

# SAMA-ΑΩΑ Student Honors Day: Abstracts of Scientific Presentations

Medical College of Virginia, May 17, 1968

## Histochemical and Fine Structural Studies of Lymphocyte Transformation with Phytohemagglutinin and Pokeweed Mitogen\*

PETER F. HOFFMAN (M-11A)

During a study of the effects of pokeweed mitogen extract (PWM) on cultured human peripheral lymphocytes, several distinct morphological differences were noted when these cells were compared with cultures of lymphocytes treated with phytohemagglutinin (PHA). The number of blast cells which appeared in both PHA- and PWM-treated cultures was maximal at 72 hours, not only as seen by light and phase contrast microscopy, but also as determined by the incorporation of tritiated thymidine into DNA, which reached its peak at this time. Giemsa- and acetic orcein-stained preparations of the 72-hour cultures showed that the transformation induced by PWM differs from that induced by PHA, in that cell nuclei are more eccentric, nucleoli are fewer, and heterochromatin is more prominent in the former. PWM-transformed cells showed maximum PAS staining after 72 hours, while the PAS staining of the PHA cells was diminished by this time. Both PHA- and PWM-treated cells showed similar ability to bind rabbit anti-human gamma globulin conjugated to fluorescein isothiocyanate, which cross-reacts with IgG, IgA, and IgM. Histochemical and electron microscopic studies revealed a significant increase in the number of lysosome-like bodies in PHA- and PWM-stimulated cells when compared to control cultures at 72 hours. A comparative fine structural study of the cells present in culture after 72 hours revealed differences in the populations of cells in PHA- as compared to PWM-treated cultures. In PHA cultures, there was a predominance of large blast-like cells, with smaller numbers of normal-appearing small lymphocytes, as described previously. In PWM cultures, three cell types were distinguishable: normal appearing lymphocytes of varying size, large blast-like cells, and a distinct type of intermediate-sized cell. The latter type of cell contained significantly more rough-surfaced endoplasmic reticulum than PHA-transformed cells or 72-hour control cultures. In addition, the cells of intermediate size contained numerous ribosomes in various stages of aggregation. These fine structural features were comparable to those of cells which have been described by other investigators as early antibody-producing cells.

Preceptors: STEVEN D. DOUGLAS, JAN BORJESON, LAWRENCE N. CHESSIN, *National Institute of Arthritis and Metabolic Diseases, National Institutes of Health*

\* Originally published in *Anatomical Record* 154: 340, 1966.

## Plasma Pressor Activity in Normal and Stressed Newborns

KENTON R. HOLDEN (M-111B)

Plasma pressor activity (PPA) was measured in umbilical plasma of 29 full-term infants using a modified *in vitro* bioassay which reflected, primarily, plasma levels of epinephrine and norepinephrine. Of this group, seven infants with asphyxia neonatorum demonstrated significant hypercapnia ( $p_aCO_2 = 78.3 \pm 5.2$  S.E.M.), acidemia ( $pH_a = 7.06 \pm 0.05$ ), elevation of PPA ( $42.7 \pm 19.9$   $\mu\text{g/ml}$ ), and lowered Apgar scores (one minute =  $4.4 \pm 0.7$ ; five minutes =  $7.6 \pm 0.7$ ) as compared to 14 normal newborns ( $p_aCO_2 = 49.8 \pm 2.2$ ,  $pH_a = 7.27 \pm 0.01$ , PPA =  $1.6 \pm 0.4$   $\mu\text{g/ml}$ ), Apgar scores (one minute =  $8.6 \pm 0.1$ ; five minutes =  $9.9 \pm 0.1$ ). Eight newborns of mothers with mild pre-eclampsia showed a trend similar to the newborns with asphyxia neonatorum. Pooled data on all infants revealed a highly significant correlation between umbilical artery PPA and  $p_aCO_2$  and  $pH_a$ , respectively, as well as Apgar scores at one and five minutes. However, no correlation of umbilical artery PPA could be demonstrated with  $p_aO_2$ , free fatty acids, blood glucose or maternal PPA at time of delivery. All infants and their mothers were discharged in normal condition except for one infant with asphyxia neonatorum who had persistent tracheomalacia on discharge.

Preceptor: REUBEN B. YOUNG, *Department of Pediatrics, Medical College of Virginia*

## Adrenal Cortical Responsiveness in Patients with Renal Homotransplants Receiving Prednisone

JOSEPH D. LINEHAN (M-11)

It is known that large doses of prednisone, taken daily, produce adrenal atrophy and inhibit the adrenal response to ACTH or insulin-induced hypoglycemia. All the patients in the MCV transplant series received daily doses of prednisone from the time of transplantation. Because these patients were subjected to normal stresses as well as stresses peculiar to transplantation, such as multiple surgical procedures, infection, and rejection, the adrenal responsiveness was tested in these patients on various dosage

schedules and at relatively stable periods up to five years following transplantation. Plasma levels of 17-hydroxycorticosteroids were determined by the Nelson-Samuels method before and after a four-hour infusion of 25 units of ACTH. The following results were obtained in 38 patients: The responsiveness of the adrenal was related to the dose of prednisone given, to the extent that seven out of nine patients on 5 mg/day responded normally, while only seven out of 29 patients on 7.5 mg/day or more responded normally. There was no correlation between the presence or the absence of a response and the resting levels of 17-hydroxycorticosteroids, nor was there any correlation with the presence or the absence of obvious changes as described by Cushing. There was no statistically significant correlation between the response and the length of time the patients had been on corticosteroids, as nine out of 29 patients on prednisone for over two years responded normally. These rather surprising results indicate that the majority of patients receiving doses as low as 7.5 to 10 mg per day showed suppression of their adrenal response. As long-term patients in our series were in excess of 5 mg/day, they should be considered adrenal deficient in stress states. It was also concluded that, while small dosages do suppress the adrenal gland, prolonged prednisone treatment at suppressing dosages may not produce permanent adrenal insufficiency.

Preceptor: HYUNG M. LEE, *Department of Surgery, Medical College of Virginia*

#### Cortical Influences on Midbrain Evoked Activity in Cat

JOHN H. OSTRICH (M-III A)  
DAVID F. POLSTER (M-III A)

This study was designed to investigate cortical influence on evoked afferent activity at discrete thalamic and midbrain loci in cats. Animals anesthetized with nitrous oxide were placed in a stereotaxic instrument. Monopolar electrodes were positioned at two midbrain loci, one in the periaqueductal grey at the level of the superior colliculus, the other in the ventral tegmentum at a point characterized physiologically by Collins and O'Leary. A third electrode was placed in the nucleus ventralis posterolateralis of the thalamus. Evoked potentials in these areas, produced by contralateral peripheral nerve stimulation, were monitored by capacity-coupled amplifiers and a triple-beam oscilloscope. Cortical somatosensory areas I (SI) and II (SII), ipsilateral to the deep recording loci, were identified. Physiological saline ice slush was applied to SI, SII, or both areas simultaneously. Following either a demonstration of alteration in evoked responses at the deep loci or an elapsed time of five to ten minutes, the cortical areas were warmed with saline and covered with warm mineral oil. During the experiment, the animal was immobilized with Flaxedil and maintained by positive pressure ventilation through a tracheostomy. Rectal temperature was monitored and was kept above 36°C, using electric heating pads. Systolic blood pressure, recorded continuously by an indwelling arterial catheter, stayed above 120 mm Hg at all times. Animals were sacrificed with barbiturate overdose, and recording loci were marked by electrolytic deposition of iron at the electrode tips. Subsequent arterial perfusion with Potassium Ferrocyanide solution allowed microscopic eval-

uation of recording sites to be made by histological brain sections. Data were drawn from 24 experiments which met the criteria of reproducible baseline potentials and accurate electrode tip placement. Evoked activity in the periaqueductal grey was always depressed—never augmented—during cooling of the somatosensory cortex. The tegmental and thalamic responses were always stable. Cooling SII alone produced greater depressions than cooling SI alone. Simultaneous cooling of both areas caused the most marked depression of the periaqueductal response. Such results could be obtained as many as four times during a single experiment. These data suggest a physiological substrate for cortical control over discrete areas of the reticular formation. Anatomically separate cortical areas, although sharing a similar input, may exert varying degrees of control at specific sites even in the histologically diffuse reticular brain stem. One can conjecture that subtle cortical malfunctions, not only in the sites we have studied, but also in others, might lead to severe disturbances in the modulation and channeling of sub-cortical information, and that these disturbances, in turn, might be manifested by gross behavioral changes in the animal not satisfactorily explained by a consideration of cortical zonal physiology alone.

Preceptor: WILLIAM F. COLLINS, *Department of Surgery, Medical College of Virginia*

#### The Ultrastructure of the Vibratory Muscle of *Crotalus horridus*

LOUIS T. PASTORE (M-1)

A comparison of the vibratory and epaxial muscles of *Crotalus horridus* (timber rattlesnake) was made. Muscle fibers, fibrils, filaments, mitochondria and sarcoplasmic reticula were described in this study. The sarcomeres of the epaxial muscle were almost three times the length of those of the vibratory muscle. The A band was observed to be about twice the length of the I band in the vibratory muscle. As seen in cross section, the vibratory muscle had fibrils arranged in discrete packets with a limited number (less than 100) of thick myofilaments. In contrast, the fibrils of epaxial muscle were less conspicuously separated from each other and contained about six times as many myofilaments. The vibratory muscle was profusely provided with large spherical mitochondria having highly developed cristae. The epaxial muscle had relatively fewer mitochondria; these were ellipsoidal and smaller in size, with less-developed cristae. The sarcoplasmic reticulum of the vibratory muscle was highly developed with numerous tubules, which were continuous between fibrils. A reduced development of reticulum was found in the epaxial muscle. It was concluded that the vibratory muscle is highly specialized in structures related to energy production or dissemination of the contraction impulse to the interior of the fiber.

Preceptors: FRANCIS B. LEFTWICH, *Department of Biology, University of Richmond*; THOMAS M. HARRIS, *Department of Anatomy, Medical College of Virginia*

### Beta-Adrenergic Receptors in the Human Distal Esophagus\*

RICHARD F. PRINCE (M-II)

The body of the esophagus and the lower esophageal sphincter (LES) are thought to be under different neural control. The following study was performed to test the hypothesis that adrenergic receptor activity may be important in control of the motor function of the esophagus. In 11 normal volunteers, three open-tip esophageal catheters were placed 5 cm apart, locating the distal tip in the LES. Each catheter was perfused with microliter quantities of water, and pressures were recorded. Response to deglutition was evaluated by measuring duration and amplitude of contraction, velocity of the peristaltic wave and sphincter tone while BP and EKG were being monitored. Phenylephrine Hydrochloride (alpha stimulant) and Phentolamine (alpha blocker) were administered I.V. to four subjects; no alpha-adrenergic response was observed. Isoproterenol (beta stimulant) and H-56/28 (Astra; beta blocker) were administered I.V. to seven subjects. No effects were noted in the body of the esophagus; in six of seven subjects, beta blockade caused a marked increase in amplitude and duration of sphincteric contraction; beta stimulation caused decrease in sphincteric tone, an effect which was abolished by beta blockade. These findings indicate that beta-adrenergic inhibitory receptors are present in the LES, and blockade of these receptors results in exaggerated sphincteric contraction. Such a mechanism may be operative in the "hypertensive" or "hypercontracting" sphincter.

Preceptor: ALVIN M. ZFASS, *Department of Medicine, Medical College of Virginia*

\*Originally published in *Clinical Research* 16: 291, 1968.

### In Vitro and in Vivo Activity of Hamycin Against Blastomyces dermatitidis

GILES M. ROBERTSON, JR. (M-IIIB)

Variable responses to hamycin, a new polyene antifungal agent, have been observed in patients with North American blastomycosis. Possible mechanisms for such differences were studied with five strains of *Blastomyces dermatitidis*. *In vitro* studies revealed only a twofold difference in minima inhibitory concentration values (0.008 $\mu$ g to 0.016 $\mu$ g per ml) for hamycin. *In vivo* responses were measured in fatally infected mice treated, intraperitoneally, for 28 days with daily doses of 0.001 to 0.033 mg per mouse. Differences in response to treatment were significant among the five strains ( $p = < 0.001$ ), with median protective dose values ranging from 0.001 to 0.033 mg per mouse per day. Surviving mice were further observed, without treat-

ment, for 34 days, and marked relapse rates and renewed deaths were seen with several strains. At the termination of the studies, all remaining mice were killed and grossly examined; cultures of tissues revealed high levels of persistent infections with several strains. Deaths or histopathological changes due to the drug were not observed. These results suggest that variations in response to hamycin therapy in North American blastomycosis reflect differences in pathogenesis and host response *in vivo*, rather than differences in sensitivity of *B. dermatitidis* as measured *in vitro*.

Preceptor: SMITH SHADOMY, *Department of Medicine, Medical College of Virginia*

### Sinus Arrhythmia in the Canine Cardiac Transplant

MARC D. THAMES (M-II)

The recurrence of sinus arrhythmia was investigated in 13 mongrel dogs submitted to cardiac autotransplantation or homotransplantation. The animals were studied at various times postoperatively in an attempt to demonstrate vagal and sympathetic reinnervation and recurrence of sinus arrhythmia. Electrical stimulation of the vagus nerve and stellate ganglion was used to elicit proof of parasympathetic and sympathetic reinnervation, respectively. Further proof of reinnervation was the reflex response to acute, pharmacologically induced changes in blood pressure: bradycardia, following intravenous injection of phenylephrine, and the hypertension thereby produced provided proof of vagal reinnervation; tachycardia, following intravenous injection of nitroglycerine, and the hypotension thereby induced provided proof of sympathetic reinnervation. Simultaneous cardiachometric and pneumographic recordings were employed to detect sinus arrhythmia. Thirty-two studies were performed on 11 dogs without anesthesia, and ten studies were done on ten dogs using chloralose and urethane anesthesia. Most of the 13 dogs were shown to have both vagal and sympathetic reinnervation by four months postoperatively. No sinus arrhythmia was demonstrable during the period prior to cardiac reinnervation. Sinus arrhythmia was found to recur as early as 100 days postoperatively, ten of the 13 dogs demonstrating the arrhythmia by 455 days postoperatively. Sinus arrhythmia failed to recur in three animals, all of which had demonstrable reinnervation. It is thus concluded that, within a few months following canine cardiac transplantation, cardiac reinnervation is sufficiently developed to elicit sinus arrhythmia.

Preceptor: RICHARD R. LOWER, *Department of Thoracic Surgery, Medical College of Virginia*