 2- and 5-month-old mice of each strain.

In summary, morphine-stimulated whole-brain histamine patterns show an age-dependent relationship in both strains tested, yet their locomotor response remains unchanged with age. There appears to be no causal relationship between morphine-induced locomotor activity and brain histamine levels.

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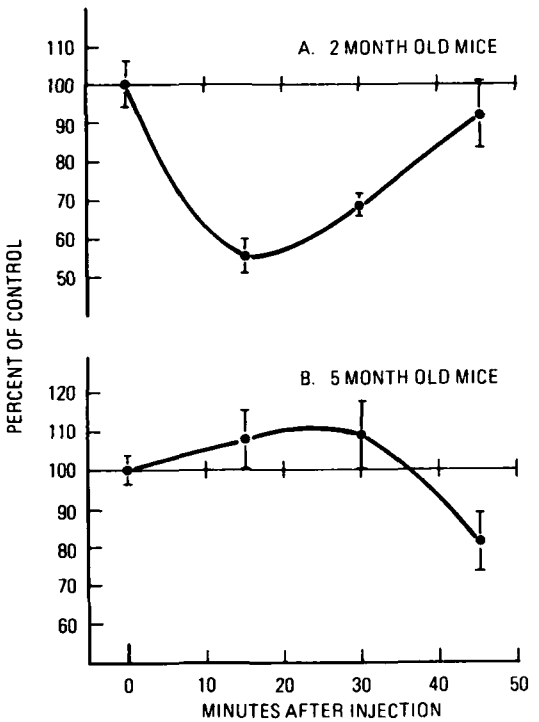

Figure 1. Whole-brain histamine vs time after 20 mg/kg morphine in C57BL/6J mouse. Each point represents the mean ± S.E.M. of a minimum of six animals.

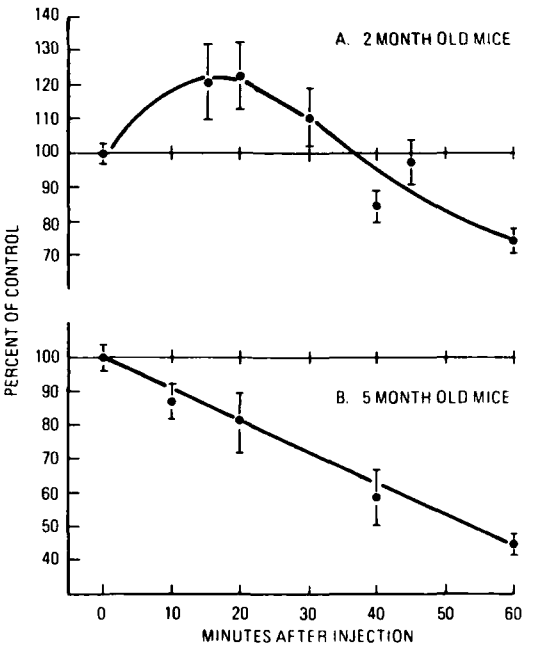

Figure 2. Whole-brain histamine vs time after 20 mg/kg morphine in DBA/2J mouse. Each point represents the mean ± S.E.M. of a minimum of six animals.

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QUANTITATION OF PROTEIN ZONES STAINED WITH COOMASSIE BLUE R250 AFTER PORE-LIMIT ELECTROPHORESIS

Principal Investigators: G. M. Oosta and N. S. Mathewson
Technical Assistance: K. M. Hartley

Polyacrylamide gel electrophoresis is a sensitive method for separating the components of complex protein mixtures. In most cases, the separated zones are visualized by staining, frequently with Amido Black 10B or Coomassie Blue R250. Quantitation of stained zones can be accomplished by densitometry or dye-extraction methods. The latter methods are operationally simple, avoid the necessity of measuring peak areas, and allow direct measurement of bound dye.

A method is presented for quantitating electrophoretically separated protein after staining with Coomassie Blue R250 based on extraction of dye into a mixture of 1-butanol and 0.1 M phosphate buffer (pH = 7.8) (1). The method is rapid, with extraction complete in 1 hour. The equilibrium constant for the distribution of dye between the butanol and aqueous phases is estimated to be 538 ± 168. Using this method, the dye protein molar ratio for increasing protein loads in pore-limit electrophoresis was found to be constant for ovalbumin, to decrease for bovine serum albumin, and to increase for catalase. These data indicate the importance of known dye-binding curves in quantitative electrophoresis. Further, at a fixed protein load, optimum dye binding for bovine serum albumin, ovalbumin, and catalase occurred at different stain concentrations between 0.5 and 2 mM. The results suggest that maximum dye binding occurs at a unique stain concentration for each polyacrylamide gel concentration and that the dye concentration for maximum binding by protein decreases with increasing acrylamide concentration (Figure 1).

Preliminary experiments using bovine serum albumin, ovalbumin, and cytochrome C separated by sodium dodecyl sulfate (SDS) electrophoresis and stained with Coomassie Blue R250 suggest that this dye-extraction method is also useful for quantitating protein zones after SDS electrophoresis. This extraction method may be applicable to quantitation of all forms of electrophoresis in which staining is used.

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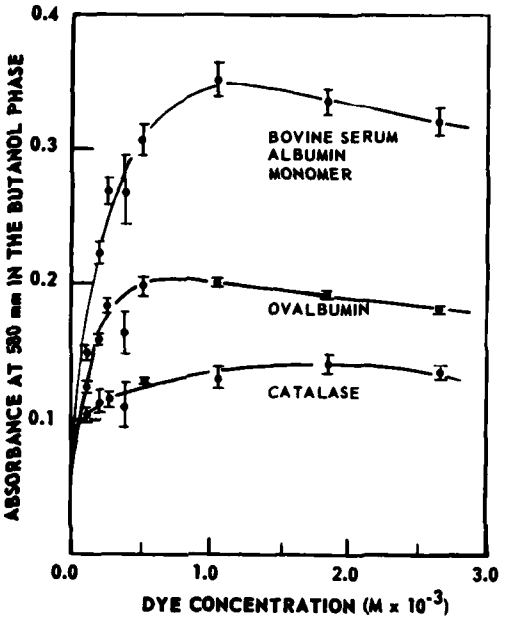

Figure 1. Effect of dye concentration on Coomassie Blue R250 bound by fixed protein load [bovine serum albumin (monomer), 44.9 x 10^{-11} mole; ovalbumin, 44.7 x 10^{-11} mole; catalase, 12.5 x 10^{-11} mole] after pore-limit electrophoresis. Each point represents the mean ± S.D. of five separate measurements.

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EFFECT OF HIGH-POWER-DENSITY MICROWAVE IRRADIATION ON THE SOLUBLE PROTEINS OF THE RABBIT LENS

Principal Investigators: G. M. Oosta and N. S. Mathewson
Technical Assistance: K. M. Hartley, C. J. Morrisey, and A. E. Cummings

Both ionizing and nonionizing radiations at sufficient exposure levels can result in the formation of cataracts. In both cases, the dose-response relationship for lens damage is not well-known, particularly in the low-dose region. Establishment of precise dose-response relationships is hampered by insufficient understanding of the molecular events (lens damage) that precede the development of an opacity. It has been proposed

that opacity formation results from either lens protein aggregation or changes in the long-range order of lens proteins.

In the present study (1), New Zealand rabbits were irradiated on the left side of the head by microwaves (2.45 GHz) at 300 mw/cm^2 for 20 min on each of 2 consecutive days. Lens changes in irradiated animals, observed by biomicroscopy, ranged from no changes to small posterior subcapsular opacities. Pore-limit electrophoresis was used to examine the distribution of soluble lens proteins. Marked differences in soluble protein distribution of the lens cortex and nucleus were observed. Comparison of irradiated and control lenses revealed an apparent shift toward higher-molecular-weight components in the cortex samples of the microwave-irradiated lenses. These observations are discussed in terms of the current models of lens opacity formation, and tend to support the idea that aggregation is an important factor in the microwave cataract.

The results of our study indicate that pore-limit electrophoresis is a technique that can be applied to detect lens protein alterations even if changes are in localized regions of the lens. Using this technique, a more detailed understanding of the early stages of protein involvement in opacity formation can be obtained.

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OPTIMIZATION OF FOLIN-CIOCALTEAU REAGENT CONCENTRATION IN AN AUTOMATED LOWRY PROTEIN ASSAY

Principal Investigators: G. M. Oosta, N. S. Mathewson, and G. N. Catravas

Measurement of protein concentration by the method of Lowry *et al.* (1) has proven useful for samples with low protein concentrations, and a number of modified or automated procedures are available (2,3). In the assay, color is produced when the Folin phenol reagent is mixed with protein solution previously treated with alkaline Cu(II) tartrate solution. Despite the wide use of the Lowry *et al.* assay, we found that the concentration of the Folin phenol reagent had not been adequately optimized. We report an automated method for determining protein concentration in which the final concentration of Folin phenol reagent in the assay mixture is reduced by 45% from the conditions used by Lowry *et al.*

The manifold for the automated Lowry assay is shown in Figure 1. Using this assay procedure, samples with concentrations as high as 400 μg/ml or as low as 1 μg/ml can be reproducibly assayed.

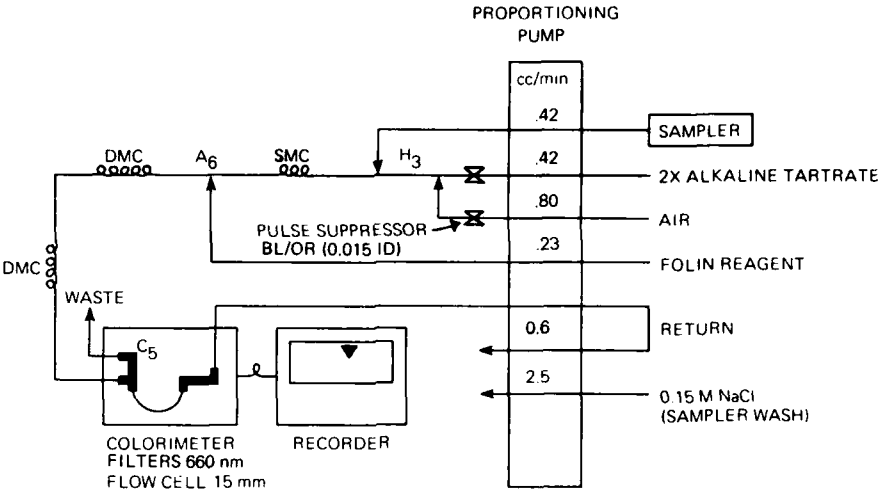

Figure 1. Flow manifold for the automated Lowry assay. Assay uses components of the Technicon AutoAnalyzer I system. The sampling rate is 50/hour with a sample-to-wash ratio of 2:1. All glass connections were used after connector H3. Both pulse suppressors were 0.005 inch inner diameter.

The dependence of assay color yield on the concentration of phenol reagent for solutions of bovine serum albumin, catalase, and Monitrol I, a serum substitute, is shown in Figure 2. Extrapolation of the linear segments suggests that maximum color development occurs when the phenol reagent concentration is 11% of the stock concentration. Maximum color development is decreased to 10% of stock phenol reagent concentration when the protein sample concentration is lowered from 400 μg/ml to 40 μg/ml. For this reason, a phenol reagent/water mixture of 3:23 is recommended for routine measurement of protein concentration.

In order to assay samples with low protein concentration, Lowry *et al.* (1) recommended in their assay protocol mixing 0.5 ml sample, 0.5 ml double-strength alkaline-Cu(II)-tartrate solution, and 0.1 ml phenol reagent (1 N). Under these conditions, the final concentration of the phenol reagent is 0.091 N. We find that maximum color development occurs when the final concentration of phenol reagent is 0.05 N. In addition, we find that the phenol reagent concentration for maximum color development is

the same for three diverse sample types. Thus, it seems likely that once the phenol reagent concentration is optimized for one sample, the same conditions are applicable to a wide variety of other protein samples.

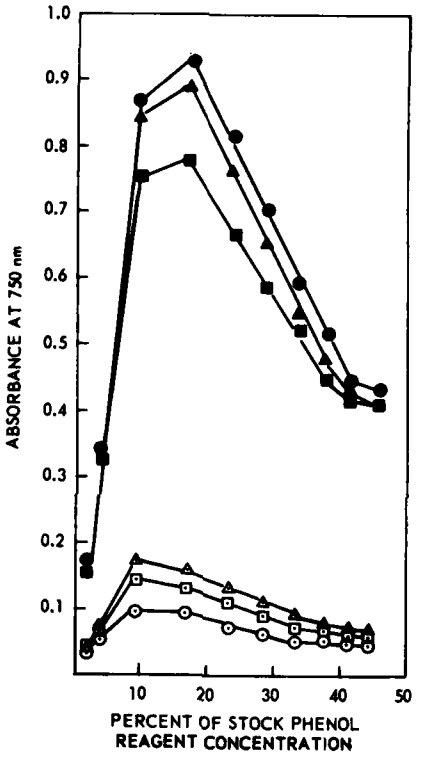

Figure 2. Variation in color yield vs phenol reagent concentration. Stock phenol reagent concentration is 2 N. Samples are Monitrol I (squares), catalase (circles), and bovine serum albumin (triangles). Protein concentration for solid figures is 400 μg/ml and 40 μg/ml for open figures. Samples were run at the rate of 20/hour. Steady-state values were achieved under these conditions.

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MICROWAVE EXPOSURE ARRAY: IMPROVED FIELD MEASUREMENTS

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

Previously we reported the results of testing quinine-coated Styrofoam and Plexiglas cages in a microwave field (1). We also described the design of a microwave exposure array for multiple animal exposure at equal power density (2). The physical size of the probe used in the original evaluation of the cages prohibited extensive field measurements. The recent availability of a miniature isotropic field probe (3) has permitted more complete measurements of the fields in the interior of the cages and also allowed improved measurements of the entire microwave exposure array.

In this study the probe was oriented so that its center dipole was parallel to the E field vector. It was inserted using slide assemblies through 1-cm holes placed in the walls of the cage for this purpose. Measurements were conducted in the same anechoic chamber previously used at the Walter Reed Army Institute of Research. Characteristics of the microwave source and the anechoic chamber have been previously described (2).

After the ten cages were positioned (Figure 1), measurement was made of each cage with all cages empty in order to make baseline readings. Then nine 200-g Sprague-Dawley rats were introduced, one to a cage; the tenth cage contained only the probe. As microwave was directed to all cages, the probe measured exposure to the one empty cage. This procedure was repeated so that each cage 1 through 6 could be measured as the empty cage. Measurements were not made in cages 7 through 10 because they were positioned symmetrically to cages 3 through 6 and thus received exposures identical to them.

Results of the field measurements of the complete array are shown in Figure 2. Although the power of the exposure array varied by as much as 23% from the average power density in the cages farthest from the antenna (due to scattering from the moving rats in other cages), the average value in any cage varied by no more than 5% from the composite averages of all cages.

The improved measurements made possible by the miniature isotropic electric field probe show that the array described provides significant advantages to many of the facilities for multiple animal exposure currently in use by providing equal average power density exposures to multiple animals.

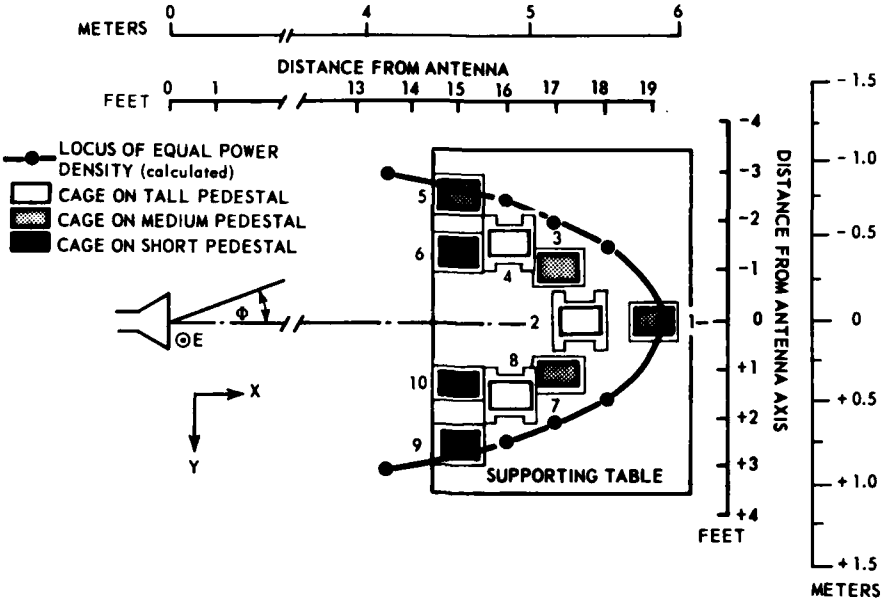

Figure 1. Locus of equal power density in the H plane through the axis of the transmitting antenna

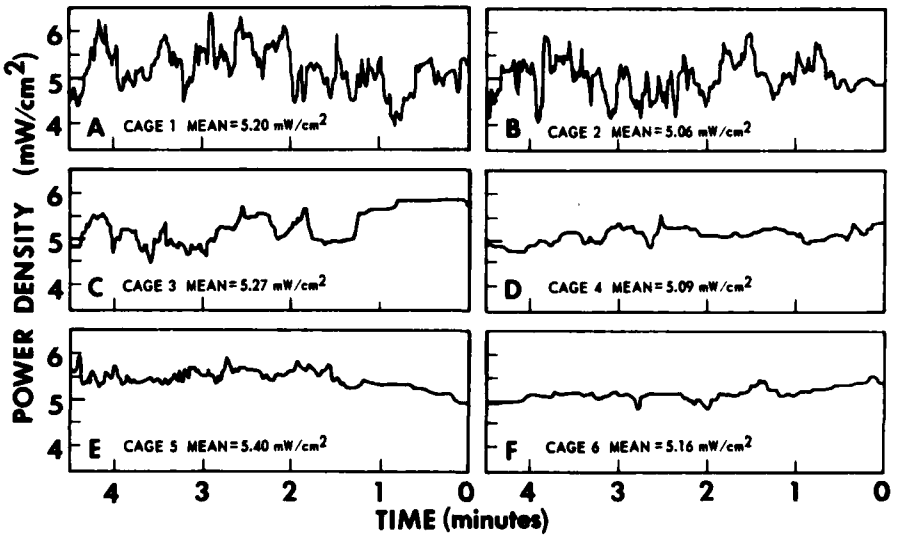

Figure 2. Power density in given cage with rats in all other cages

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EXPERIMENTAL HEMATOLOGY DEPARTMENT

The Experimental Hematology Department studies the blood cell-forming system as affected by ionizing radiation alone or in combination with other injuries. Personnel exposed to radiation doses of up to 1000 rads are injured or die, depending primarily on the extent of damage to this biological system. In addition, recovery from other injuries sustained concurrently may be greatly affected, particularly if such recovery depends on the availability of bone marrow cells or their products. The objectives of the Department are to conduct research to (a) determine the precise interaction of radiation with blood cell precursors, (b) assess the decreased capability of damaged bone marrow to recover from secondary injuries, and (c) develop treatment to ameliorate radiation and combined injury.

Department hematologists have discovered additional evidence that indicates more clearly that production of the highly radiosensitive white blood cells depends on a dual positive and negative feedback system. It is hoped that this information will permit development of therapeutic measures for improved cellular production postirradiation. Studies have been initiated to determine the precise involvement of the various blood cells in injuries such as wounds, burns, infection, and intoxication. Once this has been determined, procedures will be developed to make possible the increased production of specific cells after radiation and combined injury.

Microbiologists in the Department have continued to progress in their efforts to delineate the involvement of blood elements in the fight against infection after radiation exposure. With the assistance of physiologists in the Department, they are presently testing the efficacy of transfused, cryopreserved, white cells in decreasing bacterial infection.

The most difficult and complex task is to discover means of transplanting bone marrow from genetically unrelated donor animals or humans. Immunologists have learned a great deal about cellular markers that enable specific immune cells to differentiate their own cells from foreign cells, which they destroy. The immunologists must now discover ways to change the recognition system so that certain transplanted beneficial cells will be accepted. It must be remembered that once radiation destroys all bone marrow blood stem cells, the transplantation of donor bone marrow is the only therapy. In this direction, promising experimental results were obtained during the year.

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DETECTION OF MONOCYTE-MACROPHAGE COLONY-FORMING CELLS IN MURINE BONE MARROW, SPLEEN, AND PERIPHERAL BLOOD

Principal Investigators: T. J. MacVittie and M. Porvaznik

Recently another class of murine *in vitro* colony-forming cell was detected that is assumed to be a progenitor cell solely committed to the monocyte-macrophage line of differentiation. This unique colony-forming cell has been detected in stimulated peritoneal exudate and pleural effusion as well as the alveolar spaces (1-3). We recently reported (4) the detection of these cells within thymus and lymph node tissue through the specific use of a factor(s) within an extract of pregnant mouse uterine tissue. We hypothesize that these colony-forming cells migrate through the hematopoietic system and therefore are present in the bone marrow, peripheral blood, and splenic tissue.

Cell suspensions were prepared from femoral-derived bone marrow and splenic tissue of adult, male and female, B6D2F1/Cum BR mice. Peripheral blood mononuclear cells were obtained by the Ficoll-Hypaque technique. Triplicate cell cultures were grown using the double-layer agar technique in the presence of pregnant mouse uterine extract or mouse L-cell-conditioned medium as a source of colony-stimulating activity.

Monocyte-macrophage colony-forming cells were detected in bone marrow, splenic tissue, and peripheral blood cell suspensions. They exhibited several characteristic parameters similar to those reported for colony-forming cells derived from peritoneal exudate, pleural effusion, alveolar spaces, thymus, and lymph node cell suspensions. Bone marrow-, splenic tissue-, and peripheral blood-derived colony-stimulating cells did not initiate colony formation until after 16-18 days of culture. Maximum values in colony formation were reached after 28-30 days of incubation. The relative concentrations of colony-forming cells were: bone marrow, 4010 ± 160; peripheral blood, 2430 ± 960; splenic tissue, 491 ± 130 per 10^6 cells cultured. Respective total colony-forming cells per organ were 8×10^4/femur and 7.4×10^4/splenic tissue. Colony formation was linearly related to the number of bone marrow, splenic tissue, or peripheral blood cells cultured. Morphologically, by both light microscopy and electron microscopy, all the cells examined in bone marrow-, splenic tissue-, and peripheral blood-derived colonies were mononuclear, monocyte-macrophage type. Pregnant mouse uterine extract had significantly greater colony-stimulating activity than did mouse L-cell-conditioned medium. These data supported the hypothesis and indicated the presence of a factor(s) in pregnant mouse uterine extract capable of inducing colony formation from a heretofore undetected population of monocyte-macrophage progenitor cells within mouse bone marrow, splenic tissue, and peripheral blood.

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IN VIVO COLONY-FORMING UNIT POPULATION SIZES IN HYPERTRANSFUSED *Sl/Sl*^{d} MICE

Principal Investigator: K. F. McCarthy

As a result of a genetic defect expressed in the stroma of the tissues supporting hematopoiesis rather than in the hematopoietic cells themselves, mice of genotype *Sl/Sl*^{d} (Steel, Steel-Dickie mutant mice) suffer a chronic macrocytic anemia and are extremely sensitive to ionizing radiation (1). Previously it was hypothesized that the genetic defect disturbs erythropoiesis very early in the erythron, perhaps at the point of commitment of *in vivo* colony-forming units to the erythrocytic cellular line of differentiation (2,3). In an earlier study (4), this hypothesis was tested by measuring and comparing, in *Sl/Sl*^{d} mice and their congenic +/+ littermates, population sizes of high self-renewal potential and low self-renewal potential colony-forming unit. It was reasoned that a block in stem cell differentiation occurring early in the erythron would result in a deficiency of the latter but not of the former. However, our study did not bear out this hypothesis. Rather, it led to the unexpected observation that all the stem cell populations in *Sl/Sl*^{d} mice, with the exception of the splenic colony-forming unit population, were reduced in size.

However, anemia and other forms of hematopoietic stress are known to initiate substantial increases in extramedullary but not medullary colony-forming unit population sizes (5,6). Also, *Sl/Sl*^{d} mice suffer a chronic macrocytic anemia. So it was reasoned that a comparison of the effects of *Sl* and + genes on colony-forming unit population sizes might be more meaningful if the comparison were undertaken not only on the same genetic background but also under similar physiologic conditions in which the red blood cell concentrations of +/+ and *Sl/Sl*^{d} mice are approximately the same. Therefore, in the present study, anemic *Sl/Sl*^{d} mice and normal +/+ mice were rendered polycythemic by hypertransfusion, and the sizes of their colony-forming unit population were determined.

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COLONY-STIMULATING ACTIVITY PRODUCTION IN IDIOPATHIC APLASTIC ANEMIA

Principal Investigators: T. L. Weatherly, T. A. Fleisher, and D. M. Strong

The ability of mononuclear cells from seven patients with idiopathic aplastic anemia to produce colony-stimulating activity was studied. Colony formation in agar gel by cryopreserved human bone marrow cells from a single bone marrow transplant donor was used to assess colony-stimulating activity of paired patient- and control-conditioned media. Conditioned media were added to the cultures in a concentration of 7.5% (v/v). To prepare conditioned media, mononuclear cells were separated from 10-30 ml of peripheral blood by Ficoll-Hypaque sedimentation and incubated in suspension culture (10^{6} cells/ml) with 0.1% phytohemagglutinin for 4 days. Eleven experiments to compare colony-stimulating activity of seven pairs of conditioned media were performed. Shown in Table 1 are average results of colony formation in the agar cultures performed in triplicate. Using the t-statistic for paired data, the differences in colony

formation stimulated by patient- and control-conditioned media were highly significant ($p > 0.002$). Reduced colony-stimulating activity production by patient mononuclear cells was not due to impaired mitogen responsiveness, since ^{3}H-thymidine uptake in the presence of phytohemagglutinin, pokeweed, and Con-A were normal in five of seven patients tested. Cocultures of patient and control mononuclear cells or bone marrow with normal bone marrow did not inhibit colony formation. The relevance of these findings to the pathobiology of idiopathic aplastic anemia remains to be determined.

Table 1. *In Vitro* Colony Formation* Stimulated by PHA-Activated Lymphocyte-Conditioned Medium (ALCM) Derived From Patients With Aplastic Anemia Versus Controls†

Patient	No. Colonies ± 1 S.D.	Control ± 1 S.D.	Patient %
1	13.7 ± 2.5	20.0 ± 5.0	68.5
2	21.0 ± 3.6	83.3 ± 11.0	25.2
3‡	21.0 ± 7.0	36.3 ± 10.8	64.8
3	4.7 ± 2.5	21.7 ± 6.0	21.7
4‡	28.0 ± 10.1	50.0 ± 6.9	56.0
4	10.3 ± 4.5	41.3 ± 5.5	24.0
5‡	60.3 ± 2.3	57.0 ± 7.5	105.8
5	14.3 ± 1.2	31.7 ± 0.6	45.1
6‡	36.3 ± 4.0	57.0 ± 7.5	63.7
6	13.7 ± 3.5	31.7 ± 0.6	43.2
7	42.7 ± 5.5	67.3 ± 1.5	63.4
8	33.0 ± 5.3	62.7 ± 2.5	52.6

* Colonies per 10^5 nucleated bone marrow cells

† Mean values ± S.D. of triplicate cultures

‡ Double assay of patient ALCM against additional control ALCM

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MEMBRANE POTENTIAL CHANGES IN LEUKOCYTES EXPOSED TO CHEMOTACTIC FACTORS

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

Both macrophages and polymorphonuclear leukocytes are important in host defense reactions. During exposure to toxic agents such as irradiation, these cells help initiate and participate in inflammatory and immune responses necessary for recovery of the exposed aremol. We have been interested in studying leukocyte physiology during activation of the immune response and, in particular, in studying membrane potential and permeability changes.

The electrophysiology of chemotactic factor interaction with cultured human macrophages was investigated using standard intracellular recording techniques (1,2). In initial studies, when *E. coli* endotoxin-activated serum was added to cell cultures during intracellular recordings, it caused membrane hyperpolarizations greater than 30 sec in duration and 10-50 mV in amplitude, which were associated with decreased membrane resistance. Fractionation of normal activated serum by molecular sieve chromatography (G-75 Sephadex) indicated that only fractions that elute with an estimated molecular weight of 12,500 produced membrane potential changes. The active material that was chemotactic for the macrophages was identified as the small-molecular-weight cleavage product of C5, C5a, by heat stability (30 min at 56°C) and inactivation by goat anti-sera to human C5 but not C3. Mg (5 mM)-EGTA (1.5 mM) blocked the C5a-evoked potential changes, whereas colchine (10^{-6}M) and cytochalasin B (3.0 μg/ml) did not. Hydrocortisone sodium succinate (0.5 mg/ml) decreased the percentage of cells responding to C5a. In related studies, synthetic N-formyl methionyl peptide (f-met-leu-phe), which had chemotactic activity for cultured macrophages, produced similar membrane potential changes. These observations demonstrate that ion fluxes associated with membrane potential changes are early events in macrophage activation by chemotactic factors.

In addition to the studies using electrophysiological techniques to monitor membrane potential changes in leukocytes, we have also used the fluorescent dye depentyloxacoibocyanese [di-O-C5(3)] to measure membrane potential changes during exposure to activating substances such as chemotactic factor (3). A biphasic fluorescence change (a decrease in fluorescence followed by an increase in fluorescence) occurred immediately after exposure to concentrations of peptide, which produce chemotaxis in these cells. Both phases of the fluorescent response were inhibited by 5 mg/ml cytochalasin B, which inhibits cell motility, and 10^{-5}M formyl-phe-met, which inhibits the chemotactic response to FMLP. The second phase of the fluorescent response was blocked by

5 mM EGTA and was sensitive to changes in external K^+. Our data support the view that exposure of PMN's to chemotactic factors results in changes in the ionic permeabilities and membrane potential of these cells.

We hypothesize that membrane potential changes produced by chemotactic factors in leukocytes are the triggering mechanism for immunologically activating the cell (4). It is our contention that understanding the process of leukocyte activation can lead to the ability to manipulate leukocyte function in order to enhance the immune response of an animal during inflammation.

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RECOVERY, STRUCTURE, AND FUNCTION OF DOG GRANULOCYTES AFTER FREEZE PRESERVATION WITH DIMETHYLSULFOXIDE

Principal Investigators: J. E. French, W. J. Flor, and M. P. Grissom
Technical Assistance: J. L. Parker, G. Sajko, and W. G. Ewald

The recovery, structure, and function of dog granulocytes were determined before and after freeze preservation (1). Leukocytes were isolated from defibrinated or anticoagulated whole blood and subsequent erythrocyte sedimentation on a column of 2:1 Dextran-Isopaque (6% and 33.9%). Granulocytes isolated by these procedures were examined for changes in oxygen consumption associated with phagocytosis, *in vitro* directed migration (chemotaxis), bactericidal activity, and ultrastructure before and after freezing.

Granulocytes were frozen in dimethylsulfoxide (DMSO) (7.5%) and autologous serum or Hanks' balanced salt solution and 20% autologous serum at the rate of -1°C/min to -80°C and stored in liquid nitrogen vapor. After freeze preservation, oxygen consumption associated with phagocytosis was decreased by 54% and 64% for granulocytes isolated from defibrinated blood and from blood anticoagulated with acid-citrate-dextrose (ACD), respectively. Bactericidal activity was only slightly depressed in samples from either isolation method after freeze preservation when compared to the prefreeze controls, but granulocytes isolated from defibrinated blood were significantly less effective in killing bacteria than those from ACD-anticoagulated blood. Chemotactic response after freeze preservation was completely inhibited in granulocytes isolated from defibrinated blood. Exposure of granulocytes to ACD inhibited chemotaxis prior to freezing, but the granulocytes responded chemotactically after freeze-thaw and additional washing. The ultrastructures of granulocytes observed before and after freeze-thaw were similar for cells isolated by both methods. However, the nuclear, cytoplasmic, and granular changes observed were slightly greater in granulocytes isolated from defibrinated blood. Dog granulocytes isolated by either method withstood freeze preservation in DMSO to a degree not previously reported. It is concluded that dog granulocytes freeze-preserved by these methods are functional *in vitro*, but that phagocytic, directed migration as well as bactericidal functions and ultrastructure are impaired to different degrees, according to the method of isolation and preparation for storage. These results indicate the need for continued investigation of the effects of storage variables on the preservation of granulocytes.

The increased efficacy of granulocyte transfusions in conjunction with antibiotic therapy against microbial infection during agranulocytosis or granulocytopenia has been well documented. An effective method for granulocyte preservation would minimize logistical problems (typing, preservation, matching, and transportation) in increasing the availability of this therapy to combat casualties. To meet such a requirement, this model system for studying granulocyte-preservation methods was developed.

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PLATELET AGGREGATION IN RABBITS MADE TOLERANT TO ENDOTOXIN

Principal Investigator: R. I. Walker
Technical Assistance: L. J. Shields, J. R. Fletcher, and D. A. Walden

Endotoxin may cause abnormal deposition of platelet-endotoxin aggregates, and this event could have damaging effects. We compared the aggregation characteristics of platelets from rabbits that had been made tolerant to the lethal effects of endotoxin to those of platelets from normal rabbits. Platelets from tolerant rabbits aggregated more rapidly (>90 sec faster) in the presence of endotoxin than did platelets from non-tolerant animals. Furthermore, platelets from tolerant animals aggregated reversibly. We concluded that these characteristics of platelets from tolerant animals are due to humoral factors in the plasma, since 1:1 dilution of normal platelet-rich plasma with plasma from tolerant rabbits causes the normal platelets to behave similarly to those from tolerant animals. Survival after challenge with lethal quantities of endotoxin was enhanced in tolerant rabbits; this may be due to promotion of more efficient removal of endotoxin-platelet complexes from the blood by the reticuloendothelial system.

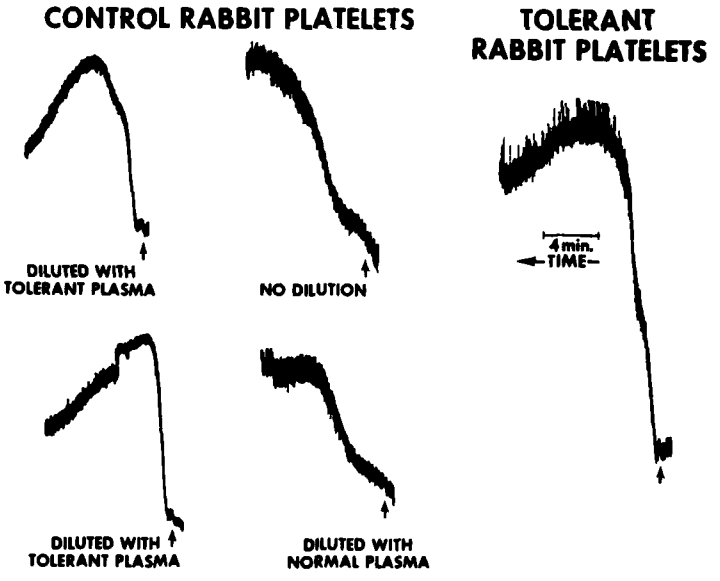

Figure 1. Alteration of aggregation characteristics of platelets from non-tolerant rabbits by dilution with plasma from tolerant rabbits. Each arrow indicates point at which endotoxin was injected into the cuvette.

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EVIDENCE FOR INHIBITION OF LEUKOCYTE CHEMOTACTIC RESPONSE TO ENDOTOXIN BY HUMAN PLATELETS

Principal Investigators: J. M. Sheil and R. I. Walker

Human blood platelets have a strong affinity for endotoxin. We tested human platelets for their ability to influence the chemotactic response of human polymorphonuclear cells to endotoxin-activated normal plasma. In the absence of endotoxin, polymorphonuclear cell chemotaxis in response to platelet-rich plasma was 43% less than that of platelet-poor plasma. When 0.025 μg of *Salmonella typhosa* endotoxin was added to platelet-poor plasma, polymorphonuclear cell chemotaxis was increased 60% above unactivated plasma. Activation of platelet-poor plasma with 0.05-100 μg endotoxin increased polymorphonuclear cell chemotaxis only around 25%. There was a biphasic increase in polymorphonuclear cell chemotaxis to endotoxin-activated platelet-rich plasma. Addition of 0.025-0.50 μg endotoxin to platelet-rich plasma increased chemotaxis by 50%-85%. However, platelet-rich plasma activated with 1-2 μg endotoxin resulted in an increased polymorphonuclear cell chemotactic response of only 30%-40%. With 5-100 μg endotoxin in platelet-rich plasma, polymorphonuclear cell chemotaxis was increased by 65%-100%. No similar decrease in polymorphonuclear chemotaxis was observed when $CrCl_3$-treated (attenuated) endotoxin was added to platelet-rich plasma. These data support the hypothesis that human platelets may influence endotoxin-induced inflammation by inhibiting the chemotactic responsiveness of polymorphonuclear cells.

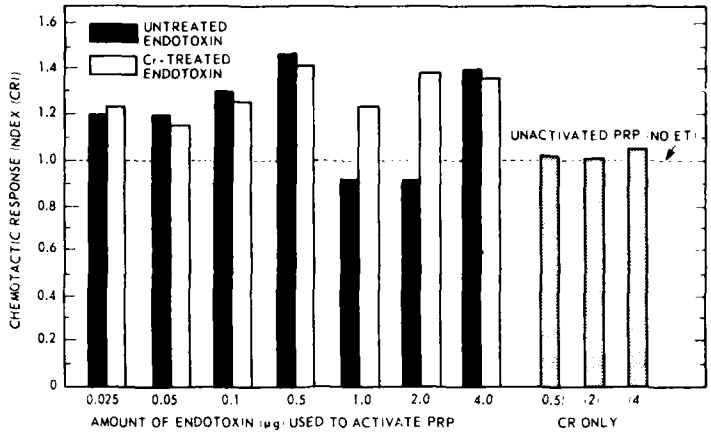

Figure 1. Chemotactic response index (CRI) for polymorphonuclear cell migration to platelet-rich plasma (PRP) activated with different amounts of either untreated or $CrCl_3$-treated endotoxin. CRI was obtained by dividing the number of polymorphonuclear cells migrating in response to activated PRP by the number responding to unactivated PRP. The CRI value of 1.0 represents the response to unactivated PRP. Addition of $CrCl_3$ alone had no effect on polymorphonuclear cell chemotaxis. Significant differences between responses to PRP activated with .ntreated or treated endotoxin were obtained at 1 and 2 μg. Each bar represents the average of five replicate experiments.

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MATURATION AND DIFFERENTIATION OF IMMUNE COMPETENCE IN MOUSE RADIATION CHIMERAS

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

Radiation chimeras (lethally irradiated animals transplanted with hemopoietic cells) will survive indefinitely if donor and host animals are genetically similar (syngeneic).

However, genetic differences between donor and host animals usually lead to complications such as graft-versus-host disease and infection, which culminate in the death of the host. Immune recovery is important to the health and survival of these animals, but it 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/6xCBA) 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 each of three successive monthly intervals following engraftment, immune reactivity of spleen cells harvested from chimeras was measured by (a) *in vitro* responsiveness to phytohemagglutinin and lipopolysaccharide, (b) *in vitro* mixed lymphocyte reaction, (c) *in vivo* graft-versus-host reaction, and (d) 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 1). 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% 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 to host (B6CBF1) C57BL/6 H-2 antigens induced in the semiallogeneic combination.

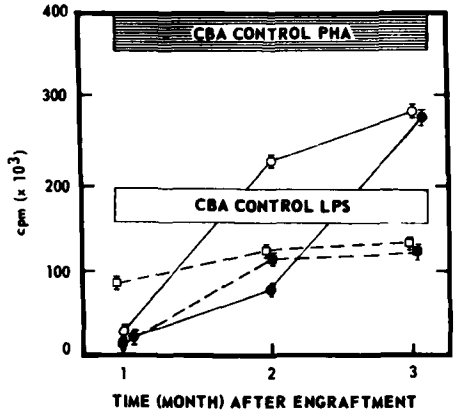

Figure 1. Counts per minute (cpm) of normal CBA or chimeric spleen cells (SPC) stimulated with either phytohemagglutinin (PHA) or lipopolysaccharide (LPS). The symbols and lines used are:
○—○, chimeric CBA spleen cells and PHA;
●—●, chimeric B6CBF1 SPC and PHA;
□—□, chimeric CBA spleen cells and LPS;
■—■, chimeric B6CBF1 spleen cells 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.

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EXPERIMENTAL MODEL OF CHRONIC COMMUNICATING HYDROCEPHALUS

Principal Investigator: W. J. Flor, *AFRRI*
Collaborator: A. E. James, Jr., *Vanderbilt University Hospital, Nashville, TN*
Technical Assistance: J. L. Parker, W. G. Ewald, and E. L. Barron, *AFRRI*

Chronic communicating hydrocephalus (also known as normal-pressure hydrocephalus) is an adult form of hydrocephalus that can develop secondarily to head trauma, central nervous system infection, intracranial surgery, or spontaneous subarachnoid hemorrhage. The military population is as much at risk to these cerebral insults as the civilian population, and often more so. This disease process is a progressively debilitating one in which the ventricles within the brain (which contain cerebrospinal fluid) expand at

the expense of the surrounding neural tissue. Surgical ventricular shunting is the current method of treatment, but it is successful in only a limited subpopulation of patients, and only a minimal number of surgical successes survive for 5 years.

To investigate the pathophysiology underlying the development of the disease, we have established two animal models of this syndrome in which to study the mechanisms responsible for disease progression and to evaluate alternative methods of treatment after the disease mechanisms are understood. These models have been reported previously (1).

We have recently published an extensive discussion of our research results to date (2). We hypothesize that the basic underlying mechanism in development of chronic communicating hydrocephalus is an imbalance of cerebrospinal fluid production and absorption or drainage. The pathophysiological changes observed in this disease process appear to be attempts to resist the derangements caused by diminished absorption of cerebrospinal fluid. The mechanisms by which these compensations occur and the time course of their development are not completely understood. In this report, we present the gross pathology and histopathology of the animal model as well as results of our experiments to date in the following areas: (a) changes in cerebrospinal fluid pressure during disease development and in cerebrospinal fluid flow by cisternographic imaging, (b) alteration in cerebrospinal fluid production at various times during disease progression, (c) metabolism of choroid plexus cells from normal and from hydrocephalic animals *in vitro*, (d) gross and microscopic studies of the distribution of radionuclide tracers injected into the cerebrospinal fluid spaces, and (e) clearance of radionuclide tracers from the cerebrospinal fluid space. This animal model successfully recreates the clinical disease as seen in the human. It develops over a chronic time course, it can be evaluated by the diagnostic studies used with human patients, and it makes possible the study of early phases of disease development before gross clinical abnormalities are observed in the animals.

As an adjunct to the research described above, we have developed a chronic technique for sampling and monitoring cerebrospinal fluid (3). It is necessary to have reliable access to the cerebrospinal fluid spaces of animals used for a variety of neurophysiological, neuropharmacological, and neurotoxicological studies. This is to record cerebrospinal fluid pressures, to sample cerebrospinal fluid over a chronic time frame, or to administer drugs on test compounds. The technique of fourth ventricular catheterization using subcutaneous cerebrospinal fluid reservoirs in rhesus monkeys does the following: (a) provides chronic access to sterile cerebrospinal fluid without chronic immobilizations, (b) enables mixing of injected drugs with lateral ventricular cerebrospinal fluid, (c) permits sensitive monitoring of intraventricular pressure, and (d) does not produce tissue damage during cannula implantation or breakdown of the blood-brain barrier.

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NEUROBIOLOGY DEPARTMENT

The Neurobiology Department was established in late 1972. It has been comprised of three divisions with different approaches to the study of the nervous system. The Neurophysics Division uses conventional electrophysiology techniques to study simple nervous systems such as those from snails, lobsters, and frogs. The Cellular Neurobiology Division uses tissue culture techniques to study the nerve cells from mammals but in isolation from the animal and growing in an artificial medium. The Neurological Sciences Division, on the other hand, primarily investigates the intact brain of several mammalian species. The objective of the Neurobiology Department is to describe and elucidate the mechanisms whereby ionizing radiation interferes with nervous system function.

The nervous system is relatively resistant to the effects of ionizing radiation. However, at high doses the nervous system is quite dramatically affected, and the primary symptoms and the cause of death at sufficiently high doses are attributable to the direct action of radiation on the brain.

The effects of radiation on nervous tissue fall into three major areas. At relatively low doses, including levels that are used for therapeutic treatment of malignancies in humans, there is a pronounced fatigue following radiation exposure, which may last for many months. This fatigue is often very debilitating, even after exposure at doses that are not fatal, through secondary effects on gastrointestinal or hemopoietic systems. At present the mechanisms underlying fatigue are unknown, and indeed, it is not clear whether this is a central nervous system fatigue or some effects of the radiation at the neuromuscular junction. At exposures of 1000 rads or greater, which are lethal, the primary effects fall into two broad categories and are due to different mechanisms. In a variety of mammals, and particularly in primates, a period of early transient incapacitation (ETI) occurs shortly after exposure. In the monkey, ETI has a latency of 2 to 5 min and lasts for only a matter of minutes. During this transient episode, animals that were trained to perform tasks can function no longer. However, at the end of the period of ETI, the animal recovers function and can continue to perform efficiently until a final deterioration which leads to death. This final deterioration is known as the central nervous system (CNS) syndrome and is usually accompanied by a very rapid decompensation, coma, and death. The CNS syndrome may occur from a few to 48 hours postexposure, with considerable variation from animal to animal, but with a general dependency on radiation dose.

Although the mechanisms causing these effects of radiation on the central nervous system are not clearly understood, there are experimental leads for each of them. Both fatigue and the CNS syndrome may result from disturbances in regulation of intracellular calcium ion concentration. This conclusion is suggested by observations indicating that the effect of high doses of radiation on nerve cell membrane potential and resistance is identical to that occurring when calcium concentration is raised, although

it is not presently possible to measure the calcium concentration directly in an irradiated neuron. The study of fatigue and the mechanisms of control of intracellular calcium are major areas of investigation in the Neurophysics Division.

ETI, by virtue of its latency and its transient nature, appears most likely to result from the action of some humoral agent on the central nervous system. One possibility is histamine. It is a very active substance that affects the bronchi of the lungs, blood vessels in the brain and elsewhere in the body, and also has direct effects on neurons. Previous work in this laboratory has shown that histamine is released upon exposure to radiation. We are continuing to study the effects of histamine and are investigating the presence of receptors for histamine on smooth muscle cells, isolated neurons, and neurons in the central nervous system. Although it seems very likely that histamine is the causative agent, it is still not clear whether the primary site of action of histamine in causing ETI is the smooth muscle cells of the cerebral blood vessels or the neurons of the brain.

In whole-animal exposure to radiation, it is impossible to determine the relative radiosensitivity of most cellular elements of the body. This is particularly true for the various cellular elements in the central nervous system, which include nerve cells of different types, glial cells, and the smooth muscle cells of the cerebral blood vessels. A primary aim of the Cellular Neurobiology Division is to develop dividing cell lines of each of these types and to determine their relative sensitivities to radiation.

In an actual nuclear detonation, the damaging forces include not only radiation but also injury caused by blast and high temperatures. We have in the Neurological Sciences Division a number of investigations that approach various aspects of injury such as might result from blasts, with additional investigations on the combined effects of mechanical injuries and radiation. A major site of injury in a blast is the head and brain. Our laboratory for the study of experimental head injury is investigating questions such as: What kinds of movements of the head upon the neck are most likely to cause injury? Is the nervous system or the vascular system more susceptible to injury? How is the injury reflected in terms of alterations of cerebral electrical activity, cerebral blood flow, and the generation of cerebral edema? Other laboratories are concerned with the study of the cerebral regulation of the circulatory system and the disturbances that result in this system after radiation exposure alone or in combination with mechanical injury.

The Neurobiology Department sponsors and/or participates in a number of educational activities for its members and other interested personnel. These include the weekly AFRRI Neurosciences Seminar, a weekly research seminar, a weekly journal club, and often evening graduate seminar courses. At present the Department has six professionals who are supported by NIH postdoctoral fellowships and two who are supported by

National Research Council fellowships. During this year we have had four masters or Ph.D. graduate students doing their thesis research at AFRRI and receiving their degrees through the George Washington University School of Medicine, with which our Department is affiliated.

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EARLY TRANSIENT INCAPACITATION: A REVIEW WITH CONSIDERATION OF UNDERLYING MECHANISMS

Principal Investigator: D. O. Carpenter

Nervous tissue is relatively more resistant than other organ systems to damage by ionizing radiation probably because, unlike most other cells, neurons do not divide in adult mammals. However, exposure to ionizing radiation at high doses does affect nerve cell activity and can, at sufficiently high doses, lead to death from central nervous system depression within hours or a very few days of exposure. In addition, there are effects of large exposures to radiation that occur soon after exposure (within minutes) and are transient in nature. This phenomenon, called early transient incapacitation (ETI), has been the subject of extensive investigation in awake and behaving animals.

ETI has been observed in a number of (but not all) animal species after exposure to supralethal doses of radiation. The experience of several laboratories studying primates is summarized in Table 1. In some species ETI may be elicited by trunk-only irradiation, and it probably results from a direct effect of some released agent (possibly histamine) on the brain. ETI is accompanied by and may be caused by a fall of blood pressure and cerebral blood flow. Since radiation causes a release of histamine from mast cells and since histamine causes a fall in blood pressure and cerebral blood flow, ETI is probably a result of a direct action of histamine on blood vessel smooth muscle cells. In humans, injected histamine elicits an intense headache. Histamine headache may contribute to the incapacitation. However, the primary source of incapacitation in experimental animals is probably faintness caused by the fall in cerebral blood flow. An understanding of the mechanism of this phenomenon is essential for the development of a rational prevention of incapacitation of combat troups exposed to radiation.

Table 1. Behavioral Decrements of Primates Following Exposure to Ionizing Radiation

Dose	Task	NE	ETI*	Incap†	Ref
1,100 R	VD	9/10	1/10	0/10	1
1,700 R	VD	2/12	10/12	0/12	1
2,000 R	VD	5/14	5/14	4/14	2
2,500 R	VD	1/6	4/6	1/6	3
2,600 R	VD	9/39	23/39	7/39	1
4,200 R	SB	2/6	3/6	1/6	4
4,900 R	VD	1/15	12/15	2/15	1
5,000 R	VD	2/6	4/6	0/6	5
8,900 R	VD	0/5	2/5	3/5	1
10,000 R	VD	1/6	5/6	0/6	6
15,000 R	VD	0/7	2/7	5/7	7

* Performance below 80% within first 30 min; recovery to above 80% within 1 hr.

† Performance below 50% within 20 min and no recovery within 60 min.

Numbers under NE (no effects), ETI (early transient incapacitation), and Incap (incapacitation) show the number demonstrating that response over the total number irradiated. VD indicates a visual discrimination task, and SB indicates a shuttle box task.

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EFFECTS OF IRRADIATION OF *APLYSIA* PACEMAKER NEURONS WITH 20-MeV ELECTRONS

Principal Investigators: D. O. Carpenter, J. A. Willis, and R. Severance
Technical Assistance: G. Gaubatz

Aplysia pacemaker neurons are excited by irradiation with 20-MeV electrons. The response is an increase in discharge occurring immediately after exposure and decaying within a few minutes except at very high exposures (Figure 1). The threshold is of the order of 1000 rads, and cell inactivation and death occur acutely only at doses of the order of 200,000 rads. Within these limits the excitatory effect is more or less linear with dose. The effect is not associated with dramatic resistance changes, although the resulting depolarization indicates an increase in Na^+ permeability. Synaptic transmission in this preparation does not appear to be more sensitive than impulse propagation. At very high doses, spike generation is blocked. Neurons recorded for a number of hours following irradiation show a hyperpolarization prior to death, suggesting that one terminal event may be an accumulation of intracellular Ca^{++}.

These studies confirm and extend previous observations on the relative radioresistance of *Aplysia* neurons. With respect to mammalian studies on nervous system susceptibility to high doses of radiation, no events were found that correlate in time with early transient incapacitation (ETI). However, the depressed excitability occurring several hours after exposure may correlate with the occurrence of death due to the central nervous system (CNS) syndrome seen in higher animals. The understanding of these elementary mechanisms after exposure to large doses of ionizing radiation may aid in the prevention and treatment of early CNS symptoms following exposure to high doses of radiation.

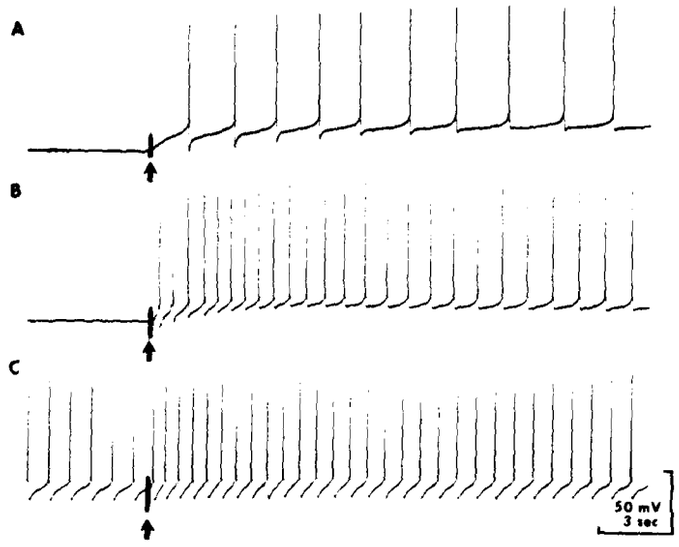

Figure 1. Excitation of pacemaker neurons by 20-MeV electrons. Intracellular recording was made in cell R_6. At the arrow the preparation was exposed to 10 pulses of 2600 rads focused radiation. *B* was taken 14 min later with the same exposure. After 15 min, discharge continued regularly at about 1 spike/2 sec. At this point ouabain (10^{-4} M) was added, which caused frequency to increase to about 1/sec. Record *C* was taken 15 min after ouabain addition. Although there is now spontaneous discharge, the effect of exposure is not changed.

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IONIC AND METABOLIC BASES OF NEURONAL THERMOSENSITIVITY

Principal Investigator: D. O. Carpenter

All passive ionic and metabolic processes of cells vary with temperature. In some neurons the temperature dependence of these processes is sufficient to cause dramatic changes in the neuronal activity as a function of temperature, although such neurons are not necessarily involved in temperature sensitivity or thermal regulation (Figure 1).

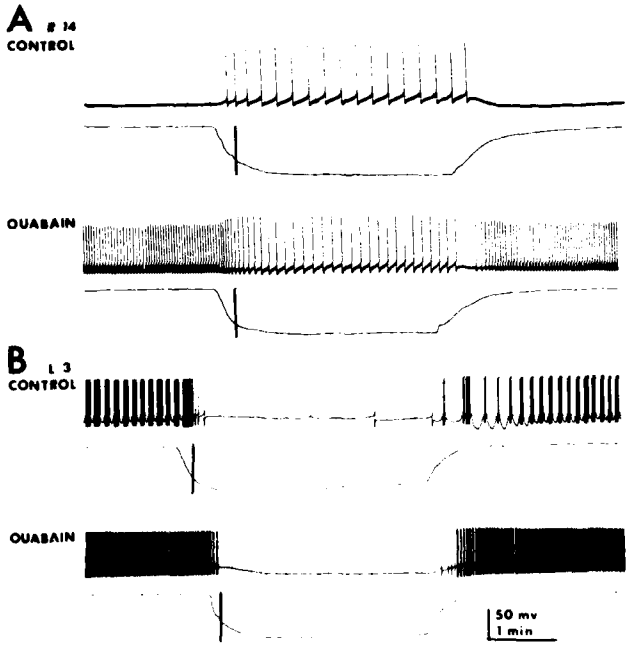

Figure 1. Discharge patterns on sudden temperature changes before and after ouabain in cells R_{14} and L_3. In each case the upper trace is the intracellular recording. The lower trace is temperature, where the vertical bar indicates 5°C and 20°C at lower and upper ends, respectively. In R_{14} the response changes from that of a "cold receptor" to that of a "warm receptor" following 10^{-4} M ouabain for 10 min. Cell L_3 in the control illustrates its normal bursting pattern. After ouabain, this pattern is altered, and discharge is more regular.

Three prominent temperature-dependent processes have been identified in invertebrate neurons. These include:

(a) Activity of the electrogenic sodium pump, which causes a membrane hyperpolarization with increasing temperature. In some neurons this may be as great as 2 millivolts/°C. Pump activity tends to decrease discharge with increasing temperature and is therefore a mechanism that may cause a neuron to function as a "cold receptor."

(b) Temperature dependence of passive ionic permeabilities and, in particular, temperature dependence of the $G_{Na}+/G_K+$ ratio. In *Aplysia* neurons, the $G_{Na}+$ has a higher Q_{10} than does the G_K+. As a result, the neuron tends to depolarize with increasing temperature. If this mechanism predominates, the neuron has properties of a "warm receptor."

(c) Dramatic temperature sensitivity shown by some synaptic potentials and responses to ionophoretic transmitter application. Thus the potassium conductance transmitter responses on *Aplysia* neurons are nearly abolished by cooling below 10°C whereas Na^+ and $C1^-$ responses are not. This mechanism might result in a selective blockade of some inhibitory inputs by cooling.

Several observations suggest that similar mechanisms may apply to mammalian neurons involved in thermal regulation and thermosensitivity. Experiments on specific cold-sensitive afferent fibers from rats' scrotal skin have demonstrated that the cold sensitivity is abolished by blockade of the electrogenic sodium pump. Consequently, this mechanism, although not unique to neurons involved in thermosensitivity, probably is the generator potential mechanism in at least some specific afferent fibers. However, other observations suggest the possibility that many neurons are thermosensitive even though not involved in central temperature regulation or detection. In a study of the temperature sensitivity of neurons in somatosensory motor cortex of the cat, up to 50% of unselected neurons were found to respond to temperature changes with Q_{10} greater than expected of passive membrane properties. These observations suggest that temperature sensitivity is a characteristic of many, and perhaps all, neurons. Consequently, great caution must be exercised before concluding that temperature-dependent changes in neuronal activity reflect utilization of this information in thermosensitive or thermoregulatory pathways.

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FUNCTIONAL MAPPING OF THE CENTRAL PROJECTIONS OF THE PIT VIPER THERMORECEPTOR AND VISUAL SYSTEMS USING ^{14}C-DEOXYGLUCOSE

Principal Investigators: C. R. Auker, R. M. Meszler, and D. O. Carpenter

The central projections of the infrared receptor and visual systems of two pit vipers, *Agkistrodon p. piscivorus* and *Crotalus horridus,* were mapped using ^{14}C-deoxyglucose after the method of Kennedy *et al.* (1). In addition to unaltered controls, snakes were prepared by surgical excision, under ketamine anesthesia (50 mg/kg), of eyes and/or facial pits, leaving: (a) one eye intact, (b) one facial pit intact, (c) one eye and the contralateral facial pit intact, or (d) no eyes or facial pits. After at least 2 days for recovery, the snakes were given 50 μCi 2-deoxy-D-glucose-1-C^{14} by intracardiac

injection and were exposed to visual and infrared stimuli for 75 min. The snakes were then decapitated, the brains immediately removed, frozen, and cut into 20-μ sections. Alternate sections were used for autoradiography or histology.

Autoradiographs of brains from snakes with one intact eye revealed projections of the visual system consistent with those described by others. In particular, there was strong asymmetry in the tectum with the greatest density over the contralateral side (Figure 1*B*).

In the infrared receptor pathway there was marked asymmetry over the nucleus of the lateral descending tract of V with the greatest density on the ipsilateral side. However, a similar asymmetry was not apparent in the tectum (Figure 1*A*).

This result is surprising in light of evidence from other laboratories indicating a projection of the infrared detectors to deep lazer of the tectum. We are clearly exciting the primary afferents since there is dense labeling in the descending nucleus. This procedure may be of great value in functionally mapping other afferent pathways.

Figure 1*A*. Autoradiogram of pit viper brain stem showing accumulation of ^{14}C-deoxyglucose in the nucleus of the lateral descending tract of V with infrared stimulation of the ipsilateral pit organ. *B*. Autoradiogram of pit viper tectum showing accumulation of ^{14}C-deoxyglucose in the superficial layers with stimulation of the contralateral eye.

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ORGANIZATION FOR RECEPTORS FOR NEUROTRANSMITTERS ON *APLYSIA* NEURONS

Principal Investigator: D. O. Carpenter

The neurons in the nervous system of the marine mollusc *Aplysia* have receptors for at least nine small putative neurotransmitter substances, including acetylcholine, dopamine, octopamine, phenylethanolamine, serotonin, histamine, glutamate, aspartate, and GABA. We have studied these cells using electrophysiological techniques with ionophoretic application of these putative neurotransmitters. At least three different types of response can be elicited on different specific neurons to each of these substances. These common responses are Na^+, $C1^-$, or K^+ conductance increases, respectively. Although all cells have receptors for acetylcholine, fewer than 50% of neurons have receptors for any one of the other substances, and for several of these substances, receptors are rare. However, identified single neurons (identified by size, color, position, and connections) always show the same profile of receptors. We propose a model of organization of these receptors in which the receptor (the transmitter-binding site) is one functional entity in the membrane that can be coupled to any of three functional ionophores (the membrane structures mediating the conductance changes). In this model, at least nine different and specific receptor proteins can be coupled with any of at least three ionophores. The evidence supporting this model consists of the following observations: (a) the structure-activity relations of agonists for one type of receptor is the same whether the response is an Na^+, $C1^-$, or K^+ conductance; (b) alpha-bungarotoxin blocks all three acetylcholine responses, binds to membrane homogenates with single K_D, and has the same K_D on identified neurons that show different acetylcholine responses; (c) the time course of the voltage changes is a function of the ionic conductance and not which transmitter activates its receptor (Table 1); and (d) some inhibitors appear to be blockers of the ionophore(s) rather than the receptors.

Table 1. Mean Time to Peak* of Responses of *Aplysia* Neurons to Different Putative Neurotransmitters

	IONIC REPONSE				
	Fast $\uparrow G_{Na^+}$	Slow $\uparrow G_{Na^+}$	$\uparrow G_{Cl^-}$	$\uparrow G_{K^+}$	$\downarrow G_{K^+}$
Ach	1.8	–	2.4	12.2	–
DA	2.3	–	2.5	15.1	–
GABA	2.0	12.3	2.4	13.1	9.8
Glu	2.3	–	2.5	15.2	–
Asp	2.2	–	2.5	–	–

*Time to peak expressed in seconds

Thus picrotoxin and bicuculline block all Cl^- conductance increase responses, and curare blocks all Na^+ and Cl^- but not K^+ conductance increase responses. Some observations from the mammalian central nervous system are consistent with the view that this model of organization of receptors is a general feature of the nervous systems of both vertebrates and invertebrates.

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INTERCHANGEABLE ASSOCIATION OF NEUROTRANSMITTER RECEPTORS AND IONOPHORES IN VERTEBRATE AND INVERTEBRATE CELLS

Principal Investigators: D. O. Carpenter, P. R. Myers, W. Shain, C. N. Sinback, and J. W. Swann

There are major technical difficulties in the study of receptor organization in the central nervous system of mammals. To minimize these difficulties we have studied receptors not only on invertebrate neurons but also on mammalian cells in culture and *in vivo*. On the basis of experiments with the marine mollusc *Aplysia*, we have developed a model of organization for receptors of neurotransmitters that suggests that the binding sites for putative neurotransmitters and the ionophores mediating different ionic conductances are independent entities in cell membranes which can be functionally assembled in any combination. In this report we present evidence from cultured neural cells and human smooth muscle cells that are consistent with this model.

We have begun to test this hypothesis by electrophysiologic recordings from a continuous somatic cell hybrid line (TCX11) resulting from fusion of mouse sympathetic ganglion cells with mouse neuroblastoma cells and from a continuous smooth muscle line derived from human oviduct. Figure 1 illustrates properties of a depolarizing response to DA obtained from TCX11 cells. In *A*, the DA response consists of a depolarization associated with a conductance increase. NA elicits a similar but smaller response which cross-desensitizes with the DA response. In *B*, the DA response is reversibly blocked by curare. *C* shows the effects of polarization on response amplitude, and gives a reversal potential of about −15 mV. This value is similar to the reversal potential of the ACh response at neuromuscular junction, and suggests that the depolarization results from a simultaneous conductance increase to Na^+ and K^+.

In addition, we have recorded responses to acetylcholine, histamine, and norepinephrine on human smooth muscle cells in culture. For all of these substances, hyperpolarizing, depolarizing, and biphasic responses have been found on different cells.

These observations, obtained from three very different types of cells, are consistent with the hypotheses that (a) the receptor complexes for neurotransmitter substances are composed of at least two structural and functional entities: the transmitter binding site (receptor) and the ionic channel (ionophore); and (b) the receptors and ionophores are interchangeable, in that one receptor may be associated with several different ionophores, and, conversely, one ionophore, mediating (for example) a Cl^- conductance, is the same whether associated with an ACh, DA, or GABA receptor. These similarities in ionophores are indicated by the time course of responses, the similarities of ionic selectivity, and (in the case of at least curare) the pharmacologic sensitivities. The DA response in the somatic cell hybrid, for instance, is similar to the response at neuromuscular junction in ionic selectivity and sensitivity to curare. The DA receptors in TCX11 appear to be similar to those in *Aplysia* neurons in that antiserum developed against the cultured neurons blocks the DA response in these cells and in *Aplysia*. Although it has not been possible yet to test this model directly on central nervous system neurons, the present observations indicate a general pattern of organization of receptors.

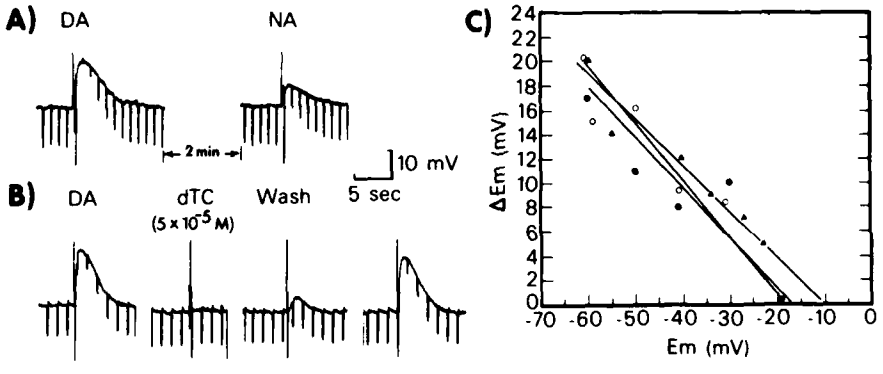

Figure 1. Response to DA and NA of TCX11 cells. Record *A* shows intracellular recordings while passing 0.5-namp current pulses with extracellular ionophoresis of DA and NA. *B* shows that in 5 x 10^{-5}M curare (dTC), both voltage and conductance changes are reversibly abolished. In *C* the response amplitude (ΔEm) is plotted as a function of membrane potential for experiments on three different cells.

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ACTIONS OF SEVERAL PUTATIVE NEUROTRANSMITTERS ON THE GILL OF *APLYSIA*

Principal Investigators: P. C. Ruben, J. W. Swann, and D. O. Carpenter

Nerve-muscle interaction in the gill of *Aplysia* constitutes a highly suitable preparation for study of the presence of receptors for specific neurotransmitters. Biochemical assays have shown the presence of high concentrations of dopamine (DA) in the *Aplysia* gill. We have studied the effects of DA and other putative transmitters on spontaneous and induced contractions in the isolated gil pinnule of *Aplysia californica* in an effort to determine which of these act on the gill muscle and which may be the neuromuscular transmitters for identified gill motor neurons.

A pinnule was removed from the gill and suspended by a ligature through the efferent vessel and attached to a Grass tension transducer. The afferent vessel was tied to a fixed anchor in the bath. Contractions were amplified and recorded on a Brush recorder. Drugs were applied by bath perfusion in either normal seawater, high-Mg^{++} (150 mM) seawater, or 30 mM $CoC1_2$ seawater to depress synaptic activity. Reproducible contractions were elicited by a speaker-driven tactile stimulator. Figure 1 shows the experimental apparatus.

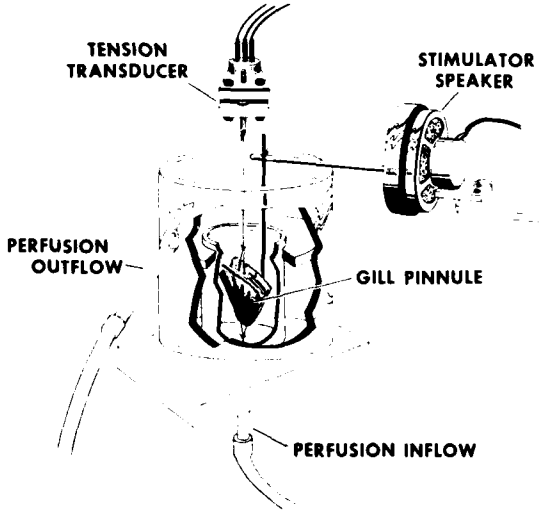

Figure 1. Single pinnule preparation and apparatus. The pinnule was secured to a Grass tension transducer by a ligature through the efferent vessel, and was anchored to the bath bottom by a ligature through the afferent vessel. The double wall chamber allowed for maintenance of temperature at 16°C.

Our studies focused on three types of contractions: spontaneous pinnule movements, contractions induced by tactile stimulation, and those induced by the perfusion of DA at 10^{-4}M. High-Mg^{++} seawater blocked spontaneous and stimulus-induced contractions, but had only a slight reducing effect on DA-induced contractions. Seawater containing $CoCl_2$ blocked spontaneous and stimulus-induced contractions and had little or no effect on DA-induced contractions. Contrary to expectations, neither acetylcholine nor glutamate caused contractions in this preparation. These substances induce contractions when perfused through the gill in an intact preparation including the PVG and the gill ganglion. GABA, aspartate, histamine, epinephrine, and norepinephrine had neither excitatory nor inhibitory effects. Carbachol and DA were inhibitory on specific muscles in stimulus-induced contractions. Octopamine and phenylethanolamine were also inhibitory to spontaneous and stimulus-induced contractions. Serotonin, which is present in peripheral gill neurons (1), was inhibitory on spontaneous and stimulus-induced contractions as well as on DA-induced contractions.

Our results suggest that there are excitatory DA receptors on some smooth muscle cells in the gill of *Aplysia*. In addition, these experiments confirm the functional role of a peripheral nerve net which mediates spontaneous and stimulus-induced contractions. The dominant effect of several putative neurotransmitters on the peripheral nerve net is inhibitory.

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CYCLIC AMP IN *APLYSIA* GILL: INCREASES BY PUTATIVE NEUROTRANSMITTERS

Principal Investigators: P. R. Kebabian, J. W. Swann, and D. O. Carpenter, *AFRRI*
Collaborator: J. W. Kebabian, *National Institutes of Health*

Dopamine (DA) is found in substantial amounts in the gill of the marine mollusc *Aplysia*. When perfused, it induces contractions and also greatly facilitates transmission from identified motor neurons, some of which are clearly not dopaminergic (1). Because of the possibility that at least the modulation of neuromuscular transmission

might be mediated through cyclic adenosine monophosphate (cAMP), we have determined the ability of several putative neurotransmitters to affect cAMP levels in gill tissue.

Slices of gill tissue were incubated in seawater (30°C) for 20 min. The amines were added immediately before addition of the tissue to the seawater. At the end of the incubation period, the tissue was rapidly removed and frozen on dry ice. Subsequently the tissue was homogenized, and cyclic AMP was measured with the method of Brown *et al.* (2).

Both DA (30 μM) and serotonin (5-HT) (100 μM) caused increases in cAMP levels to values 10 times control (Figure 1). Carbachol (100 μM) showed no significant effect. The half-maximal concentration of DA for this effect was 10 μM. While the 5-HT stimulation was immediate and approached maximal within 5 min, the stimulation by DA was slower and approached maximal only at about 20 min. Neither the DA- nor 5-HT-induced increases in cAMP levels were decreased when the experiment was performed in the presence of elevated Mg^{++} concentration (200 mM), which is known to depress synaptic transmission. These results suggest that both transmitters are acting directly and not through activation of some intermediate receptor. In addition, the maximally stimulating effects of DA and 5-HT were additive, indicating the presence of two independent receptor types.

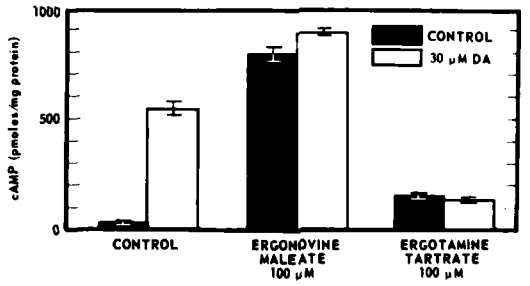

Figure 1. Stimulation of cAMP by 30 μM dopamine (DA) in *Aplysia* gill slices, and the effects of ergonovine and ergotamine on resting cAMP leads and cAMP stimulation by DA

[illegible] increases in cAMP induced by both DA and 5-HT were blocked by both fluphenazine and cis fluphenthixol. These drugs were essentially equipotent and gave nearly [illegible] at 100 μm. While neither fluphenazine nor cis-fluphenthixol had [illegible] resting cAMP levels, trans-fluphenthixol was unexpectedly found to stimulate cAMP levels and not block the action of either DA or 5-HT. The three ergot [illegible] ergonovine, ergotamine, and lergotrile—did antagonize the effects of DA

but, unlike their action in mammalian systems, they caused an accumulation of cAMP in absence of either amine (Figure 1). Although the site of the receptors has not been identified, our observations are not inconsistent with the hypothesis that DA may cause modulation of neuromuscular transmission by a cyclic nucleotide-mediated modulation of contractility of smooth muscle fibers.

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L_9-INDUCED GILL CONTRACTIONS IN *APLYSIA* ANTAGONIZED BY THE DOPAMINE RECEPTOR BLOCKERS FLUPHENAZINE AND ERGOMETRINE

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

The gill of *Aplysia californica* contains 3 μg dopamine (DA) per gram of tissue—a very high concentration. Our aim is to determine the physiological role of DA in the gill. Experiments were performed on the semi-intact gill preparation of *Aplysia.* The preparation consists of the abdominal ganglion and the gill. The ganglion was isolated from the rest of the preparation in a vaseline-sealed chamber. The gill was cannulated and perfused. As we have previously reported, perfusion of the gill with DA at threshold concentrations of 10^{-7} to 10^{-6} M results in highly reproducible contractions of efferent vessel trunklets, pinnule longitudinal muscles, and afferent vessel. These contractions in part mimic gill contractions due to the motor neuron L_7. DA perfusion also dramatically modulates (i.e., enhances) the gill contractions of L_7 and at least one other motor neuron, LDG_1. Biochemical assay of the soma of L_7 found very little DA (1 μM). This finding suggests that L_7 may not be dopaminergic.

More recently, we have found that induced spiking of L_9 neurons causes the same contractions as DA and L_7. As many as three of these L_9 cells and L_7 have been recorded from in the same preparation. The largest of the L_9 cells causes contractions of

the whole gill (i.e., all efferent vessel trunklets, pinnule longitudinal muscles, as well as the afferent vessel). Other L_9 cells control the movement of these same muscle groups but in a more restricted area of the gill. For instance, in five preparations we have recorded from two smaller L_9 cells. One cell induced contractions of the anterior half of the gill, and the other cell controlled the posterior half. Unlike L_7, the L_9 cells show pacemaker activity, do not produce measurable EJP's in the gill (as recorded by an extracellular suction electrode), and send their axons to the gill via the siphon nerve. In addition, L_9 cells are much less effective than L_7 in producing gill movements.

Perfusion of the gill with the DA antagonist ergometrine maleate ($1-3 \times 10^{-4}$ M) produces at least partial and usually complete abolishment of L_9-induced contractions. L_7 and LDG_1 contractions are not affected (Figure 1). Fluphenazine HCl ($3-4 \times 10^{-5}$) appears to act as a mixed agonist-antagonist. That is, fluphenazine alone produces DA-like contractions, and it enhances L_7-induced contractions. But fluphenazine blocks L_9-induced contractions. LDG_1 contractions are not blocked by fluphenazine.

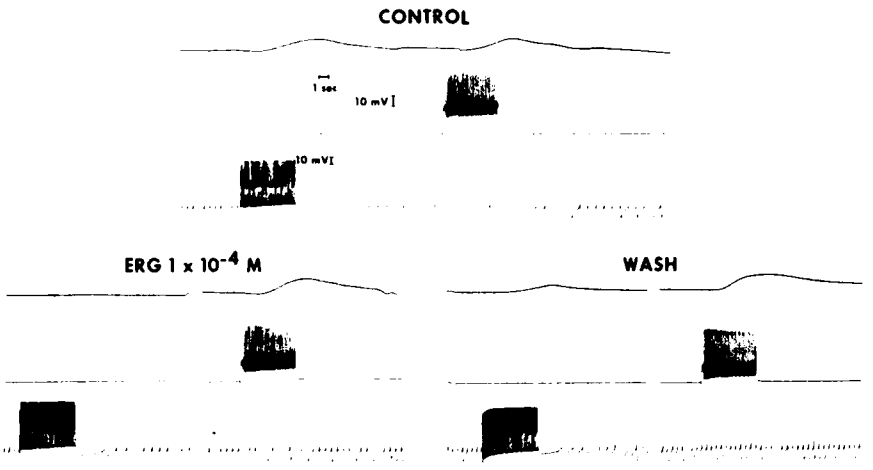

Figure 1. Effects of ergotamine on contraction caused by the identified motor neurons L_9 and L_7. In each series this upper record shows contraction measured by the movement of gill over a photocell. Middle records are intracellular recordings from L_7. Lower records are intracellular recordings from L_9, a pacemaker neuron. Contractions were elicited by passing depolarizing current through the recording electrode.

These results have led us to conclude that L_7 is not dopaminergic and to develop the working hypothesis that the L_9 cells are dopaminergic motor neurons.

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DOPAMINE: A MODULATOR OF SYNAPTIC TRANSMISSION TO GILL MUSCLE OF *APLYSIA*

Principal Investigators: D. O. Carpenter, J. W. Swann, P. R. Kebabian, C. N. Sinback, and P. C. Ruben

Dopamine (DA) is present in *Aplysia* gill in quantities (4.5 μg/g) comparable to those in the abdominal ganglion and the three major nerves innervating the gill, and the concentrations in different parts of the gill are similar. We have attempted to determine the functional role of DA in the gill. Perfusion of DA (10^{-7}–10^{-6}M) through the gill induces contractions similar to those elicited by stimulation of the identified gill motor neuron L_7. However, measurement of DA content in physiologically identified L_7 somata gave an average value of 4.7 pg/cell. Similar quantities of DA were found in all other identified cells studied, including R_2, which is known to be cholinergic. This suggests that L_7 may not be dopaminergic. Perfusion of DA (10^{-7}–10^{-6}M) does, however, dramatically enhance the gill contractions due to spiking of L_7 and LDG_1, another gill motor neuron which is thought to be cholinergic and which causes a movement different from that of L_7 (Figure 1). This effect of DA is clearly a modulation in the case of LDG_1, since the LDG_1 movement that is increased by DA is very different from the movement elicited directly by DA. Octopamine, 5-HT, GABA, acetylcholine, and glutamate did not modulate gill contractions. To test the possibility that this modulation might be mediated through cyclic nucleotides, we have measured cAMP in the gill. The level of cAMP rose from 13.3 to 55 pM/mg protein following incubation with 100 μM DA. The DA stimulation of cAMP, like DA content and modulation of movement, was not localized to any one part of the gill. Preliminary experiments suggest that DA acts in part by increasing end plate potentials in gill muscle fibers.

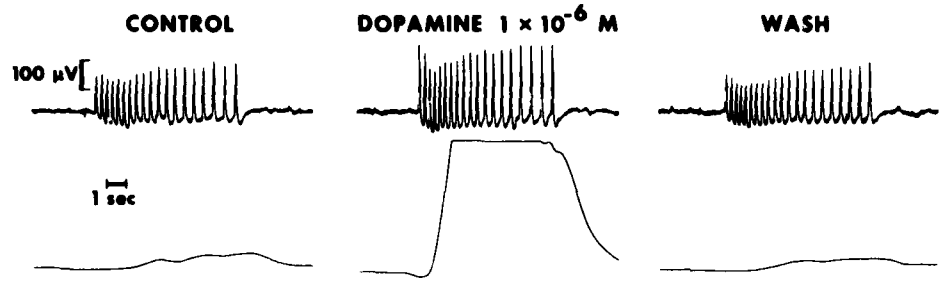

Figure 1. Modulation by dopamine of contraction induced by motor neuron L_7. Upper records show the end plate junction potential recorded using the techniques of Jacklet, with a suction electrode on a small section of pinnule. Lower records show contraction, monitored with a photocell. Perfusion of 10^{-6}M DA caused a dramatic increase in the contraction as well as an increase in the amplitude of the junction potential.

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GABA-MEDIATED EXCITATORY RESPONSES ON *APLYSIA* NEURONS

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

Gamma-aminobutyric acid (GABA), a known inhibitory neurotransmitter in mammals, can elicit two different types of excitatory response in the nervous system of the marine mollusc *Aplysia* (Figure 1). These responses are depolarizing when GABA is applied ionophoretically, and they result from either an increase in membrane conductance to Na^+ or a decrease in conductance to K^+. In addition, GABA on other neurons causes an inhibitory response similar to that commonly found in other preparations. Although not all neurons have GABA receptors, identified single cells consistently have the same type of response.

GABA must be considered a putative neurotransmitter in molluscs since it is present and is asymmetrically distributed in the nervous system (1), its synthetic enzyme is present (2), and an active uptake GABA transport system has been found (3). Because

the GABA receptors mediating at least two types of excitatory responses are specific and localized, GABA must be considered to be a putative excitatory as well as inhibitory neurotransmitter, at least in this preparation.

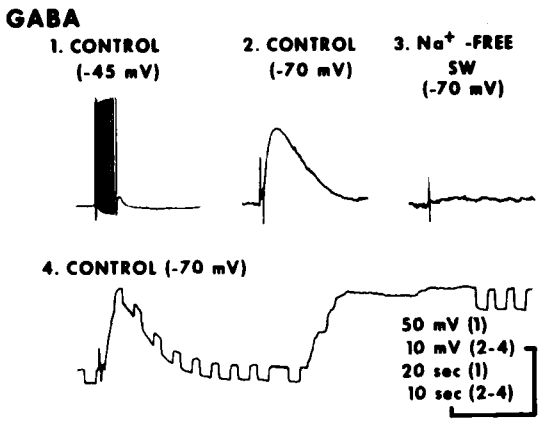

Figure 1. Depolarizing GABA response obtained from an unidentified neuron in the lower right quadrant of the abdominal ganglion, recorded with two independent microelectrodes. Record 1 was made at resting membrane potential (-45 mV) and shows that GABA causes a brisk discharge. The peak of the spike is cut off by the recorder. Records 2-4 were taken during hyperpolarization to -70 mV in order to prevent discharge after GABA application. In Record 3 the preparation was perfused with seawater in which Tris was substituted for all Na^+. In Record 4 constant current pulses of 1 namp were applied to determine the membrane conductance during the response. The pulses at the far right show that the resting conductance at the potential level corresponding to the peak of the response is greater than that at -70 mV, due to anomalous rectification. However, when such pulses are superimposed on the GABA response, they are reduced in amplitude, indicating that membrane conductance is increased. All solutions contained 100 mM added $MgSO_4$, as previously described, to depress spontaneous transmitter release. GABA ionophoretic application was 300 nC in all cases.

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INTERACTIONS OF ASPARTATE WITH GLUTAMATE RECEPTORS

Principal Investigators: D. O. Carpenter, M. J. McCreery, and P. J. Yarowsky

There are on *Aplysia* neurons several different kinds of receptors for the acidic amino acids, glutamate and aspartate. Some neurons respond to aspartate, others to glutamate, and others do not respond at all. The ionic responses to both substances are of three types (on different cells, usually): those due to Na^+, to Cl^-, or to K^+ conductance increases. There is yet another class of receptor that responds to both glutamate and aspartate, where both substances cause the same ionic response and demonstrate cross-desensitization.

We have examined an interaction of aspartate with receptors for glutamate. We have found that at some, but not all, glutamate receptors, aspartate (which does not itself cause any response) dramatically facilitates the response to glutamate (Figure 1). This observation is very similar to those observed at the lobster neuromuscular junction, first studied by Shank and Freeman (1) and later by Crawford and McBurney (2). Shank and Freeman have suggested that aspartate changes the conformation of the glutamate receptor, increasing its affinity for glutamate. Crawford and McBurney, on the other hand, have suggested that aspartate acts by blocking the active re-uptake of glutamate, thus potentiating its effect. It is of interest that Takeuchi does not find a similar interaction at the crayfish neuromuscular junction, which also utilizes glutamate as the neurotransmitter.

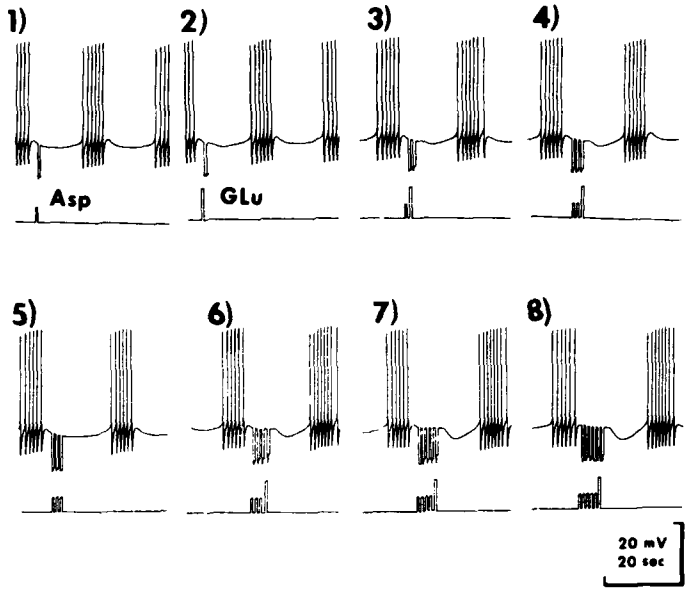

Figure 1. Facilitation of R_{15} glutamate (Glu) response by aspartate (Asp). Asp and Glu were each applied in pulses of 1000 nC as indicated in the lower records of each trace, where Asp pulses are small and Glu large. Asp alone had no effect (*1,5*), but when Asp pulses were applied before Glu pulses, the Glu response was facilitated (*3,4,6-8*).

In order to distinguish between these two possibilities, we studied the interaction in Na^+-free solutions which should block transport. In this situation the modulation by aspartate was blocked. In spite of this observation there are reasons to doubt that the phenomenon is a transport process, since not all cells with glutamate responses show interaction with aspartate, while one would expect that all cells would have active transport processes for these amino acids. Furthermore, those cells that respond to both aspartate and glutamate never show any facilitation beyond addition of responses.

We have, however, found modulation by aspartate of both Na^+ and $C1^-$ glutamate responses. This is of interest since modulation of $C1^-$ responses has not previously been seen. Although the transport explanation predicts that the modulation should not be dependent on the ion's moving, the receptor conformation thesis also leads to this expectation, since evidence has been presented (3) that different ionic responses are mediated by the same receptors coupled to different ionophores.

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RECEPTORS FOR PUTATIVE NEUROTRANSMITTERS ON THE *APLYSIA* BURSTING NEURON R_{15}

Principal Investigators: D. O. Carpenter, M. J. McCreery, K. M. Woodbury, and P. J. Yarowsky

Several laboratories have demonstrated that a single neurotransmitter substance may elicit different ionic responses on different neurons in *Aplysia*. The most frequent are fast (peak 1-3 sec) depolarizing (Na^+-dependent) and hyperpolarizing ($C1^-$-dependent) and slow (peak 12-15 sec) hyperpolarizing (K^+-dependent) conductance increase responses. In addition, slow depolarizing Na^+-conductance increase responses and depolarizing and hyperpolarizing conductance decrease responses have been found for some transmitters. At least the three common responses have been found for acetylcholine (Ach), dopamine (DA), octopamine, phenylethanolamine, histamine, serotonin, γ-aminobutyric acid (GABA), glutamate (Glu), and aspartate.

We have searched for receptors for these substances on cell R_{15} which discharges normally with an endogenous bursting rhythm. Of particular interest was the presence of and constancy of receptors from one preparation to another and the effect of the various putative neurotransmitters on the bursting rhythm.

We have found four different ionic responses to these substances on R_{15} (Figure 1). Acetylcholine excites through a fast Na^+-conductance increase, while GABA excites through a slow conductance decrease. Glutamate inhibits through a fast $C1^-$-conductance increase while dopamine inhibits through a slow K^+-conductance

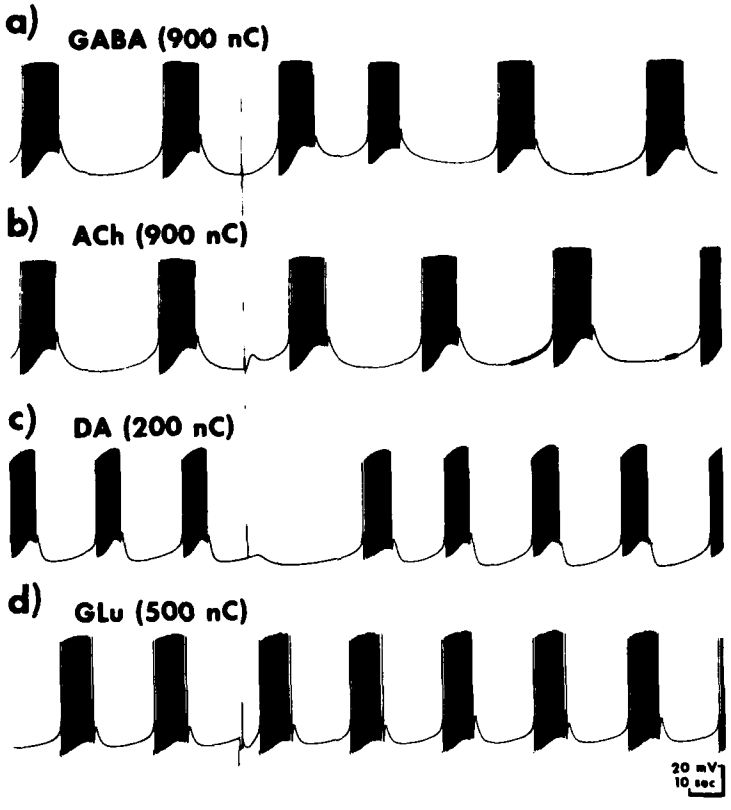

Figure 1. Responses to ionophoretic application of several putative neurotransmitters onto cell R_{15}. Records *a* and *b* are from the same cell, but *c* and *d* are different preparations.

increase. The fast responses usually do not reset the bursting discharge, while the slow responses always do.

Receptors for these substances and the ionic responses were specific and constant in different preparations. Furthermore, receptors were localized at different points in the neuropile. These observations provide a basis for understanding the variety of inputs to R_{15}.

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EFFECT OF LOW-SODIUM SOLUTIONS ON CONDUCTANCE IN THE GIANT ABDOMINAL NEURON (R_2) OF *APLYSIA*

Principal Investigators: J. P. Apland and D. R. Livengood

Low-sodium external solutions have been reported by various authors to cause an increase, a decrease, or no change in conductance of mulluscan neurons (1). It has also been reported that removal of extracellular sodium suppresses the changes in passive membrane properties induced by ionizing radiation (2). In view of these reports, a careful investigation was undertaken to establish whether a conductance change could be demonstrated consistently in the R_2 cell and, if so, to establish the ionic mechanism involved. Numerous sodium substitutes were used, including Tris, mannitol, magnesium-mannitol, glucosamine, tetraethanolammonium, tetramethylammonium, bis (2-hydroxy-ethyl) dimethylammonium, choline, and arginine. Ramp-generated current-voltage plots were used to establish slope conductances. A conductance increase was consistently observed with most of the substitutes (Figure 1). This change could be blocked by extracellular application of 30 mM cobalt chloride. These results imply that calcium is involved in the phenomenon. In some experiments no conductance change was observed in low-sodium solutions. In these experiments a conductance decrease could be demonstrated following application of cobalt. This suggests that a conductance increase which could be blocked by cobalt was masked by a decreased sodium conductance brought about by removal of external sodium. It is not yet clear whether the conductance increase in low-sodium solutions is due to a direct increase in calcium conductance or to a calcium-mediated increase in potassium conductance. It was also observed that application of cobalt abolished anomalous rectification in these cells. This suggests that anomalous rectification, which is dependent on external potassium, may involve a calcium-mediated increase in potassium conductance. Ionizing radiation has been shown to cause release of ionized calcium from intracellular stores. Carpenter et al. (3) have suggested that such a rise in intracellular ionized calcium may cause the early transient hyperpolarization they observed in irradiated *Aplysia* neurons. These processes are similar to the response to low-sodium solutions suggested by our studies. Therefore, these results are of significance in understanding the elementary mechanisms of early transient radiation injury to the central nervous system.

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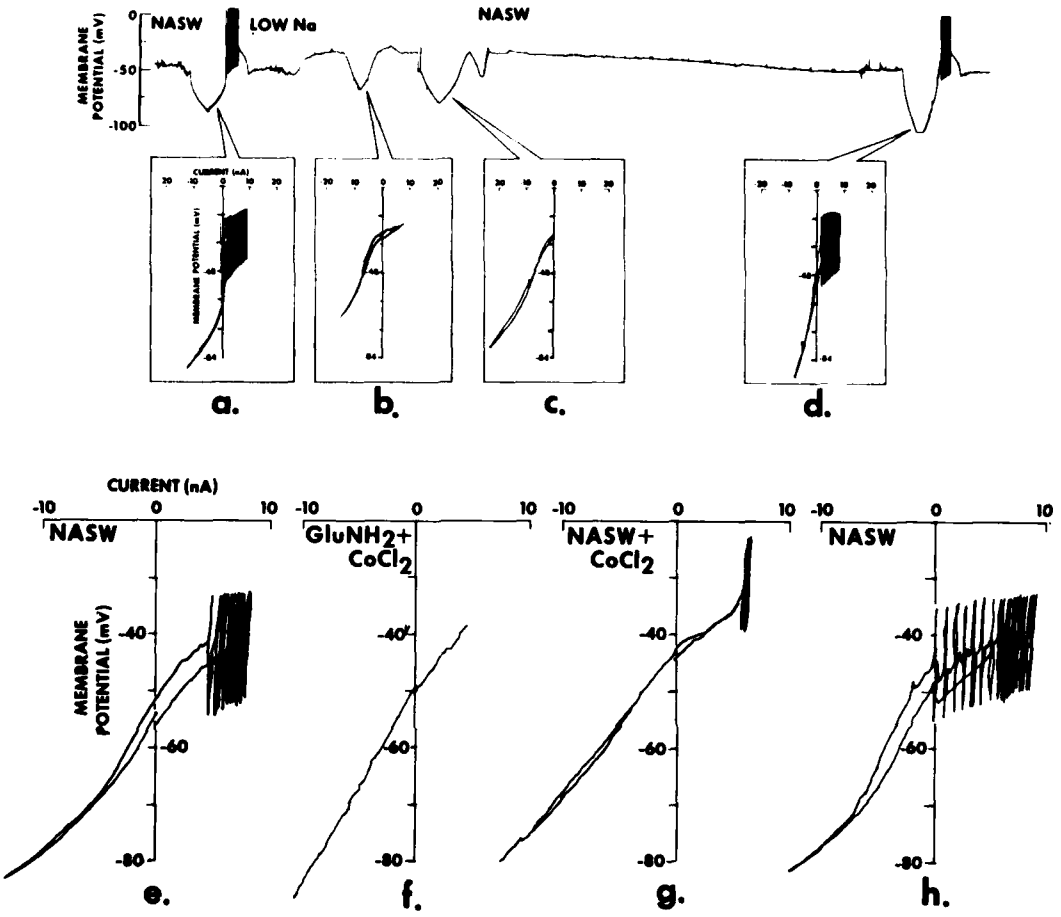

Figure 1. *Upper panel:* Conductance increase (decrease in slope resistance) induced by an external solution with 90% of the sodium replaced by glucosamine (low Na). Solution changes indicated by the arrows.

a. Current voltage plot in normal artificial seawater (NASW).

b. Current voltage plot demonstrating a conductance increase in low-sodium seawater.

c. Same cell as *b,* repolarized to resting membrane potential by current injection, showing no conductance change.

d. Recovery after return to NASW.

Lower panel: Application of cobalt chloride (C_oCl_2) prevents the conductance change in low-sodium, glucosamine-substituted seawater ($GluNH_2$).

e. Current voltage plot in NASW.
f. No conductance increase is seen in low-sodium, cobalt-containing seawater.
g. No conductance decrease is seen upon changing to NASW and cobalt.
h. Recovery upon return to NASW.

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BLOCKADE OF NEUROMUSCULAR TRANSMISSION BY ENZYMATICALLY ACTIVE AND INACTIVE BETA-BUNGAROTOXIN

Principal Investigators: D. R. Livengood, M. A. Donlon, L. M. Masukawa, G. S. Tobias, and W. Shain, *AFRRI*
R. S. Manalis, *University of Cincinnati*

Beta-bungarotoxins have been shown to be presynaptic blockers of neuromuscular transmission. This reports experiments using the most positively charged beta-bungarotoxin which elutes from a CM-Sephadex C-25 column. The toxin is a single polypeptide with a molecular weight of approximately 11,000 daltons and has phospholipase A activity. The application of the enzymatically active toxin to the frog sciatic nerve-sartorius muscle preparation results in an initial decrease in the average endplate potential (e.p.p.) amplitude followed by a temporary rebound in e.p.p. amplitude, and finally a complete inhibition of e.p.p.'s. Similarly, miniature endplate potential (m.e.p.p.) frequency is initially reduced upon toxin application but then increases dramatically. After the phospholipase A of the toxin is inactivated, treatment with the toxin results in only the initial decrease in transmitter release (Figure 1). These results suggest that this beta-bungarotoxin acts in two functionally separate steps: first, by binding to a specific presynaptic site possibly associated with calcium entry, and second, by perturbing the presynaptic membrane by its enzyme action which results in an increase and then a failure in transmitter release.

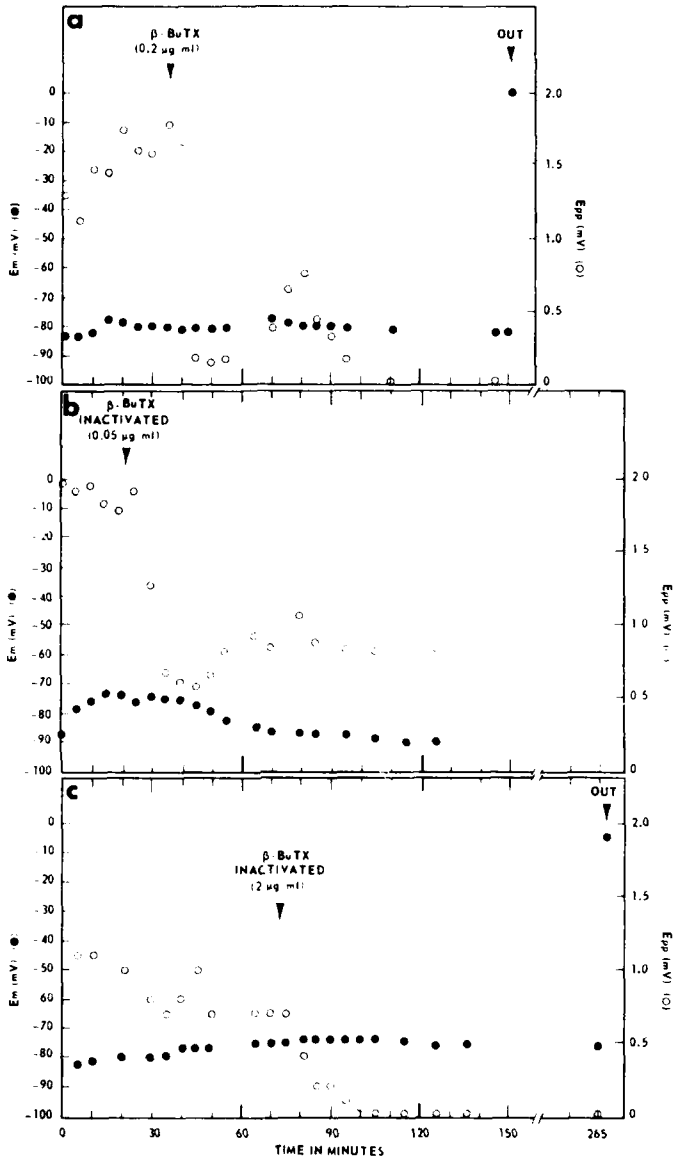

Figure 1*a*. Blockage of the e.p.p. by the application of enzymatically active beta-bungarotoxin (β-BuTX). The amplitude of the e.p.p. (open circles) is indicated by the right-hand ordinate, and the membrane potential (closed circles) is indicated by the left-hand ordinate. Time in minutes is indicated on the abscissa. Purified β-BuTX was added to the batl. (arrow) with the final concentration as shown. The e.p.p. amplitude decreased to nearly 0 mV within

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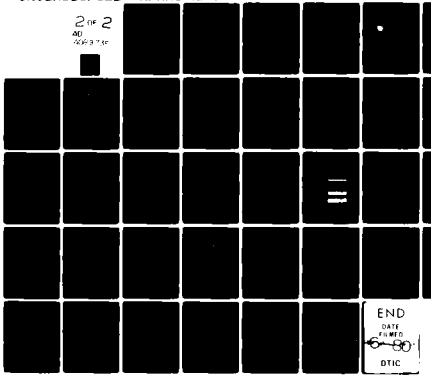

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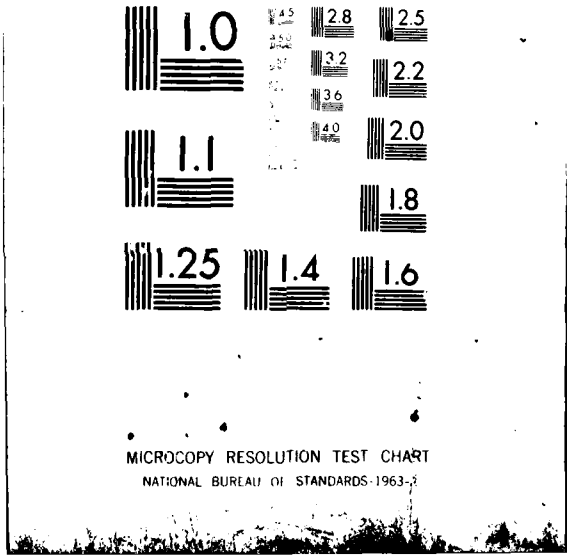
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10-15 min following application of β-BuTX. A rebound in the e.p.p. was seen beginning 60 min after the initial block. A secondary decrease in e.p.p. amplitude was followed until the microelectrode impalement was lost, 240 min into the experiment.

b. The blockade of the e.p.p. by addition of enzymatically inactive β-BuTX. An incomplete blockade of the e.p.p. was produced by the addition of 0.05 μg/ml (final concentration) of inactivated toxin.

c. A complete blockade of the e.p.p. was produced by the addition of 2 μg/ml (final concentration) (first arrow). A washout of the toxin was started 30 min after the toxin application (second arrow), but the e.p.p. showed no indication of recovery by the time the recording electrode was withdrawn 2 hours and 45 min after the start of the washout period. The experiments shown in *a* and *b* are from different preparations.

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BETA-BUNGAROTOXIN: RETENTION OF THE PRESYNAPTIC BLOCKING EFFECT FOLLOWING INACTIVATION OF THE PHOSPHOLIPASE

Principal Investigators: L. M. Masukawa, G. S. Tobias, M. A. Donlon, D. R. Livengood, and W. Shain

Beta-bungarotoxin (β-BuTX) has been shown to be a specific presynaptic blocking agent at the neuromuscular junction by a number of investigators. Its mode of action has been postulated to be due ultimately to the disruption of transmitter-release mechanisms by its phospholipase activity. The phospholipase activity can be removed by boiling the toxin at pH 8.6. After this treatment the toxin (phospholipase-inactive β-BuTX) was still able to block transmission in *in vitro* neuromuscular preparations of of the frog and rat. Blockade of evoked release of acetylcholine was examined by averaging endplate potentials that previously had been partially blocked with 3 μg/ml d-tubocurarine. The blockade due to the phospholipase-inactive β-BuTX was complete and irreversible within 20 min at high toxin concentrations (0.1 to 1 μg/ml) and was not preceded by a transient increase in evoked release as has been shown for the enzymatically active toxin (Figure 1). The degree of blockade was found to be a function of the toxin concentration. The effect was rapid in onset and reached a steady state level. This action is inconsistent with the action of a phospholipase but is characteristic of a direct binding of the toxin to sites important to transmitter release.

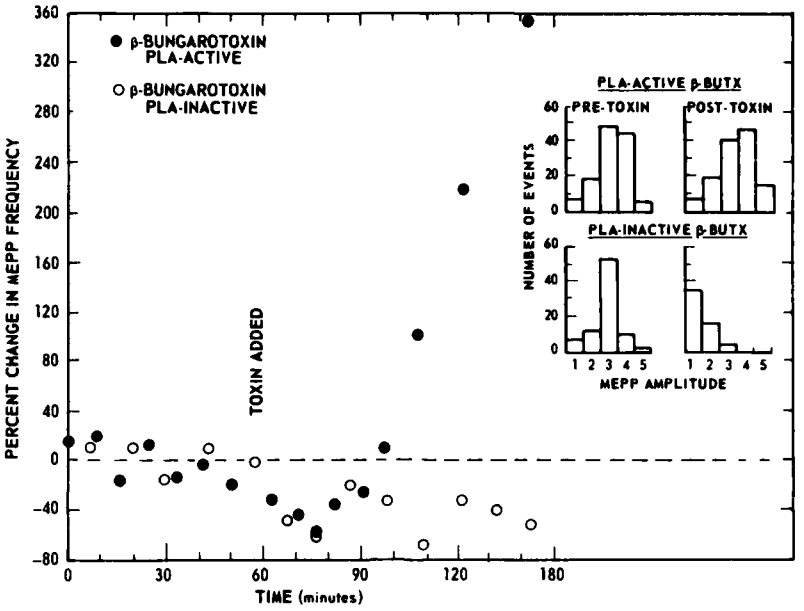

Figure 1. Alterations of miniature endplate potential (m.e.p.p.) frequency measured at the frog neuromuscular junction at various times after bath application of phospholipase A-active (PLA-active) (●) or heat-treated phospholipase A-inactive (PLA-inactive) (○) beta-bungarotoxin (0.1 μg/ml). The insert illustrates the shift in m.e.p.p. amplitude in arbitrary units before and after exposure to the two forms of the toxin.

Miniature endplate potential (m.e.p.p.) frequency was measured in the presence of phospholipase-inactive β-BuTX to study further its mode of action. The m.e.p.p. frequency appeared to increase in the presence of the toxin, and the distribution of miniature amplitudes was shifted to a population of lower amplitude. This decrease in amplitude may be due to a postsynaptic effect; therefore, acetylcholine sensitivity of extrajunctional receptors of denervated muscle was measured in the presence of phospholipase-inactive β-BuTX. We hypothesize that the fully active toxin contains two active sites: one site specific to the presynaptic terminal that can block release of transmitter, and another site that has a phospholipase action.

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EFFECT OF CHRONIC BETA-BUNGAROTOXIN TREATMENT ON THE DISTRIBUTION OF ACETYLCHOLINE RECEPTORS IN THE RAT DIAPHRAGM

Principal Investigators: G. S. Tobias, *Naval Medical Research Institute*
L. M. Masukawa, *AFRRI*

Within a few days after axotomy, the denervated muscle undergoes changes in electrical properties including the appearance of extrajunctional sensitivity to acetylcholine (ACh). This spread of ACh sensitivity following denervation can be mimicked by chronic application of botulinum toxin or beta-bungarotoxin (β-BuTX), toxins that act presynaptically to inhibit ACh release. The ability of these toxins to mimic denervation suggests that continuous ACh release is necessary and sufficient to maintain the electrical properties of the normally innervated muscle. However, β-BuTX also has phospholipase A (PLA) activity, and presynaptic endings treated with β-BuTX undergo morphological changes reminiscent of denervated nerve endings. Therefore, the ability of β-BuTX to mimic denervation may be related to its degenerative action and not to its pharmacological effects. We have endeavored to separate the morphological and pharmacological actions of β-BuTX with regard to its ability to mimic denervation effects in muscle. Rat phrenic nerve-diaphragm muscle preparations were chronically treated for 1 to 14 days by intrathoracic application of enzymatically active or inactive β-BuTX. Active β-BuTX had PLA activity whereas inactive β-BuTX had been heat-treated at high pH so that PLA activity was eliminated. After treatment *in vivo*, diaphragms were removed and assayed for distribution of ACh sensitivity by iontophoretic application of ACh and by ^{125}I-α-bungarotoxin binding. We found that chronic treatment with equivalent amounts of either active or inactive β-BuTX yields a significant enhancement of ^{125}I-α-bungarotoxin binding over the whole muscle surface compared to untreated diaphragms (Figure 1). The increase in binding was not as great as that in denervated muscle at the same time period, however. The binding results were corroborated by electrophysiological findings as well. Untreated muscles responded only to ACh iontophoresed onto end plate regions; muscles treated with either active or inactive β-BuTX had junctional and extrajunctional sensitivity to ACh. Our results support the hypothesis that ACh provides a "trophic" influence which maintains electrical properties characteristic of normally innervated muscles.

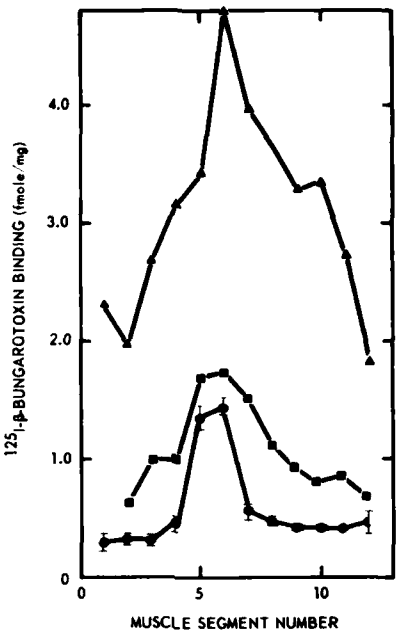

Figure 1. ^{125}I-α-Bungarotoxin-binding profiles of rat diaphragm *muscles from* (●) control (with standard error bars), (▲) 3-day denervated, and (■) 3-day enzymatically active β-bungarotoxin-injected animals.

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ELECTROPHYSIOLOGICAL RESPONSES OF SINGLE SMOOTH MUSCLE CELLS TO ACETYLCHOLINE, NORADRENALINE, AND HISTAMINE

Principal Investigators: C. N. Sinback and W. Shain

Smooth muscle cells of a continuous line derived from human oviduct retain the same electrical properties as smooth muscle cells *in vivo* including action potentials and electrotonic current spread among cells (1). Using dissociated cultures we describe the distribution of acetylcholine, noradrenaline, and histamine receptors on individual smooth muscle cells—something that cannot be studied *in vivo* due to current spread among cells.

We located receptors on 138 cells using (a) single iontophoretic electrodes containing histamine, acetylcholine, or noradrenaline, or (b) using two iontophoretic electrodes: one containing histamine and the other containing acetylcholine or noradrenaline while recording intracellularly with one or two microelectrodes (Figure 1). Single smooth muscle cells isolated from one another have receptors for acetylcholine, noradrenaline, and histamine. However, each cell did not have all three receptors. Almost all cells tested (89%) contained histamine receptors, about half the cells tested (54%) contained acetylcholine receptors, and about half the cells tested (46%) contained noradrenaline receptors.

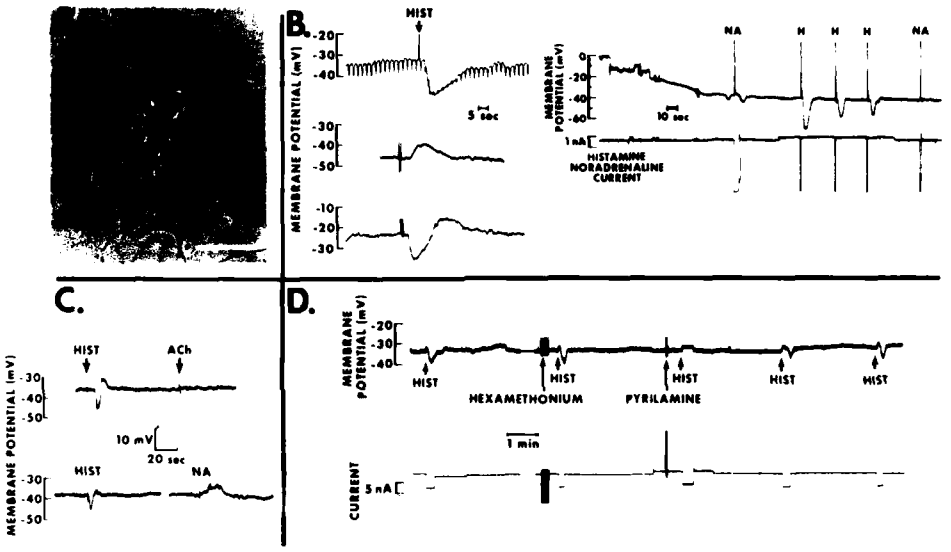

Figure 1*A*. Photo of a typical smooth muscle cell in culture. *B*. Responses to histamine (h) obtained in three different cells (left) and desentization of H response on repeated application (right). *C*. Records from two cells showing that histamine responses are distinct from those to acetylcholine (ACh) and noradrenaline (NA). *D*. Blockade of histamine response by pyrilamine, where upper records are intracellular recordings and lower show ionophoretic application.

Acetylcholine and histamine each elicited one of three electrical responses from single cells. For instance, in 68 cells, histamine elicited hyperpolarizing responses in 37 cells (54%), depolarizing responses in 13 cells (19%), and biphasic hyperpolarizing-depolarizing responses in 9 cells (13%). All three responses were due to increased ion flow, desensitized, and were blocked by antagonists. Histamine receptors did not increase the flow of the same ions in every cell since hyperpolarizing responses and depolarizing responses had different reversal potentials.

The cell dimensions (20μ x $>100\mu$) allowed mapping of receptor distribution on the membrane by iontophoresing a constant charge of histamine or acetylcholine at different positions. Receptors were concentrated on cell membranes, causing localized regions of high sensitivity to histamine and acetylcholine. Responses elicited in regions of high receptor density were >10 times the amplitude of responses in surrounding areas of low receptor density. In addition, a single cell often showed spatial segregation of receptors, causing depolarizing and hyperpolarizing responses. This segregation of hyperpolarizing and depolarizing receptors was responsible for biphasic responses.

In conclusion, these results establish that the contributions of single smooth muscle cells to neural and hormonal responses of smooth muscle tissue, which cannot be studied *in vivo*, can be studied using dissociated cultures of single smooth muscle cells.

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DISTRIBUTION OF HISTAMINE RECEPTORS ON HUMAN SMOOTH MUSCLE CELLS IN CULTURE: A MODEL SYSTEM FOR STUDY OF RADIATION EFFECTS

Principal Investigators: C. N. Sinback and W. Shain

Smooth muscle is one of the effector tissues of the autonomic nervous system. As such, changes in smooth muscle activity mediate contraction and relaxation of visceral, circulatory, and genital tract organs. A variety of agents—neurotransmitters, circulating hormones, and locally released hormones such as histamine—either excite or inhibit smooth muscle. However, it has been impossible to study the distribution of transmitter and hormone receptors on individual smooth muscle cells because autonomic organs are composed of many smooth muscle cells that are electrically coupled.

Histamine is probably the cause of early transient incapacitation (ETI) after radiation exposure. Radiation releases histamine from mast cells. The histamine so released may act on receptors on the cerebral blood vessel smooth muscle cells or perhaps even on cerebral neurons. As yet it has not been possible to study these effects directly.

In order to study the distribution of histamine receptors, we have developed a continuous line of cultured smooth muscle. Using the techniques of microiontophoresis, histamine receptors can be studied on semi-isolated smooth muscle cells.

Histamine receptors are localized on the membrane of single smooth muscle cells (Figure 1). When a constant charge of histamine was applied to different locations on a single cell, smaller and larger responses were recorded, depending on the position of the histamine electrode *(1A)*. Thus, localized aggregation of receptors exist on the cell surface. In addition, histamine receptors that cause hyperpolarizing responses and histamine receptors that cause depolarizing responses are spatially segregated on the membrane *(1B)*.

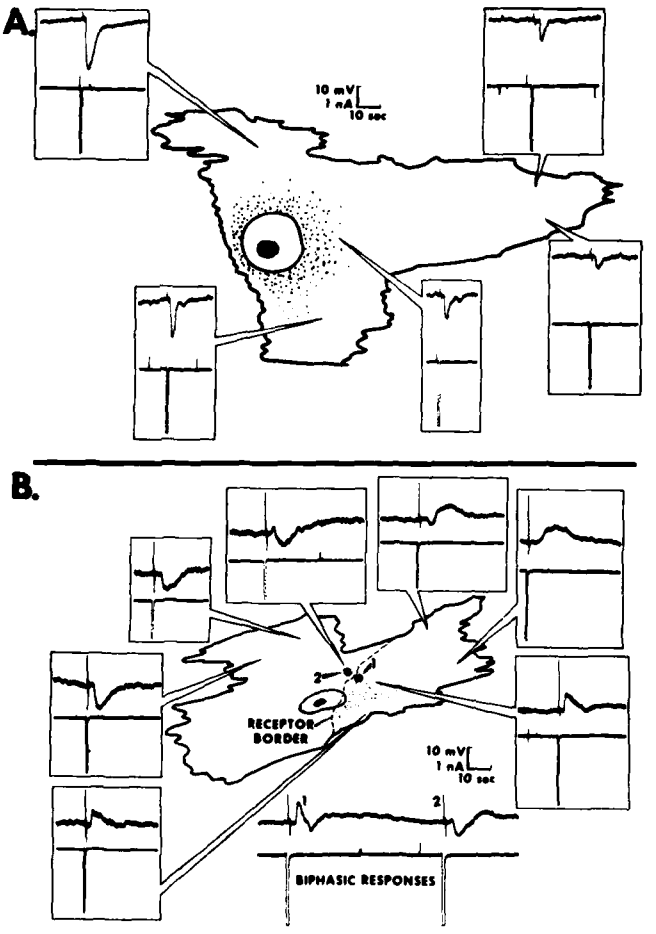

Figure 1. Distribution of receptors and different responses to histamine on single cells. *A* shows responses of different amplitude elicited from various sites on a single smooth muscle cell. *B* shows results from another experiment in which hyperpolarizing, depolarizing, and biphasic responses were recorded at different sites.

Histamine elicits a biphasic response when it is applied near the border between the hyperpolarizing receptors and the depolarizing receptors. Thus, receptor distribution determines whether histamine depolarizes, hyperpolarizes, or biphasically depolarizes and hyperpolarizes single smooth muscle cells.

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CATION ISOTOPE CONCENTRATION GRADIENTS IN SYMPATHETIC NEUROGLIAL CELLS IN TISSUE CULTURE

Principal Investigators: D. A. Brown, *University of London*
W. Shain, *AFRRI*

When intact rat superior cervical ganglia are maintained *in vitro* at 25°C in a solution containing 143 mM [Na^+] and 5.9 mM [K^+], the overall ratio of cation concentrations in the intra- and extracellular fluids (C_{in}/C_{out}) measured by flame photometry are 0.154 for Na^+ and 35 for K^+ (1). Steady-state ratios for isotopic tracers are somewhat lower: 0.07 for ^{24}Na (2), 25 for ^{42}K, and 27 for ^{86}Rb (3). These values represent the average distribution ratios in neural and glial elements. In order to assess whether their individual contributions are likely to differ appreciably, the steady-state distribution ratios of ^{24}Na and ^{86}Rb have been measured in separated neuroglial cells from rat sympathetic ganglia maintained in tissue culture.

Superior cervical sympathetic ganglia from 3-day prenatal to 5-day postnatal rats were dissociated mechanically, and the cells were maintained in Ham's F12 culture medium containing 5% added fetal calf serum at 37°C in an atmosphere of 5% carbon dioxide in air.

For isotope-exchange measurements, the culture medium was replaced with Hank's buffered saline solution containing 134 mM [Na^+] and 4.6 [K^+], pre-equilibrated with oxygen (pH 7.3-7.4), and maintained at about 30°C. The fluid volume of the cells was measured as ^{14}C-urea space: cells were allowed to equilibrate with ^{14}C-urea solution for 60 min, washed rapidly three times with nonradioactive solution, and residual ^{14}C was measured. Assuming a steady-state distribution ratio (C_{in}/C_{out}) of unity for ^{14}C-urea, uptake corresponded to a volume of 50-200 ml per chamber. (^{14}C-urea efflux measurements indicated losses of intracellular ^{14}C to be < 6% during the washing procedure.)

Cation concentration ratios were determined by adding trace amounts of the appropriate isotope to the ^{14}C-urea solution and measuring the nuclide/^{14}C ratio in the

incubation fluid and in the cells from which C_{in}/C_{out} for the nuclide could be obtained directly. This obviated errors introduced by variations in the number of cells, and hence cell fluid volume, in each chamber.

After 60 min incubation in ^{24}Na solution to steady-state uptake, C_{in}/C_{out} was 0.066 ± 0.004 (mean ± S.E., n = 5). Pre-incubation with 1 mM ouabain for 30 min raised the ratio to 0.37. Loss of intracellular ^{24}Na through active Na extrusion was reduced to <10% by washing with ice-cold solution (*cf.* 2).

The time constant for ^{86}Rb uptake was about 1 hour. C_{in}/C_{out} for ^{86}Rb after 4 hours was 29.7 ± 1.8 (n = 12).

Assuming complete isotopic exchange, $[Na^+]_{in}$ and $[K^+]_{in}$ may be estimated at 8.9 and 137 mM, respectively (E_{Na} = +71 mV; E_K = −89 mV). Comparable values were obtained using cells from a chemically induced rat peripheral glioma line, 1056A.

The calculated K^+ gradient accords with that deduced from previous electrophysiological measurements on rat sympathetic glial cells *in situ* (4). In the latter experiments, the membrane potential recorded at 5.9 mM $[K^+]_{out}$ approached the calculated value for E_K, suggesting a high P_K/P_{Na} ratio.

Comparison to the previous measurements in ganglia *in situ* suggests that the cation gradients in sympathetic neurons and glial cells are quite similar.

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NEUROTRANSMITTER MODULATION OF PROSTAGLANDIN E_1-STIMULATED INCREASES IN cAMP. I. CHARACTERIZATION OF A CULTURED NEURONAL CELL LINE IN EXPONENTIAL GROWTH PHASE

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

Two sympathetic ganglion cell X neuroblastoma somatic cell hybrids (TCX 11 and TCX 17) were found to have a PGE_1-sensitive adenyl cyclase which was inhibited in whole cells by carbachol, norepinephrine, and dopamine (Table 1). Serotonin and morphine were without effect, the latter despite the presence of opiate receptors. In the TCX 17 clone, carbachol produced a greater degree of inhibition (30% of control levels) than norepinephrine or dopamine (55% to 65% of control). The ED_{50} for inhibition was of the order norepinephrine > dopamine > carbachol (10^{-7} M, 3×10^{-7} M, 10^{-6} M, respectively). The inhibition of carbachol could be reversed by the muscarinic antagonists scopolamine and atropine whereas the nicotinic antagonists α-bungarotoxin and d-tubocurarine were without effect. The inhibition of norepinephrine and dopamine possessed the following properties: (a) the inhibition was mimicked by phenylephrine but not by isoproterenol and not by dopaminergic agonists apomorphine and ET495; (b) the α-antagonists phenoxybenzamine and phentolamine reversed the inhibition by norepinephrine and dopamine; (c) chlorpromazine reversed the inhibition of cAMP formation by dopamine, but other phenothiazines tested, trifluoperazine and fluphenazine, had no effect.

Table 1. Effect of Selected Compounds on Both Basal and PGE_1-Stimulated Increases in cAMP

Additions	Basal	PGE_1 (10^{-8} M)
None	46.75 ± 1.70	356 ± 32
Carbachol	24.90 ± 0.15	120 ± 5
ℓ-Norepinephrine	37.40 ± 1.90	195 ± 8
Dopamine	42.50 ± 0.90	215 ± 14
Serotonin	47.30 ± 3.00	394 ± 10
Histamine	60.80 ± 22.70	436 ± 13
Morphine	43.50 ± 3.50	332 ± 25

Drugs were incubated at 10^{-5} M.
Values are expressed ± S.D. for three plates of cells.

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NEUROTRANSMITTER MODULATION OF PROSTAGLANDIN E_1-STIMULATED INCREASES IN cAMP. II. CHARACTERIZATION OF A CULTURED NEURONAL CELL LINE TREATED WITH DIBUTYRYL CYCLIC AMP

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

The ability of selected neurotransmitters to modulate PGE_1-stimulated increases in cAMP was tested in the somatic cell hybrids TCX 17 and TCX 11 differentiated by growth in dibutyryl cAMP. PGE_1 was shown to cause an increase in cellular cAMP, carbachol, noradrenaline, and dopamine whereas 5-hydroxytryptamine had no effect (Table 1).

The carbachol inhibition is mediated by a muscarinic receptor since nicotinic antagonists failed to block carbachol while scopolamine reversed its effect. The noradrenaline inhibition was reversed by the antagonists phenoxybenzamine and phentolamine, but not by the β-antagonists propranolol and dichloroisoproterenol. The dopamine inhibition was reversed by chlorpromazine and trifluoperazine. The dopamine against ET495 mimicked dopamine while apomorphine had little or no effect.

These results obtained from differentiated cells are compared to those reported for exponential growth phase cells of the same cell line. Distinct differences were found with respect to the pharmacology of the noradrenaline and dopamine inhibition. Finally, the biochemical results are compared to the electrophysiological results reported for the cell lines. Neurotransmitter agents that modulate PGE_1 effects do not necessarily elicit membrane conductance charges and, similarly, neurotransmitters that elicit an electrophysiological response do not inhibit PGE_1-stimulated increases in cAMP. Dopamine elicits an electrophysiological response and inhibits the effects of PGE_1. The possibility exists that a single receptor is mediating two cellular events.

Thus, with regard to the catecholamine and endolamines, there appears to be no discernable effect between cAMP responses, passive membrane electrical properties, and electrical responses due to putative neurotransmitters. Therefore, as studied with TCX 11 neuronal cells, these compounds may not be associated with inhibitory mechanisms responsible for early transient incapacitation.

Table 1. Inhibition of PGE_1 Activity

	Exponential Growth Phase	Differentiated	Electrophysiological Response
Dopamine	+	+	+
Noradrenaline	+	+	+
5-Hydroxytryptamine	–	–	+
Carbachol	+	+	–

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PHYSICOCHEMICAL CORRELATES OF ALCOHOL INTOXICATION

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

The precise way in which alcohols interact with membranes and thus create alterations in neuronal function remains unknown. Although the literature on the biological effects of alcohols is vast, most studies have dealt with only the anesthetizing properties of this broad class of depressants. Comparison of their actions as intoxicants in intact animals has remained unaddressed largely because of the absence of a suitable animal model. Previous studies of *in vitro* systems using a limited number of alcohols have suggested that their potency is directly related to their lipid/water partition coefficients (P). In the present effort, a broad range of structurally divergent alcohols, diols, and other monofunctional alkanes were tested for their ability to intoxicate whole animals. Male Sprague-Dawley rats were injected i.p. with several doses of a given drug and evaluated for the most severe signs of intoxication using behavioral endpoints described by Majchrowicz (1). From dose-response curves, the effective dose (ED) needed to produce ataxia 2 was determined. Of over 60 compounds tested, almost all induce a behavioral spectrum of intoxication almost identical to that of ethanol. This includes amphiphilic compounds that are not alcohols, such as propyl chloride and propanethiol. A plot of [log P] vs [log ED] yields a straight line with a correlation coefficient equal to −0.9, indicating a high degree of predictability (Figure 1). The membrane concentration, the volume occupied within the membrane, and the thermodynamic activity have been successfully utilized by past workers to predict the anesthetic potency of a compound. In order to determine whether these physicochemical parameters could also be used as correlates of alcohol intoxication, partition coefficients were used to estimate the concentrations of each drug within the aqueous and

nonaqueous regions of the animal. From these calculations, each of these parameters was found to remain remarkably constant even though the ED varied over two orders of magnitude (Figure 2). Among the few exceptions were several alkanes that were ineffective in inducing intoxication even though their lipid solubility is very high. These data suggest that amphiphilicity of a compound is essential for inducing intoxication. Moreover, the three-dimensional structure of a compound is important in influencing its intoxicating efficacy only to the extent that its partition coefficient is affected.

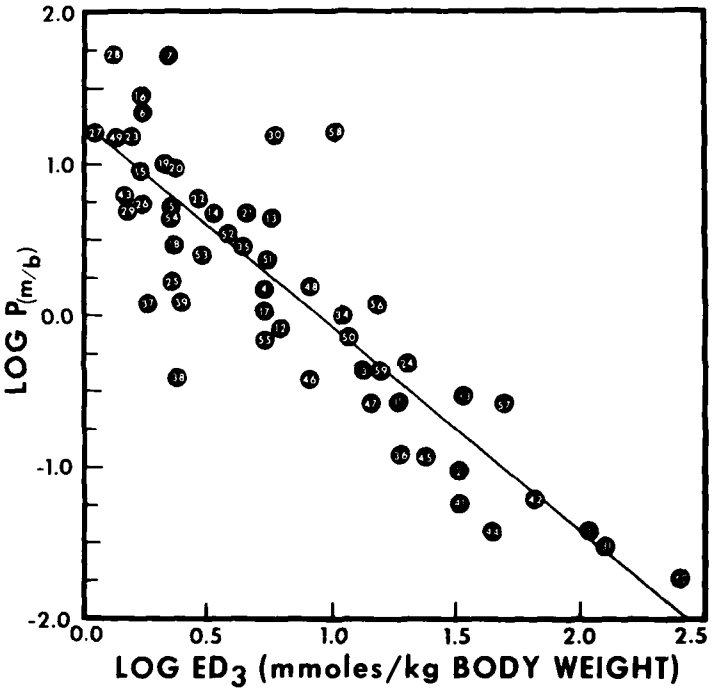

Figure 1. Plot of the logarithm of the membrane/buffer partition coefficient, $P_{m/b}$, as a function of logarithm potency, ED_3. The linearity of this plot demonstrates that the Meyer-Overton rule for anesthesia may be utilized in an analogous fashion for predicting intoxicating potency.

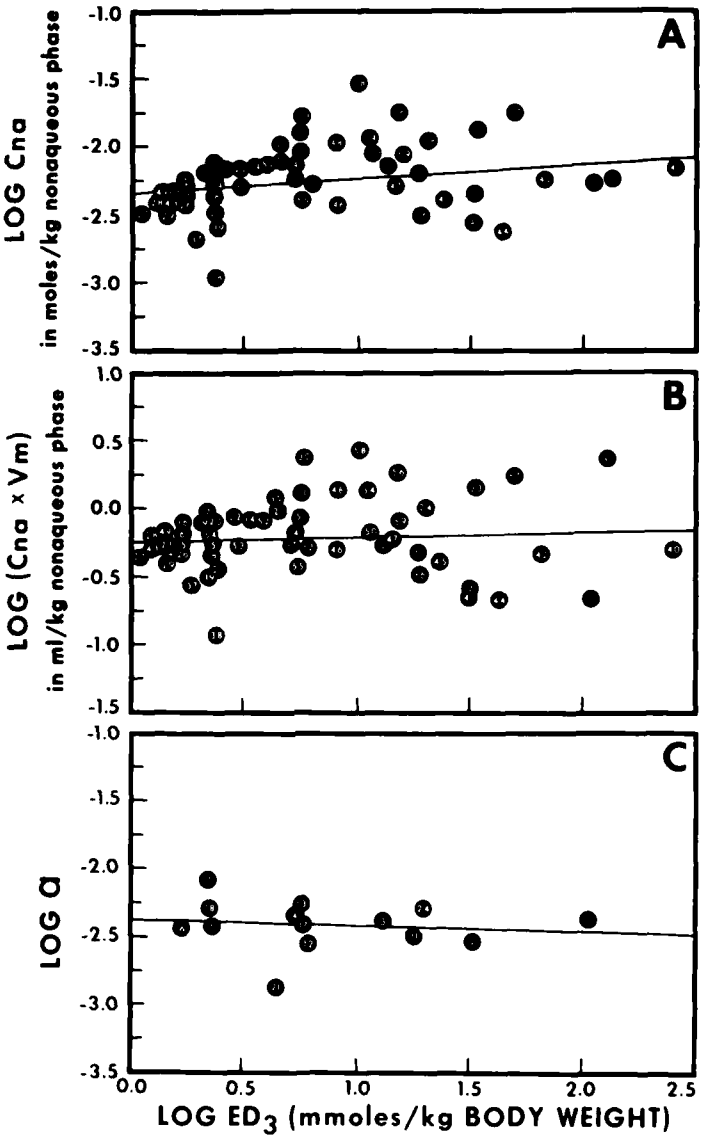

Figure 2. The logarithm of the potency is plotted vs the logarithm of each of the following physicochemical quantities necessary to induce ataxia 2. *A*. C_{na}, the drug concentration in the nonaqueous phase of the animal.

B. $C_{na} \times V_m$, a product representing the volume of the nonaqueous phase occupied by each intoxicant at the effective dose.

C. a, the thermodynamic activity. All of these parameters remain relatively constant even though there is considerable variation in structure and potency of the compounds tested. These data for intoxication thus seem to follow the hypotheses set forth by A. Meyer-Overton, B. Mullins, and C. Ferguson for correlating anesthetic potency.

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SLAB GEL ANALYSIS OF RAPIDLY TRANSPORTED PROTEIN

Principal Investigators: J. H. Neale, J. L. Barker, and W. M. Bonner

Using analytical SDS slab gel electrophoresis, fluorography, and optical scanning techniques, we have investigated the molecular weight distribution of ^{35}S-methionine-labeled, rapidly transported protein in several functionally distinct, isolated, frog neuronal systems. This technique resolved 18 major and minor peaks of labeled protein with very similar electrophoretic mobility rates that were common to the populations of transported proteins in both the central and peripheral processes of the dorsal sensory ganglion. Of these protein peaks, 14 appeared similar to those rapidly transported in the spinal motor axons and the post-ganglionic sympathetic axons. The profiles of proteins synthesized by the sensory ganglion, sympathetic ganglion, and myelinated nerve differ from the transported protein profiles in relative labeling and molecular weight distribution (Figure 1). The combined application of these techniques has increased the resolution of the complex array of rapidly transported protein beyond that obtained by liquid scintillation detection of gel radioactivity. The results support the concept that a common set of proteins are transported by functionally different neuronal systems. These results indicate, further, that the array of proteins that are rapidly transported is sufficiently complex that methods of separation based on physical characteristics such as isoelectric point should be used in conjunction with this technique in order to more effectively resolve individual protein species. The

ultimate objective of these two-dimensional separation techniques is to provide the physical basis for a variety of experimental approaches to the biologic significance of individual polypeptides within the rapidly transported protein population.

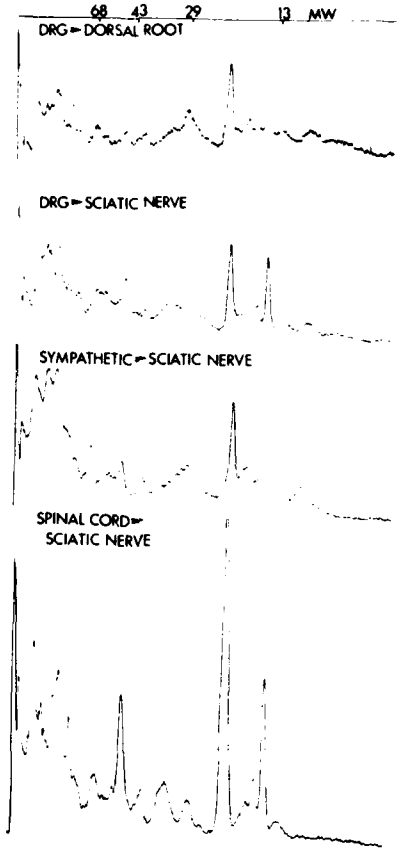

Figure 1. Densitometric tracings of slab gel autoradiogram of rapidly transported proteins. Separate experiments were conducted on the isolated frog nervous system using (^{35}S) methionine as a radioactive precursor. Following axonal transport, methionine-labeled proteins accumulating at ligations were homogenized, and different transport accumulations were separated on a polyacrylamide slab gel. A fluorogram of the slab gel was made, and the distribution of X ray film grain density was quantitatively assessed using a Joyce-Loebl densitometer set at the same gain for all tracings. (The tracings had been

aligned with reference to the start of the gel at the left.) Varying grain densities (peaks) reflect the relative radioactivity of the various protein species. The molecular weight distribution of stained standard proteins run on the same gel has been superimposed on top of the tracings. A close correspondence of peak distribution among the tracings is evident.

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IMMUNE SURVEILLANCE AND TUMORS OF THE NERVOUS SYSTEM

Principal Investigators: R. A. Morantz, *AFRRI*
W. Shain, *AFRRI*
Collaborator: H. Cravioto, *New York University Medical Center*

The theory of immune surveillance postulates that one function of the immune system is to eliminate small numbers of malignant cells that spontaneously arise within the organism. Although there has been a great deal of both clinical and experimental evidence in favor of this theory as it applies to general oncology, the question of whether or not such a surveillance system would be effective for tumors arising within the nervous system has never been studied. Rats whose mothers had been exposed to the neuro-carcinogen ethylnitrosourea (ENU) were divided into control, immunosuppressed, and immunoenhanced groups (Figure 1). These lifetime alterations of the immune system had *no* effect on the course of formation of tumors of the nervous system. We believe the most likely explanation for our results is that the "immunologic privilege" of the brain prevents the usual interaction of the neoplasm and the immune system.

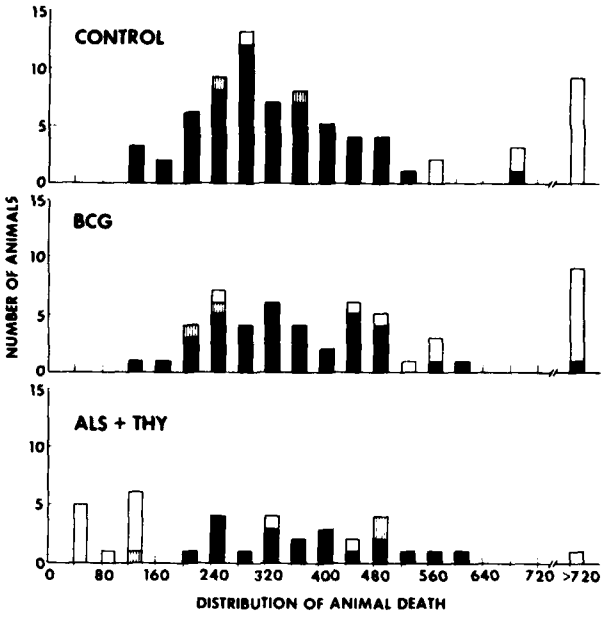

Figure 1. Distribution of deaths in rats exposed transplacentally to ENU and undergoing lifetime immunologic modulation by immunoenhancement with Calmette-Guerin bacillus (BCG) or immunosuppression by antilymphocyte serum (ALS) and thymectomy (THY).

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MEMBRANE RECTIFICATION IN A MOLLUSCAN STATOCYST

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 that responded exhibited depolarization, but this could be accompanied by either decrease, increase, or no measurable change in slope resistance, depending on resting membrane potential (Figure 1).

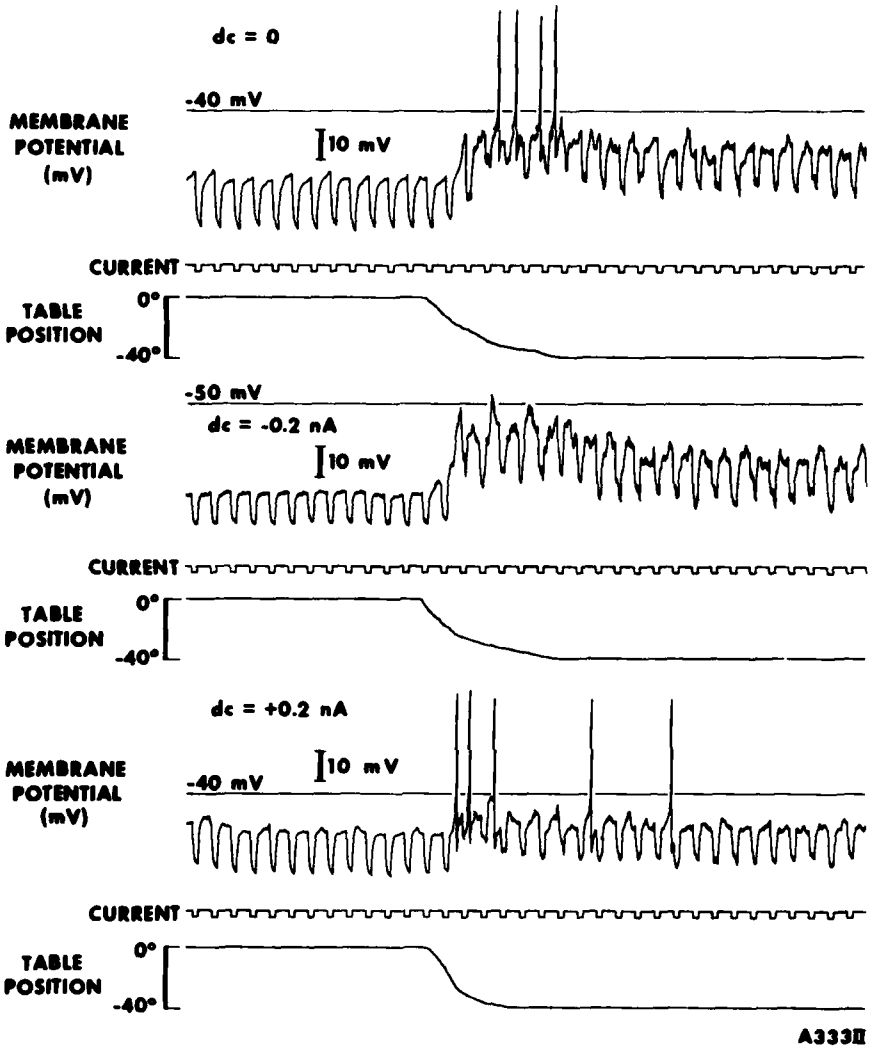

Figure 1. Effect of membrane potential on slope-resistance change caused by downward tilt of a statocyst receptor cell. With 0 d.c., the normal resting membrane potential is -62 mV, and tilting causes a 10-mV depolarization and a 14% decrease in slope resistance. With -0.2 nA d.c., the resting potential is lowered to -79 mV, and tilting causes a 12-mV depolarization but a 42% *increase* in slope resistance. With +0.2 nA d.c., resting potential is raised to -52 mV, and now tilting causes only a 2-mV depolarization but a 20% decrease in slope resistance. Thus the effects of membrane rectification have caused

the underlying resistance-decrease transduction mechanism to appear as a resistance increase when the cell was biased into the anomalously rectifying region (with −0.2 nA).

By altering membrane potential with d.c. and measuring slope resistance with constant current pulses, these cells are shown to exhibit both anomalous and delayed rectification. Either hyperpolarization or depolarization from one potential can cause the slope resistance to decrease by as much as a factor of 5.

The response to standard tilt can be changed from an increase in slope resistance to a decrease, or vice versa, by altering 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.

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CILIARY MOTILITY IS REQUIRED FOR NORMAL SENSORY FUNCTION IN A CILIATED MECHANORECEPTOR CELL

Principal Investigator: M. L. Wiederhold

The *Aplysia* statocyst is a spherical balance organ. The wall of the cyst consists mainly of 13 receptor cells with motile cilia projecting into the fluid-filled cyst lumen. Dense statoconia settle to fill the bottom third of the cyst lumen, but are kept in continual random motion by the beating cilia. A receptor cell is excited when the preparation is

tilted sufficiently to bring its cilia into contact with the statoconia. The excitation consists of a depolarization and an increase in membrane voltage noise.

An analysis of the membrane noise caused by excitation is presented (Figure 1), which indicates that the noise is a superposition of many discrete depolarizing events whose average amplitude is between 1.5 and 2.3 mV. These are interpreted as arising from collisions between the moving statoconia and the beating cilia. With a receptor cell positioned so that only occasional collisions between the statoconia and cilia would occur, isolated discrete events of the size predicted by the analysis are seen. The conductance change associated with these events is about 10 times larger than estimates of single ionic channel conductance from other studies.

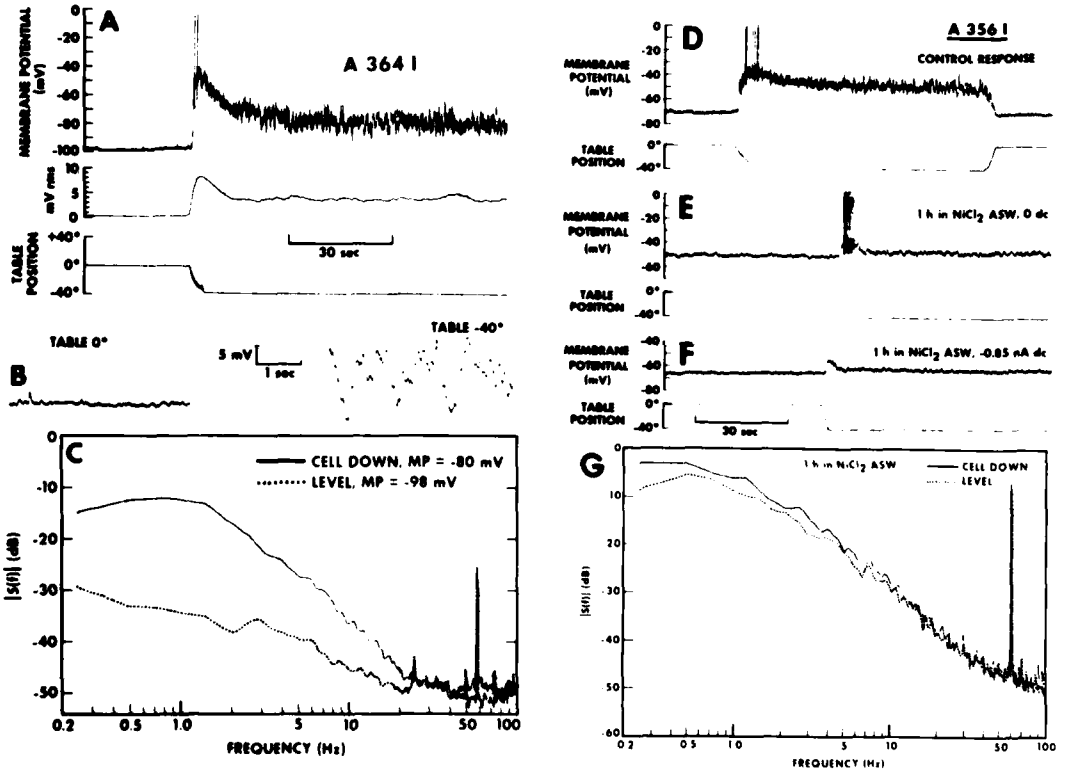

Figure 1. Response to tilting of a preparation in normal artificial seawater (ASW). *A. Upper trace:* Membrane potential as table is lowered from level (0°) to cell-down (-40°) position. Spike amplitude is distorted by pen recorder. Actual spikes are 100 mV peak to peak. Tilting indicated in lower trace. *Middle trace:* Root mean square (RMS) membrane potential measured with an a.c.-to-d.c.

converter, passing frequencies from 0.16 Hz to 110 kHz. Due to settling time of converter, readings are accurate only 25 sec after a sudden change in membrane potential. *B*. Portions of upper trace in *A* with scales expanded. Note discrete positive potentials from 0.5 to 3.5 mV amplitude. *C*. Spectral density of voltage noise at two table positions computed with a fast-Fourier transform analyzer. Spectra shown are the average of 32 in each position. Spectral level plotted in dB re an arbitrary reference (20 dB = factor of 10 in voltage or factor of 100 in power). *D*. Control response to tilting as in *A*. Actual spike amplitude 80 mV peak to peak. *E*. Decreased response after 1 hour in ASW with 10 mM $NiC1_2$ added (Ni ASW). Note that in this case, membrane depolarized 20 mV in Ni ASW. *F*. Response in Ni ASW with membrane potential restored to near its original value with −0.85 nA d.c. passed through recording electrode using an active bridge circuit. *G*. Spectral density of voltage noise in two table positions (as in *C*) after 1 hour in Ni ASW.

When a preparation is treated with seawater containing 10 mM $NiCl_2$ (which has been used in other studies to paralyze the cilia of paramecium), the motility of these cilia is reduced or blocked, and both the depolarizing and noise-increase responses to tilting are either greatly reduced or abolished. Another agent that blocks ciliary motility is a factor in the serum of cystic fibrosis patients. When such serum was mixed with seawater, results similar to those with $NiCl_2$ treatment were obtained. The mechanisms by which the ciliary motility might contribute to the sensory function of these cells are discussed.

Since ionizing radiation has been shown to decrease the beat frequency of tracheal cilia, if the results of this study can be extended to vertebrate systems, they may relate to the ototoxic effect of ionizing radiation of the ear.

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NONINVASIVE CHRONIC RECORDING OF AUDITORY NERVE POTENTIALS

Principal Investigators: M. L. Wiederhold, S. A. Martinez, D. M. Paull, M. G. Pierson, and H. O. deFries

We have developed a system that allows repeated, noninvasive recording of auditory nerve responses in cat to transient acoustic stimuli using a closed acoustic system.

N_1 responses to clicks are recorded from a stainless steel ring electrode at the end of a hollow earbar, the tapered end of which is made of insulating plastic. Acoustic stimuli are generated by a dynamic earphone coupled to the earbar. A calibrated probe microphone is also incorporated into the earbar to measure sound pressure near the tympanic membrane. This allows better stimulus control than is available with free-field systems. To facilitate insertion of the earbar, meatoplasties were performed on all animals. Responses recorded with this system in anesthetized cats are described and compared to those recorded at the round window. Good repeatability of measurements is described for an animal population of 20 domestic cats over a period of several months (Figure 1). For some of these animals, response amplitude varied from one session to another, but response latency, especially for condensation clicks, was consistent. By comparing statistics of multiple measurement of both N_1 amplitude and latency for rarefaction and condensation clicks, it is concluded that the N_1 latency vs click-level function for condensation clicks provides the most reliable measure of the cat's auditory nerve function.

Clinical studies have shown that patients whose inner ears had been exposed to ionizing radiation for the treatment of head and neck tumors suffered permanent hearing loss. Experimental studies on animals have shown that hair cells, the sensory receptor cells in the inner ear, are selectively damaged by ionizing radiation. A system such as described here can now be used to measure, on both a short-term and a long-term basis, the effects on an animal's hearing of exposure to varying total dose and dose rates of such radiation.

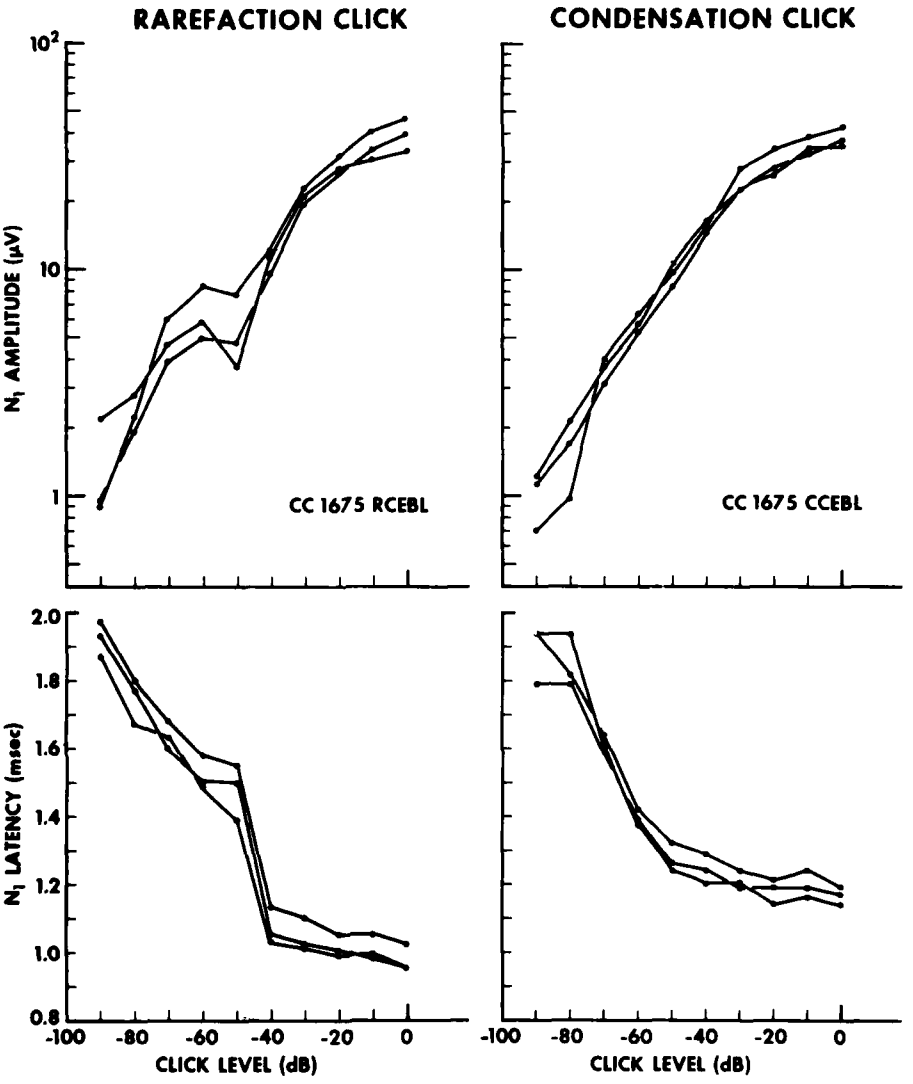

Figure 1. N_1 amplitude and latency functions for rarefaction clicks and condensation clicks on three repeated recording sessions for one chronic cat. For 0-dB rarefaction click responses, that of intermediate amplitude was recorded 3 weeks after meatoplasty, that of lower amplitude 1 week later, and that of highest amplitude 11 weeks after the second. Note good repeatability of all measures.

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EFFECTS OF EUSTACHIAN TUBE LIGATION ON AUDITORY NERVE RESPONSES TO CLICKS

Principal Investigators: M. L. Wiederhold, S. A. Martinez, R. E. C. Scott, and H. O. deFries

Auditory nerve responses to condensation and rarefaction clicks were recorded from the external ear canal of cats using a closed acoustic system. Repeated control recordings from both ears formed a baseline for each of four animals used in this study. After a baseline had been established, the eustachian tube on one side was ligated, and serial recordings of N_1 responses were performed for up to 140 days post-ligation. By comparing the shift that occurred in the N_1 latency-vs-click level plots after ligation, the equivalent hearing loss was determined. In all cases where the eustachian tube was successfully ligated, the loss was progressive for the first 20 days, then usually showed some transitory improvement (Figure 1). The loss stabilized after 60 days, varying from 15 to 40 dB in different animals. In addition to N_1 recordings, serial tympanograms were also measured. These indicated negative middle-ear pressure in the first 2 days post-ligation and the presence of middle-ear fluid by 1 week post-ligation.

These results indicate that the chronic recording system described in the preceding study is sensitive and stable enough to reliably detect hearing losses induced by environmental effects, and that the system can be used to measure the effects of ionizing radiation on hearing.

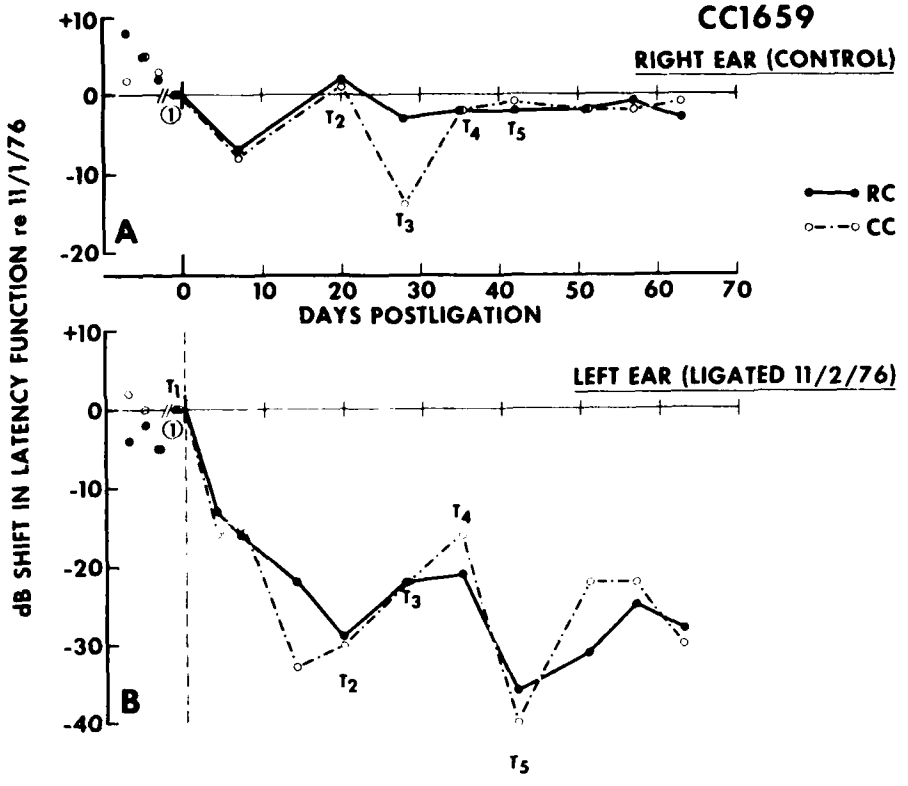

Figure 1. Shift in latency functions for rarefaction clicks (RC) and condensation clicks (CC) after ligation of the left eustachian tube of cat CC1659. Shifts are all relative to preoperative control latency functions (points labeled ① for each ear). The shifts plotted are the average displacement (in dB) in the N_1 latency-vs-click level plots for latencies between 1.1 and 2.0 msec for RC's and between 1.3 and 2.0 msec for CC's Points to the left of Day 0 are earlier preoperative controls. Points labeled T_1-T_5 correspond to recordings made immediately after tympanograms that measure middle-ear pressure.

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CARDIAC ARRHYTHMIAS RESULTING FROM EXPERIMENTAL HEAD INJURY

Principal Investigators: D. E. Evans, W. A. Alter, III, S. A. Shatsky, and E. N. Gunby

The cardiovascular events resulting from experimental head injury were studied to determine the incidence of cardiac arrhythmias and to define the autonomic mechanisms responsible for these changes. Electrocardiograms and arterial blood pressure were recorded in anesthetized monkeys before and after the animals were subjected to temporoparietal head impact. Cardiac arrhythmias and hypotension occurred immediately following impact in every animal studied. Figure 1 contains an example of the responses obtained immediately postimpact. In this animal, impact resulted in cardiac arrest for 4 sec followed by nodal bradycardia accompanied by a significant decline in blood pressure. Ninety seconds postimpact, blood pressure had returned to near preimpact level, but a complex arrhythmia was evident, consisting of sinus and nodal beats along with premature ventricular beats.

Cardiac arrhythmias occurred immediately following temporoparietal impact in each of 10 untreated animals. The abrupt decrease in heart rate following impact suggested that the arrhythmias were mediated by the parasympathetic nervous system. To test this hypothesis, five animals were subjected to impact after pretreatment with atropine (0.2 mg/kg). Vagal blockade prevented arrhythmias from occurring after temporoparietal impact in each animal tested. In contrast, adrenergic blockade failed to eliminate these impact-induced cardiac arrhythmias.

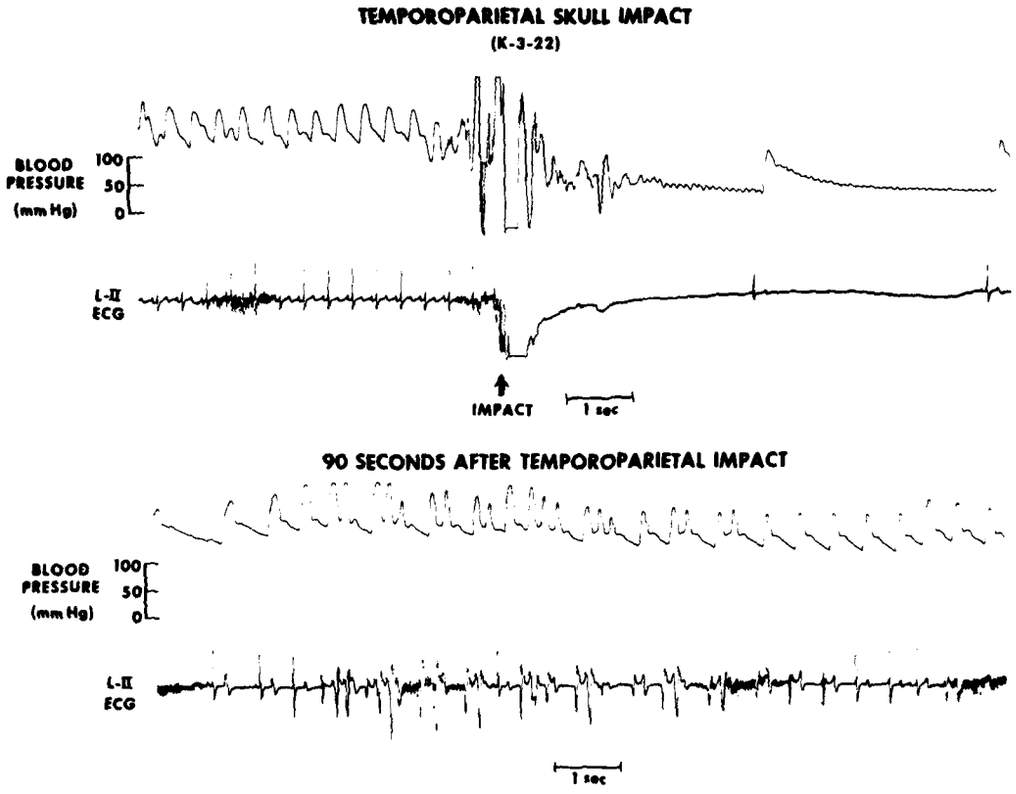

Figure 1. Effect of temporoparietal impact on the arterial blood pressure and electrocardiogram of monkey K-3-22

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CARDIAC ARRHYTHMIAS ACCOMPANYING ACUTE SPINAL CORD COMPRESSION

Principal Investigators: D. E. Evans and A. I. Kobrine

The subject of acute cardiac arrhythmias accompanying acute spinal cord injury has not received much attention and yet may be more common than is generally appreciated. Prompted by a report of a patient's (with acute spinal cord injury) developing a cardiac arrhythmia, cardiac arrest, and death, the following series of experiments were carried out in an attempt to better understand this problem.

Six *Macaca* monkeys, 3.5-4.5 kg, were initially anesthetized with phencyclidine HCl, 2 mg/kg. Catheters were inserted into the femoral artery and vein, and the animals were intubated and curarized. Anesthesia was maintained with i.v. chloralose, 100 mg/kg. Ventilation was maintained with a volume respirator; blood gases were obtained frequently and kept in the physiologic range. Laminectomies were performed at the T9 level, and a no. 3 Fogarty catheter was inserted into the epidural space so that the uninflated balloon lay at T6. EKG, arterial blood pressure, and heart rate were monitored on the polygraph. After adequate stabilization of the animal, the epidural balloon was acutely inflated with 0.25 cc saline and kept inflated for 15 min. Spinal cord-evoked response was monitored before, during, and after compression by stimulating the sciatic nerve and recording the summated evoked response from the cervicomedullary junction. The evoked response disappeared in all animals during the period of compression, indicating a complete spinal cord conduction block.

Numerous and prolonged cardiac arrhythmias, including sinus arrest, A-V dissociation, premature ventricular beats, and ventricular tachycardia, were seen in four of the six animals during spinal cord compression (Figure 1). These arrhythmias were altered or abolished by the i.v. administration of both sympatholytic and parasympatholytic drugs, indicating a role of both the sympathetic and parasympathetic components of the autonomic nervous system in the etiology of the arrhythmias.

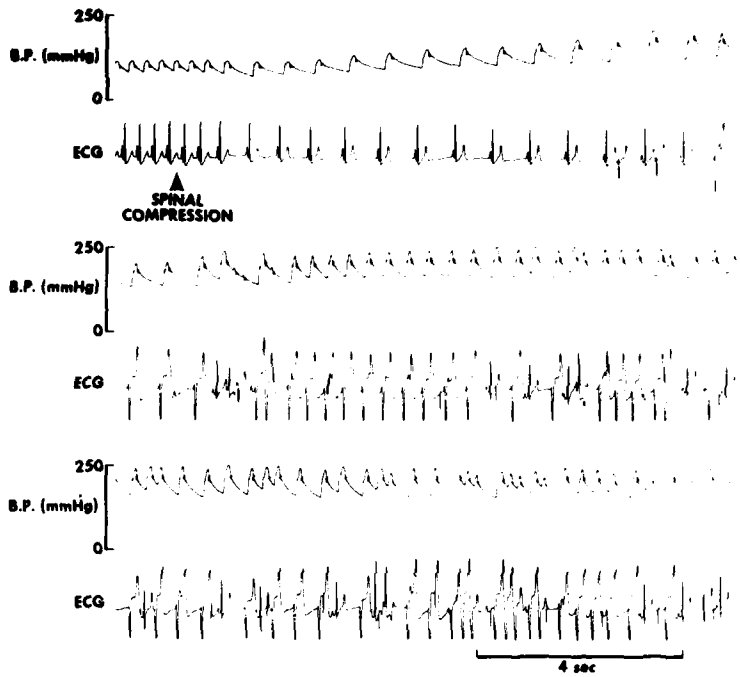

Figure 1. Cardiovascular response to acute spinal cord compression in anesthetized monkey. Responses include hypertension and complex arrhythmia including sinus bradycardia with frequent ectopic ventricular beats.

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CORRELATION OF SPINAL CORD BLOOD FLOW AND CORD FUNCTION IN SUBACUTE SPINAL CORD COMPRESSION

Principal Investigators: A. I. Kobrine and D. E. Evans

In an attempt to better understand the pathophysiologic processes involved in the development of neurologic dysfunction accompanying subacute spinal cord compression (e.g., epidural tumor, hematoma, and abscess), the following experiment was undertaken. Ten *Macaca* monkeys were initially anesthetized with phencyclidine HCl,

intubated, and curarized. Anesthesia was continued with i.v. administration of chloralose. The left sciatic nerve was exposed and a bipolar stimulating electrode was positioned. By stimulating the sciatic nerve at 10 volts, 0.1-msec duration at a frequency of one per second, the spinal cord-evoked response (ER) was easily recorded from a bipolar electrode placed on the dura over the left nucleus gracilis via a small laminectomy of C1. Data were recorded on line to a digital computer. A small laminectomy was performed at T9, and a no. 3 Fogarty catheter was slid up the epidural space so that the uninflated balloon lay at T6. A 1-mm opening was made in the ligamentum flavum at T6, and a hydrogen electrode was placed in the left dorsal column. The Fogarty balloon was slowly inflated via an infusion pump to reach a volume of 0.5 cc in 1 hour. Blood flow in the T6 segment of the cord was measured every 15 min. The physiologic integrity of the cord was monitored by ER recording every 5 min. The ER was lost after an average inflation time of 1½ hours. The balloon was deflated after a 5-min period of absent ER. The question we hoped to answer was whether the neurologic dysfunction that accompanies subacute spinal cord compression is due to ischemia or to the direct effects of compression on neural tissue.

In all cases, the ER did not disappear until the blood flow was zero. In four cases, a definite phase of hyperemia was present during compression, at which time the amplitude of the ER was decreased. A postischemia hyperemia occurred in seven cases. The ER returned in all cases. These results indicate that the loss of ER in this preparation was due to ischemia, since the effect was reversible.

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CHRONOTROPIC RESPONSES TO CORONARY ARTERY OCCLUSION IN THE RHESUS MONKEY

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

The etiology of chronotropic responses resulting from coronary artery occlusion in chloralose-anesthetized monkeys (*Macaca mulatta*) was examined. Ten monkeys were instrumented to record arterial blood pressure (BP), heart rate (HR), lead-II electrocardiogram (ECG), regional contractile force, and myocardial blood flow (using hydrogen polarographic techniques). The origins of the circumflex (CIRC) and anterior descending (LAD) branches of the left coronary artery were exposed. Baseline HR and systolic BP were 186 ± 9 beats per minute (bpm) and 128 ± 6 mm Hg, respectively. Occlusion of the LAD for 1 min resulted in a 17 ± 3% fall in systolic BP and 5 ± 1 bpm increase in HR. CIRC occlusion for 1 min resulted in a similar drop in systolic

BP (17 ± 3%) while HR declined significantly (−52 ± 8 bpm) in 8 out of 10 animals. Administration of atropine (0.5 mg/kg) and propranolol (0.5 mg/kg) failed to eliminate the bradycardia recorded during CIRC occlusion. Examination of the CIRC branches near the origin revealed one whose occlusion resulted in a bradycardia (−45 ± 8 bpm) similar to that observed during CIRC occlusion, but occlusion distal to this branch did not affect HR. Figure 1 shows that occlusion of this CIRC branch resulted in a marked bradycardia (70 bpm) without any major changes in contractile force, myocardial blood flow, or arterial blood pressure. Coronary arteries were then filled with latex, revealing that this CIRC branch perfused the sinus node region. It was concluded that chronotropic responses to CIRC occlusion were due to pacemaker ischemia.

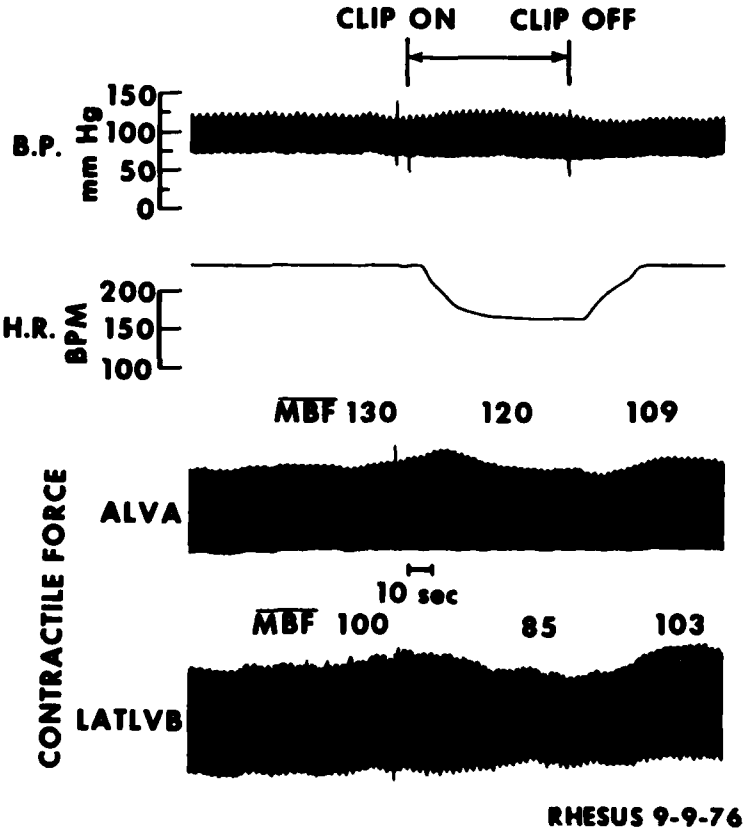

Figure 1. Cardiovascular responses to sinus node artery occlusion in the anesthetized rhesus monkey. During this 1-min occlusion, the only significant change was a 70-bpm decline in heart rate.

BP, aortic blood pressure; HR, heart rate in beats per minute (bpm). Contractile force was recorded from epicardium near the apex of the anterior wall of left ventricle (ALVA) and near base of the lateral wall of the left ventricle (LATLVB).

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EFFECT OF BETA ADRENERGIC BLOCKADE ON SPINAL CORD AUTOREGULATION IN THE MONKEY

Principal Investigators: A. I. Kobrine and D. E. Evans
Collaborator: H. V. Rizzoli

We have previously shown that autoregulation exists in the lateral white matter of the monkey spinal cord between a mean arterial pressure (MAP) of 50 and 135 mm Hg. We have also demonstrated a breakthrough of autoregulation in the intact animal when the MAP is above approximately 140 mm Hg (1). We have shown that a vascular dilatation accompanies this breakthrough, secondary to a progressive decrease in vascular tone, and we have suggested that it is this mechanism that is responsible for the observed increase in blood flow under such circumstances (2,3). We have attempted in the present experiment to study the possible mechanisms of autoregulation in the spinal cord.

In seven monkeys under N_2O and O_2 anesthesia, dorsal laminectomies were performed at T8-T10. Respiration was maintained with a small animal respirator. Several platinum electrodes, 250 μ in diameter, were then inserted into the lateral funiculus through the intact dura by techniques previously described (4). The animals were given 1 mg/kg propranolol intravenously (beta adrenergic blocker). Spinal cord blood flow (SCBF) was then measured under normocapnic conditions over a wide range of artificially varied MAP's. The MAP was either lowered by bleeding or raised by the intravenous administration of angiotensin. Using the hydrogen-clearance method (as in earlier publications), we calculated the blood flow from the monoexponential tissue desaturation curve of inhaled hydrogen gas (4). The SCBF remained in the normal range of from 50 to 175 mm Hg. There was no breakthrough of autoregulation. Below 50 mm Hg, SCBF fell as MAP was further lowered (Figure 1).

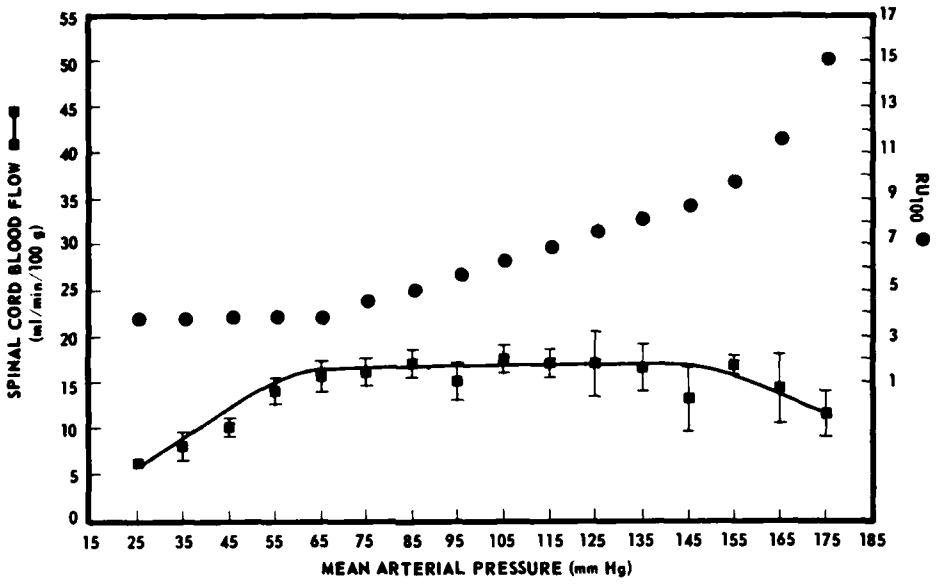

Figure 1. Relation between spinal cord blood flow and mean arterial pressure in a propanolol-treated preparation

The data from the present study suggest that this previously observed vasodilatation is a beta adrenergic-mediated phenomenon, since this phenomenon did not occur in animals after beta adrenergic blockade. In fact, at MAP levels of 170-180 mm Hg, SCBF actually decreased. This might be explained by an unchecked, continued alpha adrenergic-mediated vasoconstriction, which in the untreated animals is overcome by the beta-mediated vasodilatation discussed above.

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2. Kobrine, A. I., Doyle, T. F., and Martins, A. N. Autoregulation of spinal cord blood flow. Clin Neurosurg 22: 573-581, 1975.

3. Kobrine, A. I. and Doyle, T. F. Spinal cord blood flow as affected by changes in the systemic arterial pressure. J Neurosurg 44: 12-16, 1976.

4. Kobrine, A. I., Doyle, T. F., and Martins, A. N. Spinal cord blood flow in the rhesus monkey by the hydrogen clearance method. Surg Neurol 2: 197-200, 1974.

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EFFECT OF ALPHA ADRENERGIC BLOCKADE ON SPINAL CORD AUTOREGULATION IN THE MONKEY

Principal Investigators: A. I. Kobrine and D. E. Evans
Collaborator: H. V. Rizzoli

It has previously been shown that autoregulation exists in the lateral white matter of the monkey spinal cord between a mean arterial pressure (MAP) of 50 and 135 mm Hg, and that autoregulation remains essentially intact after high cervical cord section (1-3). Furthermore, we have demonstrated a breakthrough of autoregulation in the intact animal, when the MAP is above approximately 140 mm Hg. We have attempted in the present experiment to study the possible mechanisms of autoregulation in the spinal cord.

In seven monkeys under N_2O and O_2 anesthesia, dorsal laminectomies were performed at T8-T10. Respiration was maintained with a small animal respirator. Several platinum electrodes, 250 μ in diameter, were then inserted into the lateral funiculus through the intact dura by techniques previously described (4). The animals were given 5 mg/kg phenoxybenzamine intravenously (alpha adrenergic blocker). Spinal cord blood flow (SCBF) was then measured under normocapnic conditions over a wide range of artificially varied MAP's. The MAP was either lowered by bleeding or raised by the intravenous administration of angiotensin. Using the hydrogen-clearance method (as in earlier publications), we calculated the blood flow from the mono-exponential tissue desaturation curve of inhaled hydrogen gas (4). There was a linear relationship between SCBF and MAP, demonstrating a lack of autoregulation (Figure 1).

The data from the present experiment suggest that intact alpha adrenergic receptors are necessary for control of vessel tone and, therefore, for autoregulation in the white matter of the monkey spinal cord. In order for an organ to autoregulate its blood supply, mechanisms must exist so that changes in MAP will provoke corresponding changes in vascular resistance and the blood flow will remain constant. The progressive rise in SCBF that accompanies the induced rise in MAP in the present experiment indicates a lack of response by the resistance vessels to this induced rise in MAP. Since these animals were given phenoxybenzamine (dibenzyline), which blocks the alpha adrenergic receptors, it is suggested that the progressive rise in vascular resistance seen to accompany rises in MAP in the intact animal is an alpha adrenergic-mediated phenomenon.

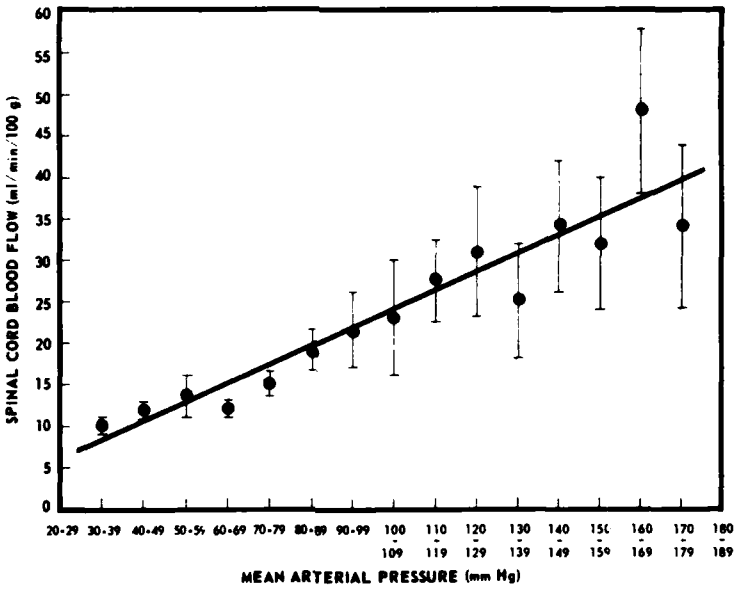

Figure 1. Relation between spinal cord blood flow and mean arterial pressure in a dibenzyline-treated preparation

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A PRIMATE, PIAL ARTERIOVENOUS FISTULA MODEL USING A BIOLOGICAL ANASTOMOTIC BOND

Principal Investigator: S. J. Wright, Jr., *AFRRI*
Collaborators: M. Gurtin and D. Doppman, *National Institutes of Health*
A. N. Martins, *Walter Reed Army Medical Center*

Anomalous cerebral blood flow through a pathophysiologically or structurally altered cerebrovascular bed may be found following the effects of combined radiation and blast injury, and can cause secondary brain damage. Therefore an effort has been made to develop and study an experimental, intracranial arteriovenous fistula in which arterial blood passes directly into veins without nourishing the surrounding brain tissue.

A means was found of firmly adhering a 0.2- to 0.3-mm artery and a 0.3- to 0.5-mm vein to one another by taking advantage of the flow expansion of the artery within the vein and the cementing action of local fibrin deposition between the two concentric vessels (see Figure 1*a-d*). Through a temporal-parietal craniectomy in two rhesus and six cynamologus monkeys, the desired arteriovenous fistulas were successfully created by this microanastomotic method. Intraoperative patency of the fistula could be assessed by the appearance of red (arterial) blood streaming within the purple (venous) blood of the draining vein. To date, post-operative patency in normal monkeys has been monitored angiographically from 1 day to 6 months, and increased diameter of the feeding artery and draining vein has been demonstrated. Further work with this model may lead to a better understanding of the secondary brain metabolism and the physiology alterations that may occur in the presence of an arteriovenous fistula.

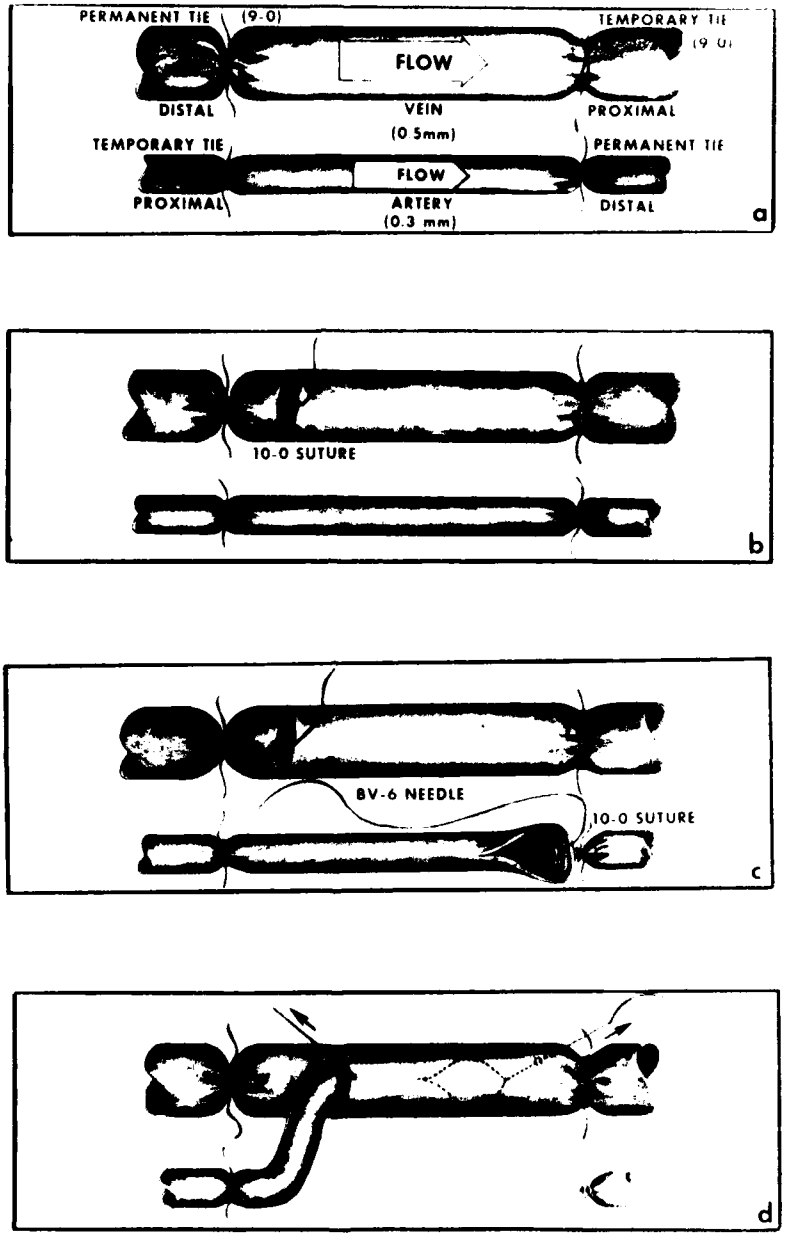

Figure 1 *a-d*. Principal steps necessary to create experimental arteriovenous fistula. Initially an artery and vein on surface of monkey's brain are brought close to one another. After each is occluded at two points, opening is made in vein, and small suture is attached to vein

near this opening. Next the artery is divided, longitudinally backcut a short distance, and suture attached to the open end. Then artery is drawn into opening of vein as shown, temporary ties of the two vessels released, and arteriovenous fistula is completed.

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MICROVASCULAR OCCLUSION MAY BE ACCOMPLISHED WITH MINIMAL INTIMAL CHANGE

Principal Investigator: S. J. Wright, Jr., *AFRRI*
Collaborators: J. McKee, *Naval Medical Research Institute*
A. N. Martins, *Walter Reed Army Medical Center*

Neurosurgical treatment of brain injury, which ensues from combined radiation-blast exposure, will occasionally require temporary occlusion of small (approximately 1 mm in diameter) cerebral vessels. Therefore, the development of the least traumatic microvascular occluding device is worthwhile. One author (S.W.) has developed a transparent microvascular occluding device (the Microblock) through which the operator may view the occlusion (blanching) of a small vessel approximately 1-2 mm in diameter or less. Since the least force possible is delivered by soft (silastic) occluding members, this device should have only a small disruptive effect on the intimal lining of a blood vessel. A series of rat common carotid arteries (n = 8) were occluded for from 1 to 30 min with the Microblock. A smaller series of arteries (n = 3) were occluded with small aneurysm clips commonly used for the same purpose. Then the animals were sacrificed and the vessels carefully removed and opened with microtechnique. After being irrigated with saline and immediately fixed in glutaraldehyde, they were submitted for scanning electron microscopy. The results indicated that the Microblock caused only localized intimal flattening with an absence of intimal tears (Figure 1*a*). Tears were seen in the intima of those vessels to which the metal clips had been applied (Figure 1*b*). It is concluded that (a) very likely the minimal amount of force needed to occlude a small vessel may create observable changes in the intima, and (2) the Microblock causes only mild compressive changes as opposed to the intimal tears that are seen following the application of some metal aneurysm clips.

The occluding device that least damages the blood vessel lining is, of course, the one that will be least likely to thrombose the vessel and harm the patient.

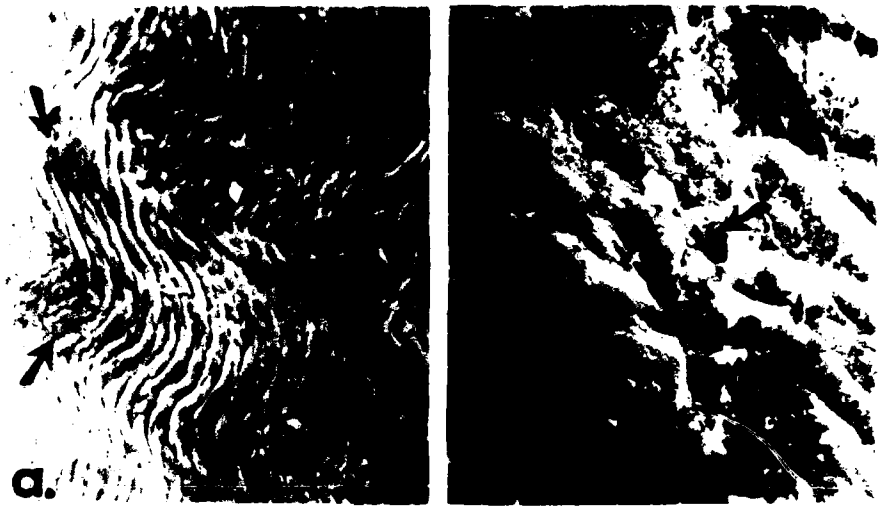

Figure 1*a*. Scanning electron micrograph (200 X) of the intimal surface of a rat common carotid artery after Microblock occlusion for 30 min. Note the intimal flattening (arrows) but the absence of intimal tears. *b*. Scanning electron micrograph (1000 X) of the intimal surface of a rat common carotid artery after metal aneurysm clip occlusion for 30 min. Note the intimal tear (arrows).

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EFFECT OF TOPICALLY APPLIED POTASSIUM ION ON REGIONAL CEREBRAL BLOOD FLOW

Principal Investigators: S. J. Wright, Jr. and T. F. Doyle, *AFRRI*
Collaborator: A. N. Martins, *Walter Reed Army Medical Center*

Normally, the cerebral blood vessels constrict or dilate in response to systemic blood pressure changes so as to maintain a nearly constant cerebral blood flow (the autoregulatory phenomenon). Following combined injury—particularly blast injury—there may

be a loss of cerebral autoregulation, and the cerebral vessels will dilate. Under these conditions, excessively high intracranial pressures occur which will further damage the brain. Therefore, a better understanding of this problem is required. Since damaged cells release high concentrations of potassium ions (K^+), and potassium ions influence tissue blood flow, the possibility exists that increased concentrations of potassium ions in the damaged brain may contribute to loss of autoregulation following combined injury.

Cynamologus monkeys were anesthetized with ketamine and maintained on a ketamine/pancuronium infusion supplemented by continuous N20 inhalation. Craniectomy sites were placed symmetrically over the left and right parietal-occipital lobes, and the regional cerebral blood flow (rCBF) was measured by the hydrogen polarographic technique. Mock CSF, containing varying concentrations of K^+ and balanced for osmolarity and pH, was applied to one hemisphere near the electrodes. The other hemisphere received only normal mock CSF.

A graded increase in rCBF was noted with increasing strengths of [K^+] (10, 20, 30, 40, 60, and 120 meq/l) (see Figure 1). The test electrodes registered a 100% or greater

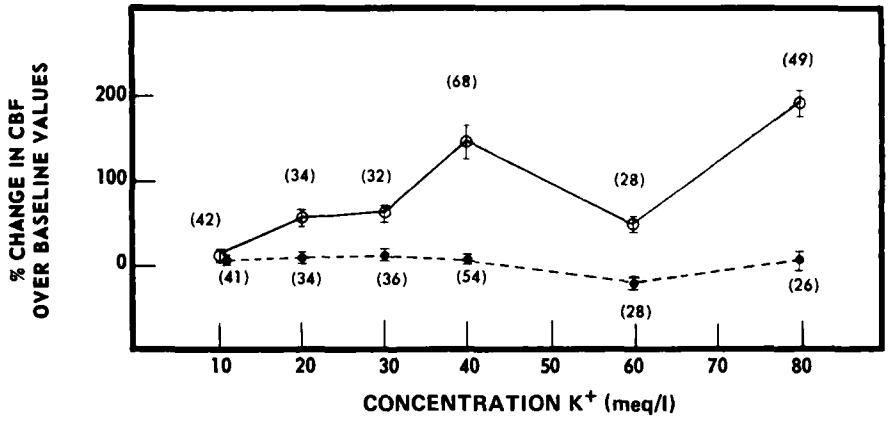

Figure 1. The effect of potassium ions (K^+) on cerebral blood flow (CBF) in the monkey brain. The solid line represents the percent change of CBF of the test hemisphere as determined by the hydrogen polarographic technique in response to increased concentrations of potassium contained in the physiological bathing solution (mock cerebrospinal fluid). The dotted line represents the percent change of CBF in the control hemisphere exposed simultaneously only to mock cerebrospinal fluid containing normal concentrations of potassium.

rise in rCBF within 30 min over baseline values when $[K^+] \geqslant 40$ meq/l (seven animals). A noticeable but less marked rCBF elevation (48%) also appeared simultaneously from the control electrodes (six animals). The pronounced flow increases induced by the higher $[K^+]$ rapidly began to subside within 15 min, when normal mock CSF replaced the test solution (five animals).

Thus, extracellular K^+ application significantly increases cerebral blood flow in a reversible manner. This mechanism might help explain flow increases found in brain damaged by combined injury.

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EFFECT OF TOPICALLY APPLIED SEROTONIN ON LOCAL CEREBRAL BLOOD FLOW

Principal Investigators: A. N. Martins, T. F. Doyle, and S. J. Wright, Jr.

It has been hypothesized that acute lesions of the brain enlarge through an auto-destructive process. Serotonin (5HT), a potent cerebral vasoconstrictor, is believed by some to mediate the process by reducing cerebral blood flow (CBF) in tissue surrounding the lesion. The hypothesis was tested in cynamologus monkeys anesthetized with ketamine and nitrous oxide. Craniectomies, 7 mm in diameter, were performed in each parietal area. The dura was opened, and polarographical electrodes of thin platinum wire were inserted into the parietal lobe cortex of each hemisphere. Mock CSF was irrigated continuously onto the brain surrounding the electrodes, from which local CBF was determined repeatedly by the hydrogen-clearance technique. After baseline CBF was established, solutions of 5HT in mock CSF (in concentrations of 5×10^{-7} M, 5×10^{-5} M, and 5×10^{-3} M) were irrigated onto one hemisphere while the opposite hemisphere served as control. 5HT failed to change CBF (Figure 1). Although 5HT is a potent vasoconstrictor, under physiologic conditions it apparently is unable to affect hemodynamically significant constriction of the peripheral cerebral vasculature of the anesthetized monkey brain.

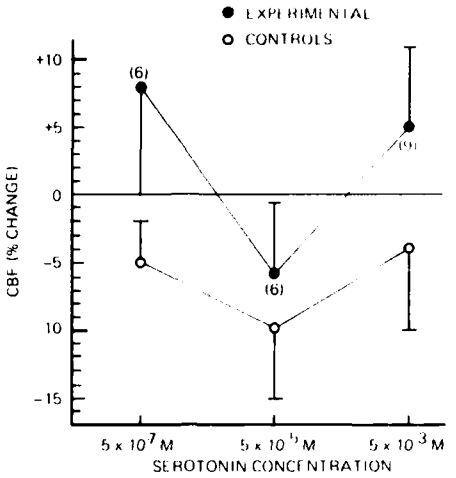

Figure 1. Effect of topically applied 5HT on local CBF as percent change from initial baseline (mean ± S.E.). Closed circles = 5HT; open circles = controls. Number of experiments in parentheses. CBF did not change significantly ($p > 0.3$, paired t test).

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BLOOD FLOW AND OXYGEN CONSUMPTION OF THE MONKEY BRAIN AFTER FOCAL CRYOGENIC TRAUMA

Principal Investigators: A. N. Martins and T. F. Doyle

A focal cryogenic lesion was made in the left superior frontal gyrus of the anesthetized Macaque brain. Cerebral blood flow (CBF) was determined by the hydrogen-clearance technique before and during the 4 hours following trauma (Figure 1). Local CBF in tissue adjacent to the lesion increased in the first half hour after the lesion was made and then decreased during the ensuing 3½ hours. Local CBF in the contralateral superior frontal gyrus as well as total CBF and oxygen consumption was unchanged by cryogenic trauma. The spread of vasogenic edema into uninjured tissue probably accounts for the observed decrease in local CBF. This experimental model may assist in discovering therapy to alter favorably the spatial and temporal profile of pathologic CBF changes in tissue surrounding an acute lesion of the brain.

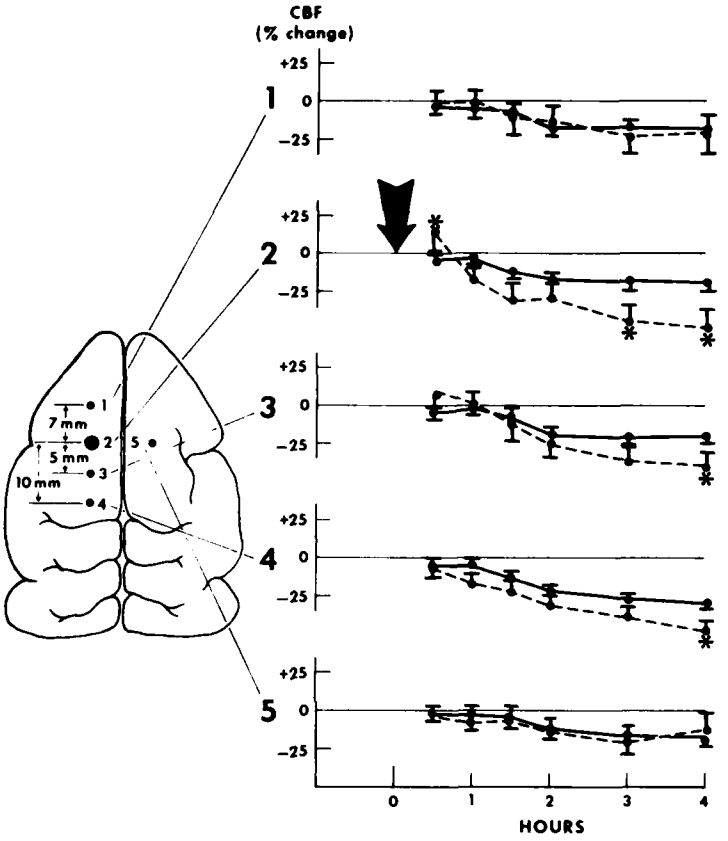

Figure 1. Graph summarizing effect of trauma on local CBF as percent change from initial baseline value:

– – – – – = Traumatized (n = 14)
——— = Controls (n = 17)
Mean ± S.E.
Trauma at arrow
* = Statistically significant difference; $p < 0.05$ (unpaired t test)

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NUCLEAR SCIENCES DEPARTMENT

The Nuclear Sciences Department is divided into two main divisions. The Nuclear Biology Division is devoted primarily to developing new uses of medical nuclear techniques in biological research, with animal models, in order to apply those techniques to military medical problems. This breaks down into four major study groups as follows: (a) *Cardiac pulmonary studies,* with three areas of interest: (i) Induction of experimental pulmonary emboli using a model perfected by Dr. P. Alderson of this Department, in which india ink is used to confirm the presence of emboli demonstrated by perfusion studies. (ii) Comparison of effects of neutrons to effects of gamma rays on pulmonary function in dogs, in collaboration with George Washington University. (iii) Studies in cardiac shunt evaluations where surgically created left-to-right shunts are quantitatively compared using radionuclides. (b) *Cellular labeling studies,* in which red cells are labeled in an attempt to image an organ. More specifically, we are trying to look at the spleen with nuclear medicine techniques. (c) *Bone studies,* approached in two ways: (i) bone-repair imaging by evaluating the healing of facial bone graft with radionuclides, which have been found to seek areas of bone growth, and (ii) irradiation effects studies aimed at looking for changes in distribution and uptake of bone-imaging radionuclides. (d) Two *tumor studies,* terminated during the year.

There are four new efforts anticipated in FY 1978 by this Division. One relates to tissue localization with labeled antibodies in normal tissue. Here the first attempt will look at the immediate effects of toxic chemicals and radiation on heart tissue using nuclear medicine techniques and cardiac gated imaging with computer analysis. This will involve the use of the PDP 11/40 Computer and radioactively labeled antibodies aimed at specific organ tissues. A second study will use thallium-201 to evaluate effects of various pharmacological interventions on heart uptake and body distribution. Thallium-201 may also be used in studies on heart function efficiency. Finally, the toxicity of oxygen in the lungs will be studied, using the nuclear medicine techniques to test total lung function in dogs in a collaborative study with the Naval Medical Research Institute.

If possible, we hope to make preliminary studies of bone necrosis that occurs as a result of exposure to more than 1 atmosphere pressure. Furthermore, the groundwork is being laid for obtaining a positron-emission study capability. Eventually this will be a major effort of the Nuclear Biology Division.

The Radiation Physics Division, by its present nature, has been primarily devoted to support functions. It has provided dosimetry of radiation for other investigators who use the cobalt facility, the nuclear reactor, and the linear accelerator. Research in radiation dosimetry per se has been limited, but one study was begun to evaluate the possibility of using the "Mossbauer" effect as a sensitive test for damage to red cells or red cell precursors due to radiation. As yet there is no conclusive evidence to either confirm or refute the usefulness of this approach *in vivo.* The Radiation Physics Division has had opportunities to advise and consult on computerized modeling of the human body with respect to neutron and gamma dose under battlefield conditions. We

were able to offer some comments about the U.S. Army's plans to develop a personnel field dosimeter. We also advised representatives of the Federal Republic of Germany. Future work for the Radiological Physics Division may be aimed at correlating *in vivo* biological dose indicators with physical dose indicators at various points in target subjects. Evaluation of any proposed field dosimetry systems could be a valuable future contribution. Work with neutron dosimetry may receive more emphasis along with the combined injury and collatoral-damage studies related to neutron-enhanced weapons.

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EXTERNAL AND INTERNAL RADIATION DOSE DETERMINATIONS

Principal Investigators: E. E. Kearsley, L. F. Winans, and E. G. Daxon

Calibration factors were updated for all radiation sources at AFRRI. Dosimetry intercomparison results were received for the cobalt and the LINAC irradiation facilities. The cobalt results were within 1% of the exposure as determined by the Regional Calibration Center at the M.D. Anderson Hospital in Houston, TX. The regular biannual high-energy electron beam intercomparison with National Bureau of Standards (NBS) completed in July 1977 indicated an agreement within 5%. Changes in the hemoglobin molecule due to cobalt-60 irradiation were demonstrated by changes in the *in vitro* iron-57 Mossbauer spectrum. The sulphur and gold foil counting system will be set up to enable the Division to do its own counting. A calibration will be made using the NBS californium-252 source. Depth dose measurement using tissue-equivalent cylindrical phantoms will be made in the reactor using 0.5 cm^3 ionization chambers with varying neutron gamma ratios. Depending on staffing, work on the Mossbauer project will continue.

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PULMONARY EMBOLISM DIAGNOSTIC MODEL

Principal Investigators: F. Vieras, K. G. Mendenhall, and P. O. Alderson

Personnel subjected to various forms of physical trauma or surgical procedures often develop pulmonary embolism, resulting in death. The objective of this work is to develop improved means of diagnosing pulmonary embolism by use of radioactive nuclides.

Pulmonary embolism will be caused in experimental animals using a modified Wessler technique. Evaluation will be accomplished using xenon-133 ventilation studies, technetium-99m-MAH perfusion studies, selective pulmonary angiography, and postmortem dissection of lungs. Results will be compared and analyzed for determination of the optimal method of detecting pulmonary embolism.

Ventilation-perfusion studies were performed in 23 dogs before and after experimental production of pulmonary embolism. Fourteen dogs had selective pulmonary angiograms. Perfusion lung scans with india ink revealed 93% of the defects larger than 2 x 2 cm and 10% of the smaller ones. Xenon-133 abnormalities were unusual (8.7%) and

occurred in areas containing large thrombi. Oblique views improved the sensitivity of the perfusion scan.

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VENTILATION-PERFUSION LUNG IMAGING AND SELECTIVE PULMONARY ANGIOGRAPHY IN ANIMALS WITH EXPERIMENTAL PULMONARY EMBOLISM

Principal Investigators: P. O. Alderson, S. S. Diamond, and K. G. Mendenhall, *AFFRI*
J. L. Doppman, *National Institutes of Health*

To determine the accuracy and limitations of xenon-133 ventilation and technetium-99m perfusion lung images in detecting pulmonary emboli, studies were performed in 23 dogs after experimental production of pulmonary embolism. Each dog had a baseline ventilation-perfusion study. Several days later venous thromboemboli were formed, using a modified Wessler technique, and released to the lungs. Each dog then had a repeat ventilation-perfusion study, and 14 also had bilateral selective pulmonary angiography. Each dog had both a 20-ml intravenous injection of india ink prior to sacrifice (to outline perfused segments of the lung) and a postmortem lung dissection. Two of 23 animals (8.7%) with normal baseline xenon-133 studies had xenon-133 abnormalities when studied immediately after embolization. These abnormalities were in regions where emboli completely obstructed large vessels (>6 mm diameter). Perfusion images revealed the location of 84% of emboli that completely obstructed pulmonary vessels but only 26% of those that partially obstructed flow. Perfusion defects were seen with 97% of emboli that completely obstructed vessels greater than 2 mm in diameter but only 65% of those that lodged in smaller vessels. Small (<2 x 2 cm) perfusion defects revealed by india ink were detected much less frequently (16%) than larger ones (93%). Ventilation-perfusion imaging is an accurate technique for detecting perfusion defects due to pulmonary embolism but has limited ability to detect very small or incompletely obstructing emboli. Pulmonary embolism-induced xenon-133 abnormalities should not significantly interfere with interpretation because they occur infrequently.

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PULMONARY IRRADIATION EFFECTS

Principal Investigators: F. Vieras and K. G. Mendenhall, *AFRRI*
E. W. Bradley, *George Washington University Medical Center*

The purpose of this research is to assess the effect of irradiation on regional pulmonary function and to compare the relative biologic effectiveness of cobalt-60 irradiation and neutron irradiation with respect to pulmonary function. Research results would be used in predicting survival of casualties after exposure to neutrons in nuclear warfare.

Changes in regional pulmonary function are assessed after right hemothorax irradiation by following the sequential changes in relative right lung perfusion, ventilation, lung volume, and radioaerosol deposition (xenon-133).

A total of 31 dogs have survived after right hemithorax irradiation. All dogs have undergone radionuclide pulmonary studies every 3 months for 12 months as well as chest radiographs at AFRRI and standard pulmonary function studies at Naval Medical Research Institute. Dogs in the high-dose photon and neutron groups and the medium-dose neutron group showed spirometric evidence of decreased lung volume, and they had decreased compliance. These dogs showed marked reductions of perfusion and aerosol deposition in the irradiated lung. Overall clearance rates for xenon-133 were increased. Relative biologic effectiveness determinations revealed that neutrons are 4 to 5 times more damaging to normal lung than are gamma rays.

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EFFECTS OF FRACTIONATED FAST NEUTRONS AND PHOTONS ON CANINE LUNG: REGIONAL PULMONARY FUNCTION STUDIES

Principal Investigators: E. W. Bradley, C. C. Rogers, and M. P. Fisher,
George Washington University Medical Center
P. O. Alderson, *AFRRI*

Thirty-nine adult, male, purebred beagles received either fast neutron or photon irradiation to the right thorax. Twenty-four dogs received fast neutrons with a mean energy of 15 MeV to total neutron doses of 1000, 1500, 2250, and 3375 rads delivered in four fractions/week for 6 weeks. Fifteen dogs received 3000, 4500, or 6750 total rads of photons (cobalt-60) in the same fractionation pattern. Radionuclide investigations of pulmonary function were performed 3 and 6 months postirradiation. These included

(a) radioaerosol deposition of an insoluble radiocolloid, technetium-99m-phytate, as well as (b) xenon-133 ventilation studies and (c) technetium-99m-macroaggregated albumin perfusion images. The data are plotted as the change in right lung value from baseline as a function of total dose for 3 and 6 months postirradiation. The 3-month data at total photon doses of 4000 rads show that the relative biologic effectiveness for the relative deposition of aerosol is 13.93 ± 3.6 (mean ± S. D.) and 6.64 ± 1.33 at 6000 rads. At 4000 rads of photons, the relative biologic effectiveness for the relative distribution of perfusion is 6.21 ± 1.41 and 4.32 ± 0.25 at 6000 rads. The relative biologic effectiveness for the relative distribution of volume is 6.25 ± 2.03 at 4000 rads of photons and 4.39 ± 0.95 at 6000 rads.

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PULMONARY VENTILATION, PERFUSION, AND RADIOAEROSOL DEPOSITION IN DOGS FOLLOWING HEMITHORAX IRRADIATION WITH COBALT-60 OR FAST NEUTRONS

Principal Investigators: P. O. Alderson, K. G. Mendenhall, and B. A. Siegel, *AFRRI*
E. W. Bradley, *George Washington University*
M. E. Bradley, *Naval Medical Research Institute*

To assess the effects of therapeutic doses of radiation on regional pulmonary function, 31 dogs had serial chest radiographs and quantitative xenon-133 ventilation, technetium-99m perfusion, and technetium-99m phytate aerosol studies following hemithorax irradiation with cobalt-60 photons or fast neutrons. Each dog also had serial spirometry and compliance measurements. All doses of radiation were delivered in a 24-treatment course at four doses per week for 6 weeks. Integral photon doses of 3000, 4500, or 6750 rads were delivered; neutron doses were 1000, 1500, or 2250 rads. All studies were repeated at 3, 6, 9, and 12 months after irradiation and compared to baseline studies obtained before irradiation. Dogs in the high-dose photon and neutron groups and the medium-dose neutron group showed spirometric evidence of decreased lung volume, and they had decreased compliance. These dogs showed marked reductions of perfusion and aerosol deposition in the irradiated lung. The single-breath xenon-133 distribution and radioaerosol deposition pattern agreed more closely with the perfusion pattern than did xenon-133 equilibrium or clearance studies. Although several dogs had focal areas of mild xenon-133 retention, overall clearance rates of xenon-133 increased. The results provide information about alterations of regional lung function in (primarily) restrictive pulmonary disease, and demonstrate that neutrons are four or five times as damaging to normal lung as photons.

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EFFECT OF IRRADIATION ON TISSUE UPTAKE OF RADIOPHARMACEUTICALS

Principal Investigators: F. Vieras and K. G. Mendenhall

This work attempts to define the time-course of radiation effects on skeletal and muscle uptake of bone-imaging radiopharmaceuticals and to evaluate the role of local bone and muscle blood flow in tracer uptake.

In this study the right lower extremity of a rat was irradiated with X or gamma rays. At varying times afterward, comparison was made of (a) the uptake of technetium-99m *disphosphonate and* (b) *the distribution of radiolabeled microspheres* in irradiated and nonirradiated tibias and in soft tissue of irradiated and "control" extremities.

Part of this project involved irradiation with X rays and was completed last year. In a new phase of the project, groups of rats have been exposed to 500, 1000, 1500, 2500, 3000, and 5000 rads of gamma ray (cobalt-60). For each exposure level, a group of rats was sacrificed at 1 and 3 days postirradiation. These data are being analyzed.

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TECHNETIUM-99m PHYTATE AS A BONE MARROW-IMAGING AGENT: BIODISTRIBUTION STUDIES IN ANIMALS

Principal Investigators: R. G. Hamilton and P. O. Alderson, *AFRRI*
P. A. McIntyre, *Johns Hopkins Medical Institutions*

Technetium-99m phytate has been suggested as a bone marrow-imaging agent. This study compared the biodistribution of technetium-99m-labeled "bone marrow" phytate, sulfur colloid, and diphosphonate in young rats and rabbits. Autoclaved bone marrow phytate revealed significant long-base deposition, but 96% of this activity was associated with compact bone and only 4% with bone marrow. This distribution is similar to that of disphosphonate but is significantly different from that of sulfur colloid. Technetium-99m-phytate is not recommended as a bone marrow-imaging agent.

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IN VIVO LABELING OF RED CELLS WITH TECHNETIUM-99m PERTECHNETATE

Principal Investigators: R. G. Hamilton and P. O. Alderson

In vivo labeling of red blood cells with technetium-99m in rats was examined to define the best stannous ion agent and stannous ion concentration for labeling, the optimum time interval between Sn(II) and $^{99m}TcO_4^-$ injections, and the effect of carrier technetium on labeling efficiency. Red cells labeled *in vivo* under optimum conditions were then compared to red cells labeled *in vitro* to determine relative labeling efficiency, *in vivo* stability, and blood pool image quality. Maximal *in vivo* labeling efficiency was obtained using a 10-μg/kg intravenous dose of Sn(II) ion followed 5-30 min later by an injection of $^{99m}TcO_4^-$. Neither the chelated form of stannous ion nor the amount of carrier technetium present at the time of labeling had a significant effect on labeling efficiency. There was no significant difference in the red cell-labeling efficiency obtained with *in vivo* or *in vitro* methods. Red cells prepared by these two methods had similar biologic half-lives and provided high-quality blood pool images.

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EVALUATION OF DAMAGED AND DISEASED TISSUE WITH RADIOPHARMACEUTICALS

Principal Investigators: F. Vieras and W. P. Bradley, *AFRRI*
W. C. Eckelman, *George Washington University*

The objective in this study is to develop clinically useful imaging techniques for defining and delineating tissues of the body that are damaged or diseased.

Various animal models, including animals with transplanted tumors, will be given newly developed or already available radiopharmaceuticals. They will be studied with gamma camera imaging for their localizing characteristics, blood clearance, urinary excretion, and ability to depic. abnormal tissue.

A series of experiments has been performed to establish the mechanism by which irradiation causes increased urinary clearance and decreased tumor localization of gallium-67 citrate. It has been established that saturation of the iron-binding capacity after irradiation results in a decrease of gallium binding to serum transferrin. Animals treated with acetyl-phenyldydrozine (which causes a hemolytic anemia with release of iron from red blood cells and saturation of transferrin) also show increased urinary excretion and decreased tumor uptake. A number of β-adrenergic derivatives have been labeled with radionuclides, and their localization in rat myocardium has been assessed. Iodine-labeled derivatives carrying tyramine as the amino moiety showed most promise as cardiac imaging agents.

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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, *AFRRI*
G. R. Mack, *National Naval Medical Center*

The right/left ratios of tibial uptake of ^{99m}Tc-diphosphonate (EHDP) and relative blood flow (based on microsphere distribution) were determined in control rats and in rats with a ligated right femoral artery or a healing right tibial fracture. Correlation between ^{99m}Tc-EHDP uptake and relative blood flow was highly significant ($r = 0.917$, $p < 0.0001$) for relative flow ratios less than 1.7. When the ratio was greater than 1.7, there was little further increase in ^{99m}Tc-EHDP uptake. Ligation of the femoral artery in rats with healing fractures resulted in a more marked reduction of blood flow than of ^{99m}Tc-EHDP uptake. These results suggest that regional bone blood flow is a major determinant of ^{99m}Tc-EHDP uptake, although changes in regional tracer extraction efficiency are also important.

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BETA-ADRENERGIC-BLOCKING AGENTS 1-(4-HYDROXY)-PHENETHYLAMINO-3-ARYLOXY-PROPAN-2-OLS FOR MYOCARDIAL IMAGING

Principal Investigators: W. L. Rzeszotarski, W. C. Eckelman, R. E. Gibson,
W. V. Jiang and R. C. Reba, *George Washington University*
F. Vieras and P. O. Alderson, *AFRRI*

Four new beta-adrenergic-blocking agents carrying tyramine as the amino moiety were synthesized, and the distribution of their iodine-125-radioiodinated derivatives were studied. A derivative of a nonspecific blocker, alprenolol, showed poor blood clearance and no cardiac selectivity. A derivative of a cardiospecific blocker, practolol, showed a promising heart/blood ratio (18.7:1) and cardioselectivity in heart/lung ratio (2:1). Two additional practolol analogs did not show any improvement over the practolol derivative. Because of their increased lipophilicity, the blood clearance and cardioselectivity were impaired. It is assumed that the partition coefficient plays a very important role in blood clearance and cardioselectivity of these beta-adrenoceptor-blocking agents.

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RADIOISOTOPIC DIAGNOSIS OF ABDOMINAL TRAUMA

Principal Investigators: F. Vieras and M. P. Grissom

Personnel subjected to physical trauma often sustain abdominal trauma and vascular damage that is difficult to detect. The purpose of this study is to develop improved noninvasive methods by use of radionuclides to image major abdominal organs and blood vessels. This would provide rapid and accurate diagnosis for trauma patients.

In the study, red blood cells will be damaged *in vitro* by several different means and then labeled with technetium-99m. A preparation will be injected into experimental animals. By use of gamma camera imaging, the distribution, accumulation, and localization of the damaged red blood cells will be determined. The optional method for labeling the red blood cells will be determined by comparing the imaging quality, localization of the material, and ease of use.

Evaluation of various methods in *in vitro* red blood cell labeling was completed. Damaging by N-ethylmaleimide (NEM) was found to be an excellent labeling method. Biodistribution studies of technetium-99m-phytate were performed in small animals to

evaluate its usefulness in nuclear hematology. In contrast to observations by other researchers, this agent was found to be a poor bone marrow label. Further work was done to develop a technique for *in vivo* labeling of red blood cells. Various techniques based on the intravenous injection of the reducing agent Sn(II) followed by pertechnetate were evaluated. Maximal labeling was obtained with 10 μg Sn(II)/kg followed by pertechnetate from 5 to 30 min later.

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SPLENIC IMAGING WITH TECHNETIUM-99m-LABELED ERYTHROCYTES: A COMPARATIVE STUDY OF CELL-DAMAGING METHODS

Principal Investigators: R. G. Hamilton, P. O. Alderson, and B. A. Siegel, *AFRRI*
J. F. Harwig, *Washington University School of Medicine*

Several methods of damaging red blood cells for splenic imaging were compared to determine the optimum approach. The 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 distributions of undamaged and damaged red blood cells were determined in rats, and splenic imaging studies were performed in rabbits. Splenic deposition and spleen-to-liver ratios with labeled red blood cells damaged by heat or sulfhydryl were significantly greater ($p < 0.001$) than the values obtained using acid-citrate-dextrose or Sn(II) ion. Damaging by heat produces good splenic localization of red blood cells labeled with technetium-99m, but requires rigidly controlled incubation conditions. Damaging by N-ethylmaleimide provides an excellent and predictable alternative approach.

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COMPARATIVE EVALUATION OF TECHNIQUES FOR RAPID AND EFFICIENT *IN VIVO* LABELING OF RED CELLS WITH [^{99m}Tc] PERTECHNETATE

Principal Investigators: R. G. Hamilton and P. O. Alderson

Red blood cells labeled *in vivo* with $^{99m}TcO_4^-$ have recently been recommended for blood pool imaging, but the optimum conditions for *in vivo* labeling of red blood cells have not been clearly defined. We therefore evaluated several stannous-ion preparations and stannous-ion concentrations to determine which provided the best labeling. The effect of the time interval between the Sn(II) and $^{99m}TcO_4^-$ injections and the effect of carrier technetium on labeling efficiency were also studied. Maximal *in vivo* labeling efficiency was obtained using an intravenous dose of 10 μg Sn(II)/kg followed 5-30 min later by an injection of $^{99m}TcO_4^-$. Neither the chelated form of stannous ion used in these studies nor the amount of carrier present had a significant effect on labeling efficiency. The biologic half-time of red blood cells labeled with technetium-99m *in vivo* was similar to that of red blood cells labeled *in vitro*. *In vivo* labeling is a rapid and efficient method for the preparation of technetium-99m red blood cells.

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QUANTITATION OF LEFT-TO-RIGHT CARDIAC SHUNTS

Principal Investigators: F. Vieras and K. G. Mendenhall

This research investigates the reproducibility and accuracy of radionuclide methods for quantitative left-to-right shunts.

The reproducibility of radionuclide quantitation of left-to-right shunts is evaluated in dogs with surgically created lateral septal defects. Results are compared to standard oximetry determinations of shunt size and direct shunt size determinations with electromagnetic flow probes.

The project, as initially outlined, was completed. Quantitative radioangiocardiography was compared to oximetry and electromagnetic flow probes. Quantitative radioangiocardiography was found to be an accurate method for determination of left-to-right shunt size and significantly more reproducible than oximetry in some atrial septal defects. Quantitative radioangiocardiography correlated closely ($r = 0.90$) to electromagnetic flow probes in the presence of experimental ventricular outflow abnormalities.

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QUANTITATIVE RADIOANGIOCARDIOGRAPHY IN ANIMALS WITH EXPERIMENTAL ATRIAL SEPTAL DEFECTS

Principal Investigators: P. O. Alderson, K. G. Mendenhall, and R. L. Donovan, *AFRRI*
V. A. Guadiani and D. C. Watson, *National Heart, Lung, and Blood Institute, NIH*

Atrial septal defects were surgically created in 11 healthy dogs. After a 4-week period of postoperative convalescence, each dog had repeated determinations (n = 10) of left-to-right shunt size using the gamma function method of quantitative radioangiocardiography (QRAC). Six dogs also had repeated determinations (n = 3) of shunt size by standard oximetry. Seven animals then underwent median sternotomy, and shunt size was determined simultaneously by QRAC and electromagnetic flow probes. During this portion of the study, the hemodynamic significance of the left-to-right shunt was altered by surgically created right or left ventricular outflow obstructions. The coefficient of variation for 110 left-to-right shunt measurements by QRAC was 11.2%. QRAC determinations of shunt size correlated closely ($r = 0.97$, $p < 0.0001$) with those of electromagnetic flow probes, but correlated poorly with oximetry ($r = 0.16$, $p > 0.10$). QRAC accurately reflected the hemodynamic significance of left-to-right shunts even in the presence of ventricular outflow abnormalities. Quantitative radioangiocardiography is an accurate and reproducible method for determining the hemodynamic significance of left-to-right shunts.

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EVALUATION OF HEALING OF TRAUMATIC BONE FRACTURES AND BONE GRAFTS

Principal Investigators: R. G. Triplett, *Naval Medical Research Institute*
F. Vieras and K. G. Mendenhall, *AFRRI*

This research is evaluating bone graft healing with radionuclide techniques and determining the effect of anemia in graft healing.

Beagle dogs with xenogenic grafts have been studied with both the previously developed radionuclide technique and radiographs. Each dog will be studied serially through 3 months postgraft. The radionuclide technique will also be applied to animals made anemic by depleting their red cell mass by approximately 40%.

Four dogs with grafts expected to fail have been serially studied with radionuclides and X rays. When data acquisition is completed, an evaluation will be made of the usefulness of the radionuclide technique in predicting graft success or failure in the early postgraft period.

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