

**XIX ANNUAL RESEARCH  
AND  
EDUCATION FORUM**



**"Integrating Research and Science Education  
in Community Health"**

**ABSTRACTS**



**A-1 Neural Tube Defects: Folate and Red Blood Cell Folate levels. (Preliminary results)** I García\*, L García, A de la Vega. Departments of Pediatrics and Obstetrics and Gynecology, UPR School of Medicine, Medical Sciences Campus.

**Background:** Neural tube defects (NTD) are considered a preventable cause of central nervous system malformation. Strong evidence supports that preconceptional and early prenatal folic acid supplementation can greatly reduce the incidence of NTD, suggesting a nutritional deficiency. Other investigators have indicated that not all causes on neural tube defect (in which a predisposing teratogen or syndrome is excluded) are folic acid preventable and that differences in folate metabolism can be involved in the pathogenesis of NTD. **Methods:** This is a case-control study in which folate and red blood cells (RBC) folate levels are being taken to a group of 30 pregnant women with an intrauterine diagnosis of NTD in their fetuses and 30 gestational age matched controls. This study was approved by the Institutional Review Board. In a Becton Dickinson Vacutainer System without additives, 5cc of maternal blood is obtained for serum folate and RBC folate levels. Cord blood is obtained for determination of serum folate and RBC folate levels. Folate levels and red blood cells folate levels are determined using the Ciba Corning Automated Chemiluminescence Systems. **Results:** We have enrolled 30 patients with a diagnosis of NTD in their fetuses. The mean gestational age is 29.5 weeks. The mean serum folate level of the mother is 16.5 ng/dl. The mean RBC folate level of the mother is 463 ng/dl. The mean serum folate of the newborn is 32.3 ng/dl and the mean serum RBC folate is 680 ng/dl. We have enrolled 13 controls. The mean serum folate level of the mother is 18.3 ng/dl and the mean RBC folate levels is 535 ng/dl. The mean folate levels for newborn is 33.5 ng/dl and the mean RBC folate levels is 851 ng/dl. **Conclusions:** With this preliminary results, mean folate levels are, as expected, higher in newborns than in mothers due to the anion effect the placenta. Red blood cells folate levels are higher in control mothers ( $p=0.06$ ) and control newborns ( $p=0.02$ ). No statistically significant difference was seen in folate levels between NTD cases and controls.

**A-2 Spinal Epidural Hematoma in a Newborn with Hemophilia A after a Lumbar Puncture.** J Rivera MD\*, M Valcarcel MD, P J Santiago MD, G Reyes MD, E Rivera MD. Department of Pediatrics, Neonatology and Hematology sections, University of Puerto Rico, Medical Sciences Campus

**Background:** Spontaneous and traumatic epidural hematomas have been reported in children with severe hemophilia, but not during the neonatal period. Intraspinous bleeding in patients with hemophilia is rare and represents 2% to 8% of central nervous system hemorrhages. Clinical manifestations include back pain, motor weakness or paralysis, bowel and bladder dysfunction, and sensory disturbance. Early diagnosis and treatment with factor VIII concentrate alone, or in combination with decompressive laminectomy, are essential in preventing neurologic sequelae. **Case report:** A 2 weeks old term male born to a mother with history of VDRL and FTA-ABS tests positive treated with Pen G. APGAR was 9/10 and birth weight 3.3 Kg. In the nursery syphilis work up was started including a lumbar puncture which was traumatic and was not obtained. He developed a large subcutaneous hematoma in the lumbosacral area, paleness, and shock. PT was normal, PTT was prolonged. He was treated with isotonic solutions, red blood cells, and fresh frozen plasma transfusions. A Spinal MRI at 3 days of age showed a large dorsal epidural hematoma involving thoracic (T12), and lumbosacral areas without cord compression, and a subcutaneous hematoma. When he was 4 days old he was transferred to our hospital and coagulation studies showed: normal PT, and prolonged PTT, Factor VIII 11% (after he had received 3 doses of Fresh Frozen Plasma). This was consistent with severe Hemophilia A and therapy with Factor VIII concentrate 50 units/Kg/dose q 12 hours was started. The patient's neurologic exam was unremarkable and no neurosurgical treatment was needed. After 4 days of Factor VIII concentrate therapy, through Factor VIII level was 17%. A Spine MRI was repeated after 6 days of therapy showing complete resolution of the epidural and subcutaneous hematoma. **Results:** Complete resolution of spinal epidural and subcutaneous hematoma after traumatic lumbar puncture was achieved in a newborn with severe Hemophilia A without neurologic sequelae. **Conclusions:** (a) a spinal epidural hematoma in a newborn with severe hemophilia may present with subtle neu-

rologic findings requiring careful neurologic monitoring; (b) complete recovery may occur after Factor VIII therapy without surgical management in patients without neurologic compromise; (c) traumatic lumbar puncture in Hemophilia is not common but awareness of its risks is necessary.

**A-3 A Case Study of a Young Autistic Latino Man with Asperger's Syndrome.** N Linares, B Lasanta, W Tapia  
Infantile Autism Project, Office for Research and Projects Development, College of Health-Related Professions, Medical Sciences Campus, University of Puerto Rico.

The DSM of the American Psychiatric Association, Fourth Edition, describes the differential characteristics of what have been termed "the autism spectrum disorders". One of these disorders is Asperger's Syndrome (AS) which is defined by its socio-communicative traits (some controversies still exists on whether these persons fall into the Asperger category or are merely of high-functioning autism). Individuals with AS can attain high educational goals but are odd in the ways they relate to people, and pedantic in their language style. This case study was aimed at obtaining preliminary data on the Hispanic social and linguistic manifestations in a Puerto Rican 26-years-old, well-mannered and healthy male with AS, who lives with his parents. JP was brought to the examining room where three examiners administered him a criterion-referenced speech and language assessment with receptive and expressive communication tasks, and an emotion-loaded evaluation protocol to assess his understanding and expression of human sentiments. All subject responses were audio and videotaped. Results indicate that JP can process linguistic information with ease, uses expressive language in stereotyped fashions that cause listeners' uneasiness, understands pictorial emotional representations with some difficulty, and demonstrates problems in explaining affective states of others. Results will be used to refine the Infantile Autism Project's assessment procedures for AS, and to initiate a long-term investigation to establish precise diagnostic criteria and particular intervention methods for Latino individuals with this syndrome.

**A-4 Relación entre el Autismo Infantil y la Incidencia de Condiciones Virales o Infecciones Durante el Segundo Trimestre de Embarazo y en la Infancia** Y Rentas, L Rovira, J Ortiz  
Proyecto Autismo Infantil, CPRS/ RCM

Recientemente se ha propuesto la teoría de que el Autismo es resultado de la suma de varios agentes que alteran el sistema inmunológico. Entre estos se menciona la posibilidad de la incidencia de condiciones virales o infecciones durante el segundo trimestre del embarazo. En varios estudios se han encontrado anomalías estructurales en los cerebelos de niños con autismo (disminución en el número de células de Purkinge, cerebelos más pequeños y otras). En el segundo trimestre de embarazo las células de Purkinge migran al cerebelo y además se ha informado de la incidencia de un número mayor de niños con autismo que han nacido en el mes de marzo (6 meses antes de la época de mayor incidencia de influenza). Con la idea de corroborar esta información, se estudiaron los expedientes de 22 niños pre-escolares adscritos al Proyecto de Autismo Infantil. Los resultados indicaron que en cuatro de los historiales (18.18%) había evidencia de condiciones virales o infecciones durante el embarazo. Estos habían ocurrido durante el primer trimestre. A la luz de estos hallazgos se continúa la investigación en relación a la incidencia de infecciones o condiciones virales o indicadores de problemas inmunológicos en la infancia. Esto con el propósito de explorar la aseveración de que la suma de varios agentes que afectan el sistema inmunológico del niño está relacionada al autismo infantil.

**A-5 Time Course of Masking Effects on the Human Brainstem Auditory Evoked Response** G.A. Owen, Sc.D., Aud. Pgm., Univ. of P.R. R.F. Burkard, Ph.D., Dept. of Com. Dis. and Sciences, SUNY - Buffalo, NY 14260

Two experiments were conducted to study the time course of onset effects of simultaneous masking on the brainstem auditory evoked response (BAER). In the first experiment, the

masker was a broadband noise of 80 dB SPL and 50 ms duration. A click of 110 dB pSPL was presented simultaneously with the masker, and the time interval between the onset of the masker and the onset of the click was varied, ranging from 5 to 40 ms. In addition, a quiet (no-noise) condition and a continuous simultaneous masking condition were included. The dependent variables were latency and amplitude of wave V. Results show the expected wave V latency increase in the presence of a masker. The entire latency increase due to masking is achieved within 5 ms., with no significant difference between the pulsed and the continuous masker conditions. The amplitude of wave V is smallest for the 5 ms post-onset interval, increasing as onset interval increases, and gradually approaching the mean continuous-masking amplitude. In experiment two the masker duration was increased to 90 ms with the onset interval ranging from 5 to 80 ms, and click level was increased to 115 dB pSPL in an effort to enhance wave I identification. Simultaneous pulsed masking results in a very rapid wave I and wave V latency increase with no apparent latency change with increasing onset interval. Wave I and V amplitude values show an initial maximum reduction with a gradual increase toward the amplitude value seen for a continuous masker. (NIH-NIDCD grant #DC00399)

**A-6 Survival and AIDS Defining Conditions of Pediatric AIDS in Puerto Rico: 1981-1996,** Pérez R, Pérez C Suárez E

**Objective:** To describe the AIDS - defining conditions ( ADCs ) and survival experience of pediatric AIDS cases ( <13 years) reported in Puerto Rico.

**Design:** Descriptive analysis of the data of the Puerto Rico AIDS Surveillance System.

**Methods:** Data for the 347 pediatric AIDS cases reported from January 1981 through September 1996 were reviewed. Survival following AIDS diagnosis was analyzed with the Kaplan-Meier method and differences between curves were assessed by the Wilcoxon test.

**Results:** As of september 30, 1996, 347 pediatric AIDS cases had been reported to the

Puerto Rico AIDS Surveillance System. The majority of them acquired the infection from their mothers. Most of the cases were diagnosed before four years of age (97%). The most common ADC was *Pneumocystis carinii pneumonia* followed by esophageal candidiasis. A considerable proportion (19.4%) of children were diagnosed with wasting syndrome. the overall median length of survival from diagnosis was 43.7 months (95% CI: 31.3-71.0). Children less than 1 year of age had a shorter survival (17.6 months; 95% CI:10.2-36-7). The worst survival rate was for children diagnosed with pulmonary candidiasis (2.4 months; 95% CI: 0.9-12.7). A significant improvement in survival was found between subcohorts based on two time periods of diagnosis, 1981-1987 vs. 1988-1996. These may be attributed to improvements in therapeutic methods or to some methodological issues such as incomplete ascertainment of deaths.

**Conclusions:** Differences in the proportion of several ADCs as well as in the median survival time was observed between Puerto Rico and the U.S. pediatric AIDS cases. This may reflect differences in diagnostic procedures or in reporting practices.

**A-7 Características Sociodemográficas y de Salud de Mujeres Fumadoras y No Fumadoras que Dieron a Luz en el Hospital Municipal de San Juan: 1995-1996** Rivera-Tubéns H, MD, MPH, Capriles Quiroz J, MD, MPH, Ramos Valencia G, DrPH, Díaz Méndez N, RN, MPH

El tabaco y sus efectos en la salud se considera un problema de salud pública (1-3). Este estudio tiene como principales objetivos describir las características sociodemográficas, de estilos de vida y de salud en mujeres que dieron a luz en el Hospital Municipal de San Juan (HMSJ) y que fumaron durante el embarazo y compararlas con aquellas que no fumaron. Además comparar las características de sus neonatos. Se recopiló información mediante entrevistas estructuradas a 1770 mujeres. Este estudio es de tipo descriptivo, se comparan las mujeres que fumaron durante el embarazo (245) con aquellas que no fumaron

(1525). Se utilizan medidas de tendencia central y se comparan proporciones. El nivel de significancia aceptado es  $p < 0.05$ . Aproximadamente un 17% de las madres fumadoras usó drogas durante el embarazo comparado con un 0.2% en las madres no fumadoras. Se encontró que entre la madres fumadoras hubo un 28.4% de bebés con bajo peso al nacer y un 19.1% en las no fumadoras. Las mujeres fumadoras presentaron con mayor frecuencia que las no fumadoras falta de cuidado prenatal, uso de alcohol y drogas y neonatos de bajo peso. Estos datos sugieren que mediante una campaña de cese de fumar previo y durante el embarazo se disminuiría significativamente el bajo peso al nacer y por ende la morbilidad y mortalidad infantil.

**A-8 Overdiagnosis of acrania by sonography**, A, de la Vega, Department of OBGYN, University of Puerto Rico, Sch of Med

The incidence of open neural tube defects (ONTD) is considered to be high in Puerto Rico. Prenatal sonographic detection is important to reduce the parental trauma associated with a delivery of a child with an unknown severe disability or a fatal condition. Adequate genetic counseling for future pregnancies depends on a correct pathologic diagnosis. In addition, current studies are underway evaluating the value of preconceptional supplementation of folic acid to prevent ONTD. Patients who are considered at risk are often given 2-5 times the folic acid dose than the rest of the population. Acrania is the complete or partial absence of cranial bones. It is a defect of mesodermal and ectodermal derivatives and not a true open neural tube defect. Etiology and recurrence risks are different from anencephaly. Sonographic diagnosis depends on the identification of cortical brain tissue in the absence of cranial bones. We have identified 4 fetuses with a prenatal diagnosis of acrania based on the identification of abundant brain like tissue. At delivery all four were proven to have anencephaly with an abundant area cerebrovasculosa which mimicked brain tissue. We believe that acrania is often misdiagnosed sonographically, and that careful histologic studies must be performed to validate such a confirmed diagnosis in order to provide

proper genetic counseling to the parents.

**A-9 Ineffectiveness of fetal monitoring in preventing intrauterine fetal death among a mixture of low and high risk obstetric population**, de la Vega, A, Vazquez-Dubeau, D, Dept of OBGYN, University of Puerto Rico, School of Medicine

Stillbirth rate, defined as fetal death occurring after the 20th wk of gestation, is 7.8-8.4/1000 of general population in U.S. We examined the incidence of stillbirth among a mixture of low and high risk private obstetrical population of 1450 patients delivered from January 1993 through February 1997. There were 12 stillbirths among 9 pregnancies (stillbirth rate of 8.2/1000 births). The average age of term at detection of death was 29.1 weeks (range 20 to 37). The most common maternal condition associated with stillbirth was diabetes mellitus with a stillbirth rate of 44%. In only one case the fetal cause of death remained unexplained. All high risk patients were followed intensively throughout the pregnancy with frequent routine sonography, biophysical profiles, amniotic fluid analysis, and "non stress" testing. Although our data cannot be used to evaluate accurately the impact of close fetal surveillance on the occurrence of fetal death, the fact that the stillbirth rate among our population was similar to that of the general population in the U.S., questions the reliability of these antepartum evaluations in the prevention of fetal wastage.

**A-10 Prevention of fetal macrosomia in patients with diabetes mellitus**, S, Schwarz, D, Vazquez, K, Adamsons, Dept of OBGYN, University of Puerto Rico School of Medicine

Insulin is a principal growth promoting hormone in the fetal period. Thus hyperinsulinemia produced by fetal hyperglycemia increases the size of all organs with the exception of the brain. Because of the prolonged release of insulin following even a short episode of hyperglycemia, fetal macrosomia is difficult, if not impossible, to prevent in patients with diabetes mellitus. Therefore the relative frequency of macrosomia of the fetus, and traumatic delivery has remained essentially unchanged in spite of intensive monitoring of blood sugar of mother, and frequent morphometry of the fetus. Fetal

hyperinsulinemia might lead to permanent liabilities such as increasing cell number in the heart, and increasing numbers of fat cells. We propose to eliminate these problems by acceleration of fetal maturation, and delivery of the fetus at or prior to 36th week of gestation. Patients with gestational diabetes were offered treatment with intraamniotic thyroxine (500 g once a week) starting at 31 or 32 weeks of gestation. Delivery was performed between the 34 to 36 weeks when amniotic fluid L-S ratio exceeded 2.5 and PG was detectable. Of the 12 patients 8 have been already delivered. All deliveries were uneventful either by the vaginal route or by cesarean section with indications other than macrosomia. Maximal fetal weight was 3523 g of a fetus at 36 weeks. No newborn required admission to intensive care, and no newborn experienced neonatal hypoglycemia. Conclusion: acceleration of fetal maturation with thyroxine, with which we have more than 15 years of experience, appears to eliminate problems created by fetal hyperinsulinemia in patients with gestational diabetes mellitus.

**A-11**      **Surgical removal of the entire trophoblastic population cures life threatening preeclampsia**, I, Perez, K, Adamsons, Dept of OBGYN, University of Puerto Rico School of Medicine

Earlier in gestation a substantial portion of the trophoblastic population resides in the myometrium detached from placenta. Therefore, the substances released from the hypoxic trophoblast, and which are responsible for causing preeclampsia, are not removed by delivery of the placenta alone if preeclampsia occurs significantly prior to term. We observed that at 23 weeks of gestation severe preeclampsia can continue for as long as one week after delivery. Therefore, if preeclampsia creates a life threatening condition, hysterectomy rather than hysterotomy should be the appropriate treatment. The following case illustrates the validity of this contention. A 16 y/o primigravida was admitted at 22 weeks of gestation with severe preeclampsia. Her main problem was pulmonary congestion which was initially misdiagnosed as

pneumonia. BP 140/90 and pulse 140 per min. In spite of increasing oncotic pressure of plasma by albumin, and reducing the epinephrine mediated arteriolar vasodilatation in pulmonary circulation with propranolol, the congestion of lungs increased requiring mechanical ventilation with progressively higher expiratory pressures. Her condition worsened with PO<sub>2</sub> decreasing to 49.8 torr. She became hypotensive with BP 80/50 in spite of maximal doses of Intropin. She was considered irretrievably moribund by the Medical Intensive Care Unit. By our staff she was taken to the operating room where a supracervical hysterectomy was performed. In one hour her arterial PO<sub>2</sub> had risen to 108 torr, and her lung edema begun to clear. She could be extubated 9 days later, and was discharged home in good condition on postoperative day 11.

**A-12**      **Alpha adrenergic receptors and cerebral vasculature in preeclampsia**, I, Colon, K, Adamsons, Dept. of OBGYN, University of Puerto Rico, School of Medicine

We have presented previously evidence regarding the importance of norepinephrine mediated cerebral vasoconstriction in preeclampsia. It was a case of a 23 y/o patient at 30 weeks of gestation transferred from a private hospital because of severe preeclampsia. Her initial BP had been 180/100 but she had no abnormal neurologic findings. She received labetalol which lowered BP to 160/100. After transfer, attempt was made to lower the BP further, and she was given additional 20 mg labetalol i.v. Within 10 min she became obtundent, and later moribund due to massive subarachnoid hemorrhage. Her BP never rose above 150/90. The two patients we are presenting now are patients with chronic hypertension and severe preeclampsia. Due to elevation of BP above 200 torr they were placed by their physicians on labetalol 600 mg t.i.d. On admission both presented severe headache and blurred vision. BP was only 160/100. Labetalol was discontinued and patients were given i.v. propranolol. In spite of rising BP to 220/120 both patients became asymptomatic. One was delivered by CS and one had vaginal delivery. Preventing the nore-

pinephrine mediated vasoconstriction by labetalol created distention of cerebral vessels and high filtration pressure which were responsible for the neurologic symptoms exhibited by both patients. Elimination of alpha adrenergic blockade freed patients of their neurologic abnormalities in spite of rising BP.

**A-13 Lethal preeclampsia occurring at 14 gestational weeks**, M, Flores, K, Adamsons, Dept of OBGYN, University of Puerto Rico School of Medicine

According to our teaching preeclampsia is caused by a yet to be identified substances, most likely polypeptides, released from the trophoblast when the PO<sub>2</sub> of the trophoblast falls below 30 torr. In most clinical situations this occurs in the third trimester of pregnancy. However, in the absence of intervillous space (IVS), as it is in cases of chorionic tumors, preeclampsia occurs substantially earlier. Decreased oxygenation of the IVS has many causes, among which a small cross sectional area, and adrenergically mediated vasoconstriction in uterine circulation, are the most frequent. The hyperviscosity of the blood is a rare cause of reduced oxygenation of IVS. The following case illustrates, however, the severity of preeclampsia that can be produced by a large trophoblastic mass of early pregnancy exposed to underperfusion. The patient was a 39 y/o g1 at 13 gestational weeks with triplets. Pregnancy was complicated by sickle cell anemia. She was admitted with signs of preeclampsia, which also included severe pulmonary congestion. She was urged to have her pregnancy terminated, but she refused. In spite the increase in plasma oncotic pressure by repeated administration of albumin, and blood transfusions her condition progressively deteriorated resulting in death at the 14 gestational week. To our knowledge this is the earliest case reported in world literature of severe preeclampsia in the presence of normal pregnancy. It illustrates the potential of the young trophoblast subjected to low PO<sub>2</sub> to liberate materials which stimulate contractile proteins of the cardiovascular system.

**A-14 Prenatal diagnosis of semilobar holoprosencephaly**, A, de la Vega, I, Garcia, Department of OBGYN, University of Puerto Rico, School of Medicine

Holoprosencephaly is caused by incomplete cleavage of the primitive prosencephalon or forebrain. It is divided into alobar, semilobar, and lobar categories based on the degree of separation of the cerebral hemispheres. Alobar holoprosencephaly is occasionally diagnosed sonographically, and is almost uniformly fatal. Semilobar holoprosencephaly has rarely been reported prenatally. Most individuals with this condition will either die or suffer from severe mental retardation. We report one of the first prenatally diagnosed cases of semilobar holoprosencephaly. The patient was referred to our institution at 31 weeks because a previous sonogram reported the presence of hydrocephaly. The mother had received genetic counseling based on this diagnosis. Careful sonographic evaluation at our institution detected incomplete division of the forebrain with absence of the anterior third of the falx cerebri and the lateral ventricles communicating at this point. Delivery near term was followed by early neonatal death. Careful identification of the falx cerebri and the lateral ventricles may avoid misdiagnosing this fetal anomaly, thus providing for better prenatal and postnatal counseling.

**A-15 Chronic hypoglycemia of the fetus and the organization of the B cells of the pancreas**, K, Adamsons, I, Colon, D, Singer, Dept. of OBGYN, University of Puerto Rico, School of Medicine and Dept. of Pathology, Women and Infants Hospital, Rhode Island, USA

The processes needed for the formation of Langerhans islands of the pancreas during the prenatal period are not known. Our hypothesis is that in order to stimulate the alpha and beta cells to aggregate, and to form Langerhans islands the fetus needs to be exposed to at least intermittent hyperglycemia. The strict control of glucose levels is the mainstay of contemporary management of patients with diabetes mellitus in pregnancy. Because of the large glucose gradient between mother and fetus the desirable glucose concentration in ma-



ternal blood could produce too low a concentration of glucose in fetal blood for optimal development of the Langerhans islands. We examined the pancreas of fetal Rhesus monkeys made hypoglycemic for 2 weeks by implantation of insulin ejecting micropumps at about 115 days of gestation (term=167 days). The mean fetal glucose at delivery in the hyperinsulinemic group was 20.8 mg (range), and 35.0 mg (range) in the age matched controls. Histologic examination of the pancreas of the hypoglycemic fetus revealed a nearly complete absence of Langerhans islands with the beta cells dispersed throughout the parenchymal tissue. Control animals showed completely formed Langerhans islands. We conclude that fetal hypoglycemia with or without fetal hyperinsulinemia disrupts the normal development of Langerhans islands in the primate.

**A-16 Tocolysis in the management of arrested labor,** G, Velazco, P, Diaz, K, Adamsons, Dept. of OBGYN, University of Puerto Rico, School of Medicine

Arrest of labor is defined as failure of the cervix to dilate completely in the presence of uterine contractions of normal frequency, duration and intensity, or the failure of the presenting part to descend. It is managed by stimulating the uterus with oxytocin, or by delivering the patient by cesarean section. The reason for a cervix not to dilate in spite of uterine contractions must be due to the return of the myometrial cell to precontraction length. This phenomenon has not been recognized previously, and progressive shortening of the contractile elements of the myometrium has not been identified as requisit for the expulsion of uterine contents. We are proposing that temporarily stopping the myometrium from contracting, the needed changes can occur in the contractile elements of the myometrium to ensure their progressive shortening. The following case supports this hypothesis. A 33-year old g6 near term was admitted in early active labor. When cervical dilations did not progress to more than 6 cm, augmentation of labor with oxytocin was instituted. In spite of it cervical dilatation re-

mained unchanged. Uterine contractions were then suppressed with magnesium (diazoxide was not available). After 2 hours contractions reappeared, and there was rapid progression of cervical dilation resulting in vaginal delivery 3 hours later. Our ongoing study has accumulated several cases in which transient tocolysis has dramatically altered the effect of uterine contractions on cervical dilatation, enabling us to achieve vaginal deliveries in situations in which traditional management would have called for cesarean section.

**A-17 Towards the Eradication of Bilharzia (Schistosomiasis) from Puerto Rico.** George V. Hillyer. Dept. Pathol. & Lab. Med. Suite 617-A, UPR School of Medicine, San Juan, PR 00936.

Isaac Gonzalez-Martinez discovered in 1904 lateral spined eggs now known to be Schistosoma mansoni in 2 Puerto Ricans who had never left the island. Later in that same year, the first Anemia Commission of Porto Rico recorded 0.4 % (21 of 5,000) infected persons with this parasite. This increased to 3.2 % (320 of 10,140 cases) from a study at the Institute of Tropical Medicine of Porto Rico in 1913, and 20 % (of 635 individuals of all ages) in a study by Hoffman in 1928. Additional studies examining 1 g of feces were those of Weller, and Dammin in 1945 (9.9 % of 19,139 selective service registrants and White *et al.* in 1953 (10 % of 8,995 students). In the ensuing 44 years no comprehensive coprologic or serologic survey for the prevalence of bilharzia has been done in PR. Because bilharzia as a disease was clearly diminishing in PR, the Health Dept abolished in 1980 the ineffective control program that relied primarily on snail control via mollusciding with chemical pollutants. Because of the paucity of information on the current status of infection, the PR Health Dept. established a "Bilharzia Commission" to evaluate the current status of infection and formulate recommendations for the development of programs to identify, treat, and follow infections for the short term control and medium term eradication of infection from the island. Utilizing algorithms such as in the diagnosis of HIV infection in which one screens by ELISA

and confirms by Western Blot for antibodies to schistosomes (99 %+ sensitivity & specificity, each) we screened 2966 blood donors island-wide and found an overall confirmation positivity of 11 % with 2 significant clusters of over 15 % comprising 10 of the 78 municipalities of PR. A second study examining over 750 individuals 25 years or younger showed an age-specific decrease in seroprevalence where, in 5-year increments, the seropositivity was 0 % (0-5 years old), 0.7 % (6-10 years), 1.2 % (11-15 years), 2.6 % (16-20 years), and 4.7 % (21-25 years). These results suggest little or no transmission of bilharzia in Puerto Rico during the decade of the 1990s. The dramatic decrease of bilharzia in PR is supported by three additional findings: (1) of over 25,000 stool examinations at the PR Medical Center from 1993-1997 only 4 egg positive cases were identified, none of which were from 1996-7; (2) we have observed a steady decrease of antibody positives referred to our laboratory for testing over the past 5 years; (3) there has been a steady decrease in antibody positives referred from the Veterans Administration Center over the past 10 years. If the mean life span of schistosomes is 5 years, then we should expect eradication of this parasite from Puerto Rico by the 100<sup>th</sup> year of its discovery by Isaac González-Martínez in 2004.

**A-18 An Update of Cancer in Puerto Rico: Analysis of Two Hospital Cancer Registries (1990-1995)** L. Herrera, E. Mora, C.M Nazario, Department of Surgery and Pathology, School of Medicine, Department of Biostatistics and Epidemiology, School of Public Health, Medical Sciences Campus, UPR

The most recent population-based cancer statistics in Puerto Rico (PR) are from 1991. Their lack affect cancer research, and the development of preventive and treatment policies. To obtained epidemiologic and treatment data from cancer patients diagnosed after this period, we proposed the merged of two hospital registries to described the disease and determine whether this data reflects recent trends of cancer in Puerto Rico. Data from the University District

Hospital (UDH) and the San Juan Oncologic Hospital (SJOH) cancer registries was joined. Descriptive analysis of epidemiologic, clinical, and survival data was performed (1990-1995). Initially, data from both hospitals was compared. Afterwards, the combined hospitals data was compared to the 1991 PR Cancer Statistics (1991 CS). A total of 11,253 cases were analyzed. The geographical distribution of the cases was representative of every municipality. Breast was the most common cancer in women (21.1%) and prostate the most common in men (11.9%). For common organs, head and neck, colorectal, lung, and leukemias/lymphomas were the leading primary sites. The most common treatment modalities were chemotherapy and radiotherapy alone. In contrast, the 1991 CS reported the most frequent common organs as: lung, colorectal, and stomach. The most common treatment modality was surgery alone. In conclusion, it is possible to merged hospital-based registries and gather important information. There are significant differences between the groups studied and the 1991 CS. This evidence further supports the need of up to date population statistics for the island. Partially support by CIDIC.

**A-19 Serotypes Prevalence in a Puerto Rican Population with Chronic Hepatitis C.** Roberto J. Firpi, MD, Fernando Castro, MD, Esther A. Torres, MD, José Rodríguez Medina, PhD, Vanessa Rios and Marina Torres. *Gastro enterology Section, U.P.R.*

Hepatitis C (HCV) is currently the most common cause of chronic liver disease in the United States. There is a marked heterogenicity in the viral genome and 6 major genotypes have been identified. These genotypes are thought to be associated with disease severity as well as response to IFN treatment. Serotyping is more rapid, convenient and cost-effective method of determining the subtypes of HCV than is genotyping. Genotypes 1,2 and 3 have been correlated with serotypes 1,2 and 3 by use of specific peptides from regions of the HCV viral genome. The objectives were to determine the predominant serotypes in a Puerto Rican population with chronic hepatitis C, and to com-

pare genotypes with the serotypes found. Fifty five patients with Hepatitis C done liver biopsy and treated with IFN were evaluated for antibodies to HCV using the CHIRON RIBA HCV Serotyping Strip Immunoblot Assay. 83% of patients were found with serotype 1, 8% serotype 2, 4.5% serotype 3 and 4.5% serotype 1,3. Serotype 1 also predominates in other reported populations, but the percentage found in our study is higher than the others.

**A-20 Depressive Symptomatology Among Puertorrican Epileptic Patients.** Margarida, M.T.Psy.D., Maysonet, T., Cruz, R.F. M.D. Neurology, Department of Medicine, University of Puerto Rico Medical School.

The purpose of this study was to assess the rate and severity of depressive symptoms among a group of patients with intractable epilepsy. Gender differences were also explored. 77 patients attending the Puerto Rico Comprehensive Epilepsy Program and the Neurology Clinics at University Hospital were evaluated with the Beck Depression Inventory, CES-D, and the Symptom Check List-36 (SCL-36). Exclusionary criteria was mental retardation, under 18 years of age and inability to read. Descriptive statistics included means, frequencies as well as factor analysis to establish gender differences. Correlation and alpha cronbach analysis were performed to measure internal consistency and reliability among instruments. High rates and severity of depressive symptoms as well as gender differences in the clinical manifestations of depression were found. On the SCL-36, the highest loading factors were generalized and phobic anxiety (35% & 7.5% respectively). Implications for medical/pharmacological treatment as well as the need for the integration of preventive strategies against depressive illness will be discussed.

**A-21 Rickets Secondary to Vitamin D Deficiency: An Uncommon Disease That Still Could Happen Nowadays.**

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Hypophosphatemic rickets remains as the most common cause of rickets since vitamin D deficiency has become unlikely with the present better nutritional practices. However, we present the case of a child with proven vitamin D deficiency. A child was referred to our service at 6 years of age for evaluation because of history of fractures at multiple sites to minimal trauma. Evaluation by social worker at that time did not disclose evidence of abuse or neglect. Past history revealed that she had been admitted previously to our institution because of multiple body trauma from a motor vehicle accident. Her hormonal evaluation was remarkable for normal serum calcium levels of 8.7 mg% (nl 8.5 - 10.5); phosphorus was low at 2.7 mg% (nl 3.5 - 6.8); inappropriately increased intact parathyroid hormone levels at 242 pg/ml (nl 10 - 65, Nichols Institute) and normal 1,25-dihydroxyvitamin D serum levels at 41 pg/ml (nl 15 - 60, Nichols Institute). However, the girl was lost to follow up and returned for re-evaluation two years later at age 8 because of marked osteopenia. This time the girl was brought by the grandmother who had become the legal guardian. The latter because parents were found negligent and became drug users. Her physical examination this time showed marked bowing of both tibiae and forearms with pain on motion. Radiological studies confirmed the osteopenia and demonstrated changes compatible with rickets. Biochemical evaluation showed an augmented alkaline phosphatase at 875 IU/L (nl up to 270), normal magnesium serum levels at 1.9 mg% (nl 1.6 - 2.3) with diminished 25-hydroxyvitamin D serum levels at 8 ng/ml (nl 9 - 52, Nichols Institute). A rapid clinical improvement was followed by the administration of calcium (i.e., calcium glubionate) and vitamin D (calcitriol) supplements concomitantly with nutritional intervention. She has maintained normal serum calcium (10.1 mg%), phosphorus (4.4 mg%) and alkaline phosphatase levels (160 IU/L) while off from calcium and vitamin D supplements. Although bowing of the bones persists, pain is no longer present. In summary, we present an instance of rickets caused by vitamin D defi-

ciency. We conclude that although vitamin D deficiency has become an unheard cause of rickets in our environment, it remains as a distinct treatable cause of rickets and most always be considered.

**A-22 Meningioma En Plaque: Diagnosis, Treatment, and Outcome** M.M. Toledo, O. De Jesús. Section of Neurosurgery, University of Puerto Rico

Meningiomas are extra-axial, slow growing, usually benign tumors that arise from arachnoid cap cells. Meningioma en plaque represents a morphological subgroup defined by a carpet or sheet-like lesion that infiltrates the dura and sometimes invades the underlying bone. A retrospective review of all the adult operative cases at the University Hospital in a seven-year period from July 1, 1990 to June 30, 1997 showed that 141 patients were operated for brain meningiomas. Of these, 46 were located adjacent to the sphenoid ridge. Six were considered to fulfill the criteria for meningioma en plaque. All six patients with meningiomas en plaque were female. Meningiomas en plaque spread along the meninges where they have a marked tendency to cause hyperostosis. For reasons that are unclear, meningiomas en plaque are more likely to provoke adjacent bony hyperostosis than the larger globular tumors. The hyperostosis is often disproportionately larger than the size of the underlying tumor. It is this bony hyperostosis what frequently produces the clinical signs and symptoms by pressing against adjacent structures. Exophthalmos is the most common presentation in patients with meningioma en plaque. Differential diagnosis of this lesion include fibrous dysplasia, osteoma, and osteoblastic metastasis. A subtotal but extensive removal combined with bony decompression of the cranial nerves at the superior orbital fissure and optic canal is frequently followed by good functional and cosmetic results.

**A-23 Fluvoxamine Maleate in Patients with Neuroacanthocytosis.** Arroyo M, M.D., Margarida M.T., Psy.D. Neurology, Department of Medicine, University of Puerto Rico Medical School.

Neuroacanthocytosis is a rare, progressive disease characterized by movement disorders, ax-

onal neuropathy, personality changes and cognitive impairment among other symptoms. The personality and cognitive problems present in these patients which are the object of our study are depression, obsessive-compulsive behaviors, frontal lobe executive dysfunction & subcortical dementia. The objective of this open label study was to look at the effects of Fluvoxamine maleate in the obsessive-compulsive symptoms, depression and neuropsychological functions of these patients. Three of eight patients with Neuroacanthocytosis and showing the above mentioned symptoms were administered the Yale-Brown Obsessive Compulsive Scale (Y-BOCS) and the CES-D to measure both obsessive and depressive symptoms before using and after twelve weeks on medication. Neuropsychological tests (Trails A & B, Wisconsin Card Sort) were used to measure executive functions associated to attention, concentration, cognitive flexibility and categorization. Medication was administered starting at 50 mg oral dose up to 300 mg or maximum tolerated dose. Following intake of Luvoxamine, all 3 patients showed mild improvements in neuropsychological measures. Obsessive-compulsive symptoms in all 3 patients showed marked improvement of compulsive self damaging behaviors, such as head banging, lip and tongue biting, as well as most obsessive-compulsive behaviors. Depressive symptoms showed little improvement. In one patient the movement disorder improved significantly. These findings are consistent with other studies which have demonstrated the effectiveness of Luvoxamine in the improvement of obsessive-compulsive behaviors. Furthermore, its use in this group of patients was also found to improve neuropsychological executive functions as well as movement disorder in one patient.

**A-24 Histologic Features of Alcoholic Liver Disease in Patients with Chronic Hepatitis C:** Correlation with Alcohol Abuse. P Costas, F Castro, E Torres, C González Keelan, A Fernández, C Pérez. University of Puerto Rico School of Medicine and School of Public Health.

**Introduction:** Histologic features characteris-

tic of both chronic hepatitis C (CHC) and alcoholic liver disease (ALD) include macrovesicular steatosis, Mallory bodies, and, in advanced stages, cirrhosis. Fibrosis around the central vein is characteristic of ALD. **Objective:** To determine the frequency of histologic features of ALD among alcohol abusers (AA) and non-alcohol abusers (NA) in a group of Puerto Rican patients with CHC. **Methods:** The population consisted of 38 patients with CHC confirmed by RIBA II and liver biopsy. The diagnosis of alcohol abuse was established from the administration of a standardized questionnaire (CIDI). Two experienced pathologists blinded to the history of alcohol abuse independently reviewed the liver biopsies. The histologic features examined were the presence of macrovesicular steatosis in greater than 50% of the specimen (FAT), fibrosis around the central vein (FV), Mallory bodies, and cirrhosis. **Results:** 12/38 (32%) were AA. Mean age of AA was  $41.7 \pm 14.1$  yrs. Mean age of NA was  $49.3 \pm 11.2$  yrs. 12/12 (100%) of AA were male, while 5/26 (19%) of NA were male ( $p < .001$ ). FAT was present in 1/12 (8.3%) of AA and 8/26 (30.8%) of NA ( $p = .22$ ). FV was present in 2/12 (16.7%) of AA and 7/26 (26.9%) of NA ( $p = .69$ ). Mallory bodies were present in 0/12 (0%) of AA and 6/26 (23.1%) of NA ( $p = .15$ ). Cirrhosis was present in 4/12 (33.3%) of AA and 14/26 (53.8%) of NA ( $p = .31$ ). **Conclusions:** Liver histopathology in CHC does not distinguish the patients with a history of alcohol abuse from the non-abusers.

**A-25 Motor Activity Program for Children with Multiple Disabilities in a Public School** K. Correa, A. Font Interdisciplinary Project of Excellence in Services, College of Health Related Professions

The Physical Therapy Clinic of the Interdisciplinary Project of Excellence in Services, is working with four children with multiple disabilities in a classroom based model. This program's main objective is to adapt the "Activity Program for Body Awareness Contact and Communication" for these children. A physical therapist evaluates and adapts the program for the children. She visits the school twice a week to train special education professionals

in using the program. This motor activity program uses music modalities to facilitate movement and relaxation in students.

After six months of demonstration and orientation the teachers and teacher assistants demonstrate moderate compliance on the application of the program. The special physical education teacher integrates the program to her class. All the children are motivated with group exercises and music.

**A-26 Psychoneurological Aspects of Normally Developing Children and Children with Brain Disturbances.** L. San José, A. González, MSC, I. Echeagaray, UPR.

The study attempts to obtain psychoneurological and developmental information of Puerto Rican children with brain disturbances (BD) and normal development (ND) through the administration of the Developmental and Psychoneurological Aspects Instrument (DPAI). The DPAI has been created to assess the prenatal and medical history of the child considering aspects of their motor, language, and affective development. Nutritional and allergy information is included. The participants are children ranging from six to nine years old selected considering neurological criteria. The DPAI has been administered to the control group to establish ND data. The paper discusses the adequacy of the DPAI and its possible use for differential diagnosis in early BD. It points out the criteria included as critical for right hemisphere functions, particularly regarding abilities to recognize familiar voices, understand and express speech prosody needed in order to behave in a socially appropriate manner. These are verified intact in children of ND. The feasibility of the DPAI is discussed as an instrument to provide early identification of right hemisphere disturbances (RHD) which could be misdiagnosed as Attentional Deficit Disorders (ADD). The presentation includes preliminary guidelines for differential diagnosis. The study attempts to provide culturally adequate information in order to develop early intervention programs and therapy in Puerto Rican children with (RHD). Funded by JARFE.

**A-27 The Use of Colposcopy in the Evaluation of Suspected Child Sexual Abuse.** B Mirabal-Colón MD; M N De Jesús MSN.

Child sexual abuse occurs when a child less than 18 years of age is engaged by an adult in acts which produce sexual gratification in the adult. It includes a wide spectrum: from fondling of genitalia to penetration of body orifices. It is a gradual process and disclosure often occurs after a prolonged time has elapsed. Thus, the medical exam reveals normal or non specific findings in over 70% of cases. The colposcope, an instrument which provides magnification, with the capability of photography has been used extensively to document physical findings. The photographic evidence can be used in the legal process, to obtain expert opinions and is helpful in follow up visits. The purpose of this study is to review the colposcopic findings of 28 children referred to the Biopsychosocial Program of the Department of Pediatrics since May 1997 for suspected child abuse. This program, supported by federal funds of the Dept. of Justice for Victims of Crime offers interdisciplinary services to child victims of family violence. There were 23 females and 5 males (1 to 13 years of age) with a total of 32 colposcopies performed. There were 16 children (57%) with normal or non specific findings. Twelve (12) patients (43%) had abnormal findings; the most common were perianal scars/healing tissue (n=5; 17.9%) and scars/ lacerations/ neovascularization of posterior fourchette/ fossa (n=4; 14.3%). In conclusion, the colposcope is useful for documentation of physical findings in these cases. However, the child's disclosure remains the most important element in the diagnosis of child sexual abuse.

**A-28 Bone Sialoprotein Acts as a Paracrine Factor in the Growth of Bone-Metastatic Breast Carcinoma Cells** E. Mora, R. Lopez, Department of Surgery and Pathology, School of Medicine, Medical Sciences Campus, UPR.

Bone-metastatic disease is very common in breast cancer patients. The role of extracellular matrix proteins in the growth of bone-metastatic breast carcinoma cells (BRCa) is unknown. Collagen IV, Osteonectin,

Osteopontin, and Bone Sialoprotein are the main extracellular matrix proteins in bone. We hypothesized that these proteins act as paracrine factors in the growth of bone-metastatic BRCa cells. To test this hypothesis bone-, pleural-, and primary BRCa cells ( $5 \times 10^3$  cells/well) were exposed to osteonectin (0.25 mg/ml), bone sialoprotein (0.5 mg/ml), and collagen IV (0.45 mg/ml). Growth was assessed by the crystal violet assay. Statistical significance was established at 0.05 ( $p < 0.05^*$ ).

Cell Line	Increase in number of cells after exposure to Osteonectin		
	BoneSialoprotein	Collagen IV	(Mean $\times 10^3 \pm$ SD)
Bone-metastatic BRCa	7.1 $\pm$ 0.2	22.4 $\pm$ 0.5 *	3.2 $\pm$ 0.3
Pleural-metastatic BRCa	6.3 $\pm$ 0.4	7.5 $\pm$ 0.6	8.4 $\pm$ 0.3
Primary BRCa	8.2 $\pm$ 0.6	4.3 $\pm$ 0.1	4.5 $\pm$ 0.2

Only bone sialoprotein significantly increased the growth of bone-metastatic BRCa cells. Conclusion: Only bone sialoprotein act as a paracrine factor in the growth of bone-metastatic BRCa cells. Whether the protein act also as an autocrine factor will be studied in the future. None of the other proteins promote the growth of these cells. Supported by the Puerto Rico Cancer Center, Office of Sponsored Research, CIDIC

**A-29 Role of Viral Load and Enhancing Antibodies in Vertical Transmission of HIV.** L.M. Meléndez-Guerrero<sup>1</sup>, C.L. Cadilla<sup>1</sup>, P. Kamara<sup>2</sup>, M.E. Vega<sup>1</sup>, G.V. Hillyer<sup>1</sup>, E. Jimenez<sup>3</sup>, E. Abreu<sup>3</sup>, I. Méndez<sup>3</sup>, J. Gandía<sup>3</sup>, and P. Jolly<sup>2</sup>. UPR-Medical Sch.<sup>1</sup>, San Juan City Hospital<sup>3</sup>, U. Alabama at Birmingham<sup>2</sup>.

Perinatal transmission of HIV accounts for all new HIV infections in children. The relationship of maternal antibodies to the V3 loop and viral load with the risk of vertical transmission of HIV has been studied by various groups, but the data obtained remained controversial. Antibody dependent enhancement (ADE) of HIV infection *in vitro* has been observed, but the *in vivo* significance of ADE has not been determined. Further, no study has addressed the

correlation of viral load with enhancing antibodies. In this study we compared the plasma viral load of mothers who transmitted HIV to their infants to non-transmitter mothers. The plasma viral load was determined for 40 HIV infected pregnant women at the San Juan City Hospital. We further examined whether high viral load correlates with high levels of enhancing antibodies and low levels of neutralizing antibodies, and whether this combination was observed more frequently in instances of HIV transmission. A wide range of viral load was observed for the mothers. Mothers of infants who were V3PCR + (n=11) had a higher mean viral load (76,731 RNA copies/ml) than mothers of infants who were V3PCR- (21,375 copies/ml). The V3PCR is a two staged amplification for the V3 loop envelope region. Only two of the infants with V3PCR+ results were HIV positive, and the mother of one of these infants had a high viral load, while the mother of the other had a low viral load during delivery. Enhancement of virus infection was observed with 14/40 maternal/infant plasma. Ten of these fourteen plasma represented five maternal-infant pairs. Viral load has been determined for three of the 5 mothers and all were high (>50,000). Interestingly, plasma from the two HIV positive infants enhanced virus infection without corresponding enhancement by the maternal plasma. Research supported by NIH CFAR (P30-AI-27767) and RCMI (G12-RR-03051) collaboration funds, and in part by NIH MBRS 2SD6GM08224-12.

A-30

**Correlation of  $\beta$ -Chemokines: MIP- $\alpha$ 1, MIP-1b and RANTES with HIV-1 Replication in Placental Macrophages.** J. Meléndez\*, R. Delgado, V. García, J. Hawyek, and L. Meléndez. Univ. of P.R. Med. Sch., San Juan, P.R. 00936.

The increase in pediatric HIV infection continue to have a substantial impact on childhood mortality, specially in developing countries where AZT treatment is not available. The mechanism by which the virus penetrates the placental barrier and enters the fetal circulation is not clear. The chemokines MIP-1alpha, MIP-1beta and RANTES have been associated

with suppression of HIV replication. Our hypothesis is that chemokine produced by placental macrophages (Hofbauer cells) suppress HIV infection in the placenta. To test this hypothesis we inoculated Hofbauer cells with T-tropic (HIV-MN) and M-Tropic (HIV-BaL) viruses. Chemokine production was measured in the supernatants of LPS stimulated and unstimulated Hofbauer cell cultures by ELISA. All cultures were tested for p24 Ag at day 5 and 10 post-infection. Stimulated Hofbauer cells produced 3 to 10x higher levels of MIP-1alpha, MIP-1beta and RANTES than unstimulated cultures. The level of MIP-1beta were the highest of the three chemokines secreted upon stimulation, while RANTES had the lowest (P<.005). We did not find differences in the levels of MIP-1alpha secreted by infected and uninfected cells. HIV-BaL infected Hofbauer cells produced half the amount of MIP-1beta than uninfected cells. HIV-BaL infected Hofbauer produced half the amount of MIP-1beta (15 ng) than HIV-MN infected (35 ng) and uninfected cells. HIV-BAL was replicating in higher amounts than HIV-MN. We did not find differences in the mean production of RANTES in uninfected and HIV-BaL infected Hofbauer cells. These results suggest that among the three chemokines, MIP-1beta may have a suppressive effect in HIV production of Hofbauer cells infected with a M-tropic virus. Research supported by NIH/MBRS 2SD6GM08224-12.

A-31

**Molecular Characterization of the SIVsmB7 mutant provirus.** Salamán, A., Rodríguez, A., Martínez, I., and Kraiselburd, E. Department of Microbiology & Medical Zoology, School of Medicine, P.O. Box 365067, SJ, PR

Simian Immunodeficiency Viruses (SIV) are primate lentiviruses closely related to Human Immunodeficiency Virus type 2 (HIV-2). The non-infectious virus-like particle (VLP) SIVsmB7 is a constitutive product of the B7 cell clone. Molecular characterization of the resident provirus in B7 clone cells is necessary to recognize which factors or mechanisms are involved in the non-replicative state of this VLP. These studies could be helpful in the de-

velopment and design of safe and effective vaccine candidates against HIV-2 and HIV-1. The SIVsmB7 provirus has a stable genome deletion in the *pol* region of about 1.6 Kb as determined by PCR. To characterize this mutation, intact sequences of the SIVsmB7 *pol* region were obtained using the proof-reading synthesis of the *Pwo* DNA polymerase. The results obtained from sequencing confirm the presence of a 1569 bp deletion from nt4653 to nt6222. The deletion includes most of the integrase, all of *vif* and *vpx*, and the first sequences of *vpr* including the ATG start site. However, RT including RNase H, *tat*, *rev* and the 5' end of *env* are unaffected. This deletion could be the result of a jump of the retrotranscriptase during viral DNA negative strand synthesis from nt6233 to nt6222 and from nt4662 to nt4651. The number of SIVsmB7 genome copies present per cell was determined by Southern blot hybridization analysis. Hybridization of EcoRI digested B7 cell DNA with an SIV LTR probe yielded two bands (5.5kb, and 4.0kb). HindIII digestion resulted in a single band (15.9kb). The data obtained is consistent with the presence of a single integrated copy of SIVsmB7 DNA in clone B7 cells. Sub-genomic libraries based on EcoRI SIVsmB7 Southern blot results were constructed with the aim of cloning the resident SIVsmB7 provirus genome. Sequencing data obtained from the EcoRI 3' end fragment showed no major changes in *env* gp43 and in the carboxy terminal end of gp130, when compared to the wild type SIVsmH4 genome sequences. However, SIVsmB7 *nef* open reading frame showed a point deletion at nt9803 which immediately creates a termination codon (TAA) at this region. The expected molecular weight of the SIVsmB7 *nef* is 30 KD (instead of the 37 KD from SIV wild type *nef* protein). However, no differences in molecular weight between wild type and SIVsmB7 *nef* protein was observed. Cloning of the SIVsmB7 EcoRI 5' end fragment is in progress. It is concluded that the resident SIVsmB7 provirus contains deletions and point mutation that may affect the expression of both structural and non-structural virus proteins.

**A-32** **The Distribution of GABA-like Immuno-reactivity in the Central Nervous System of *Aplysia californica*.** Mark W. Miller, Manuel Diaz Rios, and Eric Suess. Institute of Neurobiology, Univ. of Puerto Rico, Medical Sciences Campus.

Neurotransmitter phenotype is often used in the classification of neural systems and can sometimes provide insight into functional properties of a circuit. Gamma-aminobutyric acid (GABA) is one neurotransmitter that is widely distributed in the mammalian central nervous system where it is generally considered to be a major mediator of synaptic inhibition. GABAergic systems have been implicated in the generation of numerous psychiatric and neurological disorders, including anxiety, panic attacks, and epilepsy. Substantial evidence suggests that GABA also acts as a neurotransmitter in a wide range of invertebrate phyla, including arthropods, echinoderms, annelids, nematodes, and planarians. In molluscs, a neurotransmitter role for GABA was initially suggested by pharmacological and biochemical studies. In this investigation, we have examined the distribution of GABA-immunoreactive (GABA<sub>i</sub>) neurons in *Aplysia californica* using wholemount immunohistochemistry. Some of the most compelling illustrations of the relationships between neural circuits and behavior have emerged from intensive study using this model system during the last thirty years. Our observations indicate that GABAergic systems are confined to the central nervous system and that they appear to be specialized for the interganglionic transmission of information. In view of known circuitries, the distribution of GABA<sub>i</sub> specifically suggests that this transmitter plays a role in the regulation of central pattern generators involved in feeding and locomotion. No GABA<sub>i</sub> fibers were observed in peripheral nerves suggesting that, as in mammals, GABA is not present in motor neurons or primary sensory neurons. These structural features suggest that GABAergic neurons may play a vital role in the central organization of behavior in *Aplysia*. Supported in part with funds from the NSF (CAREER Award), Puerto Rico EPSCoR, and RCMI.



**A-33 The Role of Endopeptidase EP24.15 in Neuro-degenerative Diseases.**  
Marc J. Glucksman. Fishberg Research Center in Neurobiology, Mount Sinai School of Medicine, New York, NY 10029

Endopeptidase EC3.4.24.15 (EP24.15) is a soluble metalloendo-peptidase that is involved in neuropeptide metabolism in the brain. Processing enzymes, like EP24.15 either: inactivate peptides, convert an inert precursor to an active moiety, or in some instances, convert one bioactive peptide into another with different agonist properties. The enzyme is present in organisms as diverse as yeast, the marine mollusc *Aplysia*, and mammals. Among the peptides modulated by the action of the enzyme are: bradykinin, neurotensin, substance P, nociceptin, and gonadotropin releasing hormone (GnRH, the major reproductive peptide). EP 24.15 degrades GnRH to GnRH1-5, a highly potent glutamate NMDA receptor antagonist. Models utilizing phencyclidine (PCP, angel dust) or ketamine which antagonize glutamate NMDA receptors induce psychotomimetic symptoms resembling schizophrenia (Sz). After puberty occurs, increases in GnRH production and expansion of GnRH projections into cortical and hippocampal regions results in an increased production of GnRH1-5. Interestingly, the incidence of Sz is known to approach adult levels shortly after puberty and neuroleptics used to treat Sz also affect EP24.15 activity. Alzheimer's Disease (AD) is characterized by the deposition of proteinaceous plaques. The major component of these plaques is a 4kD amyloid b-peptide derived by proteolytic processing of a larger protein, the amyloid precursor protein (APP). The processing events that generate this "amyloidogenic" peptide result from a group of enzymes which cleave APP called secretases. Secretase activities occur primarily intracellularly and to a more limited extent at the cell surface. Several investigators have proposed that EP24.15 acts as a secretase, degrading the precursor to a neurotoxic peptide. Thus, alterations in processing enzymes may modulate the onset or severity of AD and other neurodegenerative disorders. This work is generously supported by AHAF & NARSAD.

**A-34 Inhibition of Peripheral Nervous System Regeneration.** D.P. Kuffler, A. Morales, & L. Bonilla, Inst of Neurobiology and Dept. of Physiology, Medical Sciences Campus, Univ. of Puerto Rico, 201 Blvd. Del Valle, San Juan, PR

Following injury to the PNS nerve regeneration is typically good. However, within the CNS almost no regeneration occurs following injury. This absence of CNS regeneration results from the presence of inhibitory proteins in the associated with the membranes of oligodendrocytes. In the presence of antibodies against these neurite outgrowth inhibitory proteins, CNS neurites can extend into and across lesions in the spinal cord. Although blocking the oligodendrocyte inhibitory factor is an excellent means to make the CNS more permissive to neurite outgrowth, the antibodies must be continuously infused into the spinal cord to maintain this positive environment. An important improvement to promoting neurite regeneration would be to find a means by which one could achieve a long-term down regulation of the inhibitory factors. Working with adult rat dorsal root ganglion (DRG) neurons we have found a peripheral nervous system model in which factors associated with the satellite cells of the DRG neuron somas inhibit process outgrowth from the neurons. This process outgrowth inhibition can be reduced *in vitro* by application of several known neurotrophic factors. Combinations of these factors provides a further reduction in the inhibitory influence of the satellite cells. However, addition of a cocktail of peripheral nerve-released factors appears to completely block the inhibitory effects of these cells. We are attempting to characterize the factors responsible for the process outgrowth inhibition, and understand the mechanisms by which the inhibition can be blocked. Such an understanding may lead to mechanisms by which we can induce long-term blocking of the oligodendrocyte neurite outgrowth inhibition in the CNS allowing for regeneration in the CNS. Supported by: ARO DAA03-90-G-0189, NIH 5 PO 1 NS07464-25, NSFEPSCoR EHR9108775, and a VA Grant.

- A-35 Characterization of Thienyl-cyclohexyl-piperidine Binding to the Nicotinic Acetylcholine Receptor** O.R. Pagán, (1,2) C. M. Basilio, (1) R.M. Hann, (2) and V.A. Eterovic, (2)Dept. of Biochemistry-School of Medicine UPR(1) and Dept. of Biochemistry and Center for Molecular & Behavioral Neuroscience UCC(2)

Thienyl-cyclohexyl-piperidine (TCP), an analog of the hallucinogenic drug phencyclidine (PCP), is also a non-competitive inhibitor (nCI) of the nicotinic acetylcholine receptor (nAChR). Characterization of the binding to this receptor has not, however, been reported. This work describes the binding properties of [3H]-TCP to nAChR-rich membranes isolated from *Torpedo californica*. Using the filtration assay, it was found that [3H]-TCP binds, preferentially, to the receptor in its desensitized state, achieving maximum binding within 15 min. From a saturation curve, an affinity constant of  $236 \pm 28$  was calculated. This constant is slightly lower than the one reported for PCP. A series of known nCI of the nAChR were tested for their ability to inhibit [3H]-TCP binding. These included PCP, benzoTCP, procaine, lidocaine, tetracaine, cocaine, chlorpromazine, quinacrine, and isodihydro-histronicotoxine. Six Gorgonian cembranoids analogs were also tested. All these compounds inhibited [3H]-TCP binding in a concentration-dependent manner. Inhibition experiments were also performed with alcohols and anesthetic agents. Short-chain n-alkanols (1-5 carbons) did not significantly inhibit [3H]-TCP binding, while longer chain alcohols did inhibit. Cycloheptane-methanol, cyclohexane-methanol, and propofol were also capable of inhibiting [3H]-TCP binding. These experiments provide evidence that TCP, like PCP, binds to the receptor's ion channel with high affinity. Since PCP is no longer commercially available, TCP may prove to be as useful as PCP in interaction studies of anesthetic agents with nAChR. Supported by grants NIH-RO1-GM52277, NIH-MBRS SO6GM50695 and NIH-RCMI-G12RR03035

- A-36 Effect of N-glycosylation Inhibition on Endothelial Cell Proliferation and Viability.** J A. Martinez and D K. Banerjee. Department of Biochemistry, University of Puerto Rico School of Medicine, San Juan, PR 00936-5067, USA.

Proliferation of endothelial cells is fundamental for tumor angiogenesis and wound healing. We have shown earlier that treatment of a bovine endothelial cell line with tunicamycin (TM), a potent N-glycosylation inhibitor, or with amphomycin, an inhibitor of oligosaccharide-lipid biosynthesis, inhibited cell proliferation *in vitro* by 300% and 159% respectively. In the present work we examined the effects of short and long term inhibition of N-glycosylation on endothelial cell proliferation and viability in synchronized cultures. Exposure to various TM concentrations (10 ng/ml to 10 µg/ml) for 24 hrs had no effect on cell viability and proliferation. Longer exposure time (2-4 days) inhibited cell proliferation and viability in a dose-dependent manner. This effect was reversible depending on dose and time of exposure. Continued culturing after removal of TM exhibited differential effects. TM effect was irreversible in cultures exposed to 10 µg/ml for 48 hrs or longer. The effect was reversible in cells treated with 1 µg/ml or less, up to 48 hrs. This suggests that the threshold for recovery from this TM exposure was between 48 and 72 hour exposure at 1 µg/ml. A second doubling in cell population was not observed during the recovery phase in TM treated cells. Instead there was a significant decrease in cell viability after 5 days, suggesting that there might be a long-term or cumulative effect of the exposure to TM. Cell cycle analysis by flow cytometry showed an arrest in G0/G1 during the exposure phase but normal progression during the recovery phase. Short pulses (<24 hrs) of 10 µg/ml TM had no effect on cell viability, suggesting that cells have a pool of N-glycosylated proteins essential for cell proliferation, that is neither affected by TM nor depleted in 24 hours. Supported by the CIDIC grant.

**A-37 Pharmaceutical Care and Management of Cancer Pain.** L. Hernández. School of University of Puerto Rico.

Pharmaceutical care has been adopted as the philosophy of practice of the School of Pharmacy. The objectives of the study are to provide pharmacy students the opportunity to practice pharmaceutical care, and to implement published guidelines for the management of cancer pain. Pharmacy students enrolled in the Oncology Clerkship provided pharmaceutical care to 35 cancer patients with advanced disease. Activities included the assessment of pain intensity and patients' educational needs, identification of drug related problems, intervention with patients and physicians to solve problems, and documentation of processes and outcomes measures. Thirty three patients had pain due to their disease with an average "worst pain" intensity of 5.5 on a 0-10 scale. Drug related problems were: need of pharmacotherapy, 18.2%; taking the wrong drug, 9.1%; taking too little of the correct drug, 24.2%; experiencing an adverse drug reaction, 12%; and not taking the prescribed drug, 3%. Impact measures of pharmaceutical services were: reduction of pain, increased knowledge about therapy, improved compliance, improved physician prescribing, and change in cost of medication. Students evaluated the clerkship favorably. This study provides data that supports the feasibility of a randomized trial to further study the impact of pharmaceutical care on the management of cancer pain in Hispanics. Outcomes evaluation should include clinical, humanistic, and economic measures.

**A-38 Riesgos Ocupacionales en la Investigacion con Primates no-humanos.** J. Rodriguez Rodriguez, P. Nieves y M. Kessler. Caribbean Primate Research Center, University of Puerto Rico, Medical Sciences Campus, Sabana Seca, PR 00952-1053;

Esta presentación resume brevemente los riesgos ocupacionales a los cuales se enfrentan los investigadores, tecnólogos y estudiantes al trabajar con primates no-humanos, especialmente monos Rhesus (*Macaca*

*mulatta*). Se presentara las enfermedades mas comunes en poblaciones de monos rhesus incluyendo los del Caribbean Primate Research Center. Se enfatizara en los metodos de prevención, recomendaciones y personal o lugar a referir los diferentes casos. Se presentaran temas como Herpes B, Herpes simplex, Tuberculosis, Hepatitis B, E, Retrovirus (SIV), Rabia y Tetano.

**A-39 Body Weight and Primigravidity in Rhesus Macaques.** F.B. Bercovitch<sup>1</sup> and M. J. Kessler<sup>1,2</sup> <sup>1</sup>Caribbean Primate Research Center, University of Puerto Rico, Medical Sciences Campus, Sabana Seca, PR 00952-1053; <sup>2</sup>Department of Pathology, University of Puerto Rico, School of Medicine, San Juan, PR 00936-5067.

Menarche and onset of ovarian function have been linked to surpassing a minimum body weight/fat threshold, but the relationship of body size to first conception is rarely examined. We conducted a longitudinal investigation of rhesus macaques, *Macaca mulatta*, to determine the effect of differences in body size on age at first conception and survivorship of first progeny. Young females who became pregnant weighed significantly more than their peers who remained barren. Infant mortality among primiparous females was not related to differences in their body weight. First born males tended to have lower survivorship than first born female offspring. We suggest that reproductive consequences of first birth are more likely to be modulated by immature neuroendocrine function than by inexperience, small body size, or nursing patterns. We conclude that body size influences probability of first conception, but that socioendocrine factors mediate the likelihood of infant survival.

**A-40 Organ specific scaling as a determinant of inter specific variation in body mass variability.** B.

Hallgrimsson, Dept. of Anatomy, University of Puerto Rico, San Juan, PR 00936-5067. Body size, its variability, and their ecological corre-

lates have been important topics throughout the history of evolutionary inquiry. Yet, the question of whether there is a general relationship between size and size-variability has not previously been addressed. Through an analysis of body-mass and length measurements from 65,074 individuals from 351 mammalian species, we show that size-relative variability increases significantly with mean species body size. Analysis of mean body mass and standard deviations for 237 species of birds revealed the same pattern. I present 3 plausible alternatives explanations and eliminate several others. Of these, the hypothesis that the decrease in variability with decreasing mean body mass is related to the scaling of body mass components is most strongly supported. In effect, larger mammals and birds are more variable because their body mass is composed to greater relative degree of components with higher intrinsic variability (bone, fat, and muscle). In contrast, smaller mammals and birds have lower body mass variability because they are composed to a greater relative extent of components (brain and viscera) in which size variation is more highly constrained by energetic and functional factors.

**A-41 Head and Trunk Stabilization and Spatial Orientation in Primates.**  
D.C. Dunbar. Dept. of Anatomy and Caribbean Primate Research Center, Univ. of Puerto Rico Med. Sch., San Juan, PR 00936.

In the previous UPR-MSR Research Forum (Dunbar & Badam, 1997), data were presented on the movement patterns of body segments in hanuman langurs (*Presbytis entellus*). In this monkey species, either the head or trunk is rotationally stabilized relative to absolute space during natural locomotion. It was suggested that this stabilization is necessary to provide the nervous system with a reference frame for how the body is oriented in space. However, is segmental stabilization a general phenomenon across primate species? To address this question, the natural walking and galloping behaviors of wild bonnet macaque monkeys (*Macaca radiata*) and the leaping behaviors of captive prosimian sifakas (*Propithecus tattersalli*) were cinefilmed at high-speed (100

frames/s). Kinematic analysis focused on angular movements of the head and trunk relative to absolute space (earth-horizontal reference). As with *P. entellus*, the trunk in the macaques was rotationally stabilized (~10°) in the sagittal (pitch) plane during walking, but the head was stabilized (<20°) during galloping. During sifaka leaps, both the head and trunk were stabilized (<20°). Thus, it appears that segmental stabilization is a general phenomenon among primates. Supported by the Am. Inst. of Indian Studies, Nat. Geo. Soc., RCMI Award RR-03051, and the Assoc. Deanship of Biomed. Sci., UPR Medical School.

**A-42 60 Years of Rhesus Research: The Legacy of Cayo Santiago and its Monkeys.** M. J. Kessler<sup>1,2</sup>, J.D. Berard,<sup>1</sup> F.B. Bercovitch,<sup>1</sup> J. Gonzalez Martinez,<sup>1</sup> P. Nieves,<sup>1</sup> J Rodriguez Rodriguez<sup>1</sup> and J.E. Turnquist.<sup>3,1</sup>  
<sup>1</sup>Caribbean Primate Research Center, University of Puerto Rico, Medical Sciences Campus, Sabana Seca, PR 00952-1053; <sup>2</sup>Department of Pathology, and <sup>3</sup>Department of Anatomy, University of Puerto Rico, School of Medicine, San Juan, PR 00936-5067.

This audiovisual presentation traces the history of the establishment of the world's first free-ranging colony of rhesus monkeys (*Macaca mulatta*) on Cayo Santiago in 1938 and the use of these animals over the past six decades for behavioral, biological and biomedical research. These investigations have significantly contributed to scientific knowledge, particularly in the study of nonhuman primates as models for human behavior, biology and pathology. Highlights of this presentation include Cayo Santiago "firsts" and an overview of 60 years of research on social, maternal, locomotor, reproductive, vocal communication and cognitive behavior; demographics; genetics including blood group, enzyme, DNA fingerprinting and PCR marker typing for paternity testing and development of pedigrees; naturally-occurring infectious and non-infectious diseases and hereditary conditions including genetic marker probing; and skeletal biology and pathology.

**A-43 Muscle Strength and Hormonal Levels in Adolescents: Gender Related Differences.** Ramos E., Frontera W., Llopart A., Feliciano D. Department of Physical Medicine, Rehabilitation, and Sports Medicine, University of Puerto Rico School of Medicine.

The purpose of the present investigations was to study muscle strength in adolescents and its relationship to serum levels of testosterone and growth hormone in both genders. Thirty active adolescents (15 boys; age range 11-12 y/o) participated in the first study. Isokinetic muscle strength of the dominant knee extensors (KE) was determined at 0, 12, 30, 60, 120, 180, and 240 deg/sec using a Cybex 340 dynamometer. The assessment of pubertal status was accomplished using the criteria of Tanner. Serum levels of total testosterone (T) and growth hormone (GH) were determined using radioimmunoassay techniques. Boys had higher ( $p < 0.001$ ) T levels but no differences in muscle strength were detected between genders. Fifty-seven additional subjects representing three age groups (11-12 y/o,  $n=18$ , 13-14,  $n=21$ ; 17-18,  $n=18$ ) participated in the second study. A significant increase in peak torque (absolute and corrected for body weight) with age was observed in both genders. There were no significant gender differences in strength for the two youngest age groups, but boys were stronger than girls in the oldest age group (group 3). Testosterone and GH levels increased with age in boys but not in girls. Gender related differences in T were found in groups 2 and 3. A positive correlation ( $r=0.64$  boys;  $r=0.46$  girls) between testosterone levels and absolute muscle strength was seen in both genders. Our results suggest that increases in anabolic hormones precede muscle strength gains in adolescents. In addition, gender related differences in muscle strength during adolescence cannot be explained solely on the basis of differences in body size or T levels.

**A-44 Development and Communicative Profiles of Children Enrolled in a Preschool Program for the Deaf.** N. Díaz, M. Laguna, UPR-MSI. Echegaray, UPR.

The Developmental Auditory Profile Form (DAPF) was developed as the first phase of a

study about kinesthetic aspects in non-verbal communication of deaf preschool children. The DAPF is used to obtain preliminary information about the hearing status of the child, history of the hearing problem, demographic information, medical history and developmental characteristics beginning with prenatal events, including modes of communication used within the family context. This profile form has been answered by five families, four of which include at least one child enrolled in a preschool program for deaf children in Rio Piedras. One family includes a pair of twin boys, both of whom are deaf. One of the families has two children with hearing loss of various degrees. All of the children communicate by means of a combination of signs, gestures and occasionally, speech. This presentation discusses the structure and findings using the DAPF and observations of the family settings during the interviews. Of the participating families only one lives in the Metropolitan Area. The information provided by the mothers suggests that while the children's hearing residual is minimal and the knowledge and use of sign language by the parents is scarce, adequate communication is established by family members. Funded by JARFE.

**A-45 Prevalencia de Obesidad e Hipertensión en niños entre 3 a 16 años de dos Centros Primarios** Campos, M.; González, M. R.; Burgos, R. y Rodríguez, E.; Depto. de Pediatría, Escuela de Medicina, UPR P. O. B.365067, P. R. 00936

La prevalencia de obesidad en niños latinos en Estados Unidos es de 14% y la población puertorriqueña se describe como con mayor prevalencia aunque no tenemos estadísticas. Los objetivos principales de nuestro estudio eran determinar la prevalencia de obesidad en los niños entre las edades de tres a 16 años de los centros de pediatría primaria de Cataño y Canóvanas; y determinar la relación de la hipertensión con la obesidad, y patrones de alimentación. Se encuestaron los tutores de 81 niños y se tomaron medidas de peso, estatura y presión arterial. Encontramos que la obesidad es prevalente en los niños de la muestra

estudiada. La prevalencia de la obesidad de la muestra total fue de 18.5 %; en los niños de Cataño la prevalencia fue 15 % y en los de Canóvanas 21.95%. La prevalencia de presión alta en toda la muestra fue de 4.1 %, con una prevalencia de normal alta de 2.7 %. No se observó una asociación estadísticamente significativa entre la obesidad y la hipertensión. Esto concluye que a pesar de una alta prevalencia de obesidad en los niños de la muestra estudiada, ésta no se ve asociada con elevación en la presión arterial ni con los patrones de alimentación. En base a ello recomendamos que se haga cernimiento poblacional para poder, en base a los resultados, educar a la comunidad y mejorar su estado de salud.

**A-46 The DAP is a Measure of Treatment Outcome in a Group of Puerto Rican Children.** H De Jesús PhD; B Mirabal-Colón, R Dávila,MS;I García, MS

Intended Audience: Multidisciplinary Child abuse is a major socioeconomic problem in Puerto Rico. In 1995-96, there were 50,000 active child protection cases in the PR Department of Family Services. There has been a surge of new programs directed towards child abuse evaluation and family preservation services. However, specialized treatment programs for these victims are not readily available. In October 1996, the Biopsychosocial Program, Dept. of Pediatrics, University of PR, School of Medicine developed the Therapy Center for Victims of Family Violence (Sponsored by the PR Justice Dept., with federal funds for Victims of Crime). This center provides group therapy to children 4-15 years, who have experienced family violence (negligence, physical, emotional &/ or sexual abuse), who present behavioral problems requiring treatment. The primary intervention consists of 10 sessions of abuse-focused group therapy within a cognitive/structured model. Parallel educational parenting sessions are held with the non-offending parents. Prior to and at the end of the intervention, the Draw a Person: Screening Procedure for Emotional Disturbance (DAP:SPED) is administered to each child, as a tool to evaluate progress, in addition to a clinical interview and

other instruments. This report contains the preliminary DAP:SPED results of 19 Puerto Rican Children who have completed the program. Twenty-nine children started the intervention: 19 children completed the program. There were 8 females' (42%) and 11 males (58%) with a median age of  $9.0 \pm 1.8$ . Most (78.9%) were medically indigent. Most of the children (68.4%) were in child protection and placed with relatives prior to the intervention. Three children had a diagnosis of ADD; two were on oral medication. One child required concomitant individual psychotherapy. The initial median total score (T Score) was  $59 \pm 14.1$ , suggesting evaluation for emotional disturbance was indicated. The final median T Score was  $54 \pm 10.96$ , when no evaluation was indicated. The change was not statistically significant. For statistical purpose, the age of the population was dichotomized; eight years or less ( $n=8$ ) and nine years and older ( $n=11$ ). The results show that children eight years or younger had a higher initial T Score (more emotional indicators) than older children ( $p$  value=0.02). This tendency was observed in the initial Self-drawing ( $p$  value=0.02) and also in the Woman Figure drawing in the pre and posttests, although of marginal significance ( $P=0.06$ ; 0.08). These findings suggest that the DAP: SPED is a useful screening tool, as a part of a thorough clinical evaluation of abused children. These are preliminary results and standardization of the DAP in this Puerto Rican population should be performed. Further study is needed to test its usefulness in measuring treatment outcome.

**A-47 Contribution of the School Lunch Program to Satisfaction of Nutritional Requirements: A Pilot Study from Three Types of Schools.** C. Rodríguez, I. Vargas, I. Gómez and A.M. Preston. Dept. of Biochemistry, UPR, MSC and Sch. Food Authority, PR Dept. Public Instruction, San Juan, PR 00936.

The Federally-sponsored School Lunch Program (SLP) has been designed to provide 1/3 the daily requirement (RDA's) for several nutrients as well as satisfy important Dietary Guidelines (DG) such as keeping fat below 30%

of total calories, saturated fat below 10% and cholesterol and sodium at minimal levels. To monitor use of this program in Puerto Rican schools, we have made a survey of 193 students selected from 3 types of schools: elementary, intermediate and high school being 17, 18 and 65% of the total sample respectively. An institutionally-approved 24 hr dietary recall questionnaire was administered by trained personnel and analyzed for nutrient content via the Minnesota Nutrition Data System. It was found that 58% of the students participated in the SLP, 33% brought their lunch from home and 9% skipped lunch entirely. Rate of participation by type of school was: elementary 72%, intermediate 34% and high school 60%. Both participants and non-participants consumed acceptable amounts of sodium and cholesterol but lunches exceeded 30% fat and 10% saturated fat. For RDA's participants in the SLP consumed higher amount of calcium, iron, vitamin A and vitamin E than non participants. Nutrient content of other meals eaten during the day was not different between participants and non-participants and was much lower in nutritional quality than lunches. In conclusion, it appears that in our sample of Puerto Rican school children, lunch is the "most important meal of the day" in supplying good nutrition and that participants in the SLP achieve maximum nutritional benefit. Sponsored by CIDIC, UPR Medical Sciences Campus.

**A-48      Developmental Lag and Facial Expression of Emotion: Collateral Indexes in Evaluation and Intervention.** I. Echegaray, UPR.

During the last four years several pilot studies have provided the necessary background to view facial expression of emotion as an index of complex brain information handling processes. Within this context the paper reviews the results of studies with Puerto Rican children which point to three major findings from our ongoing research: (1) Facial expression of emotion in pre-school and early school developmental ages can be considered an important component of developmental lag assessment. (2) Discrepancies in facial expression of each hemiface can be used as an additional index

associated to shortcomings in specific developmental tasks. (3) The development of valid and culturally relevant norms for language impaired and autistic children can incorporate these additional measures as an important component of developmental gain and therapeutic intervention impact. The work reported stems from a Physiological Psychology perspective. It is specifically geared to the needs of medical, health and education professionals in the pursue of collateral indexes to assess brain development, developmental lag and development of social, communicative skills and intervention with children meeting criteria for pervasive developmental disorder diagnosis. Funded by FIPI, UPR-Rio Piedras in collaboration with the Infantile Autism Project, UPR-Medical Science Campus.

**A-49      The Effectiveness of a Training Program in Interdisciplinary Rehabilitation Services.** D. Irizarry, M. Marrero. Office of Academic Affairs, College of Health Related Professions, Medical Sciences Campus, University of Puerto Rico.

The purpose of this project is to offer a certificate training program in interdisciplinary rehabilitation services to public employees providing services to individuals with physical, cognitive and psychosocial disabilities at the Administration for Vocational Rehabilitation. The goal of the program is to enhance the knowledge, skills and attitudes of participants who render services to these populations in order to improve the quality of services provided for their rehabilitation. This academic offering is the first interdisciplinary and collaborative effort between the UPR and a government agency to fulfill the training needs of employees providing services to individuals with various kinds of disabilities. Two groups of participants were selected for the two-phase project. The first group was composed of employees with high school or associate degrees; and the second group is composed of employees with bachelor's or master's degrees. Thirty-one participants finished the first phase of the seven months training program. The curriculum emphasizes attitudinal changes toward

consumers, and comprises topics in human development, health issues, laws and regulations, values and ethics; general intervention strategies and practice experiences. The project is supported with University of Puerto Rico and government funds. The program evaluation design is directed towards learning outcomes in participants as well as impact to the consumer and to the services provided. The results of the first phase of the project evaluation shows the effectiveness of the curriculum in meetings the learning and attitudinal goals established.

- A-50 The Developmental Skills of Very Young Typical Puerto Rican Children.** N. Linares, B. López, M. Santana, V. Tapia. Speech-Language Pathology Program, Graduate Programs Department, College of Health Related Professions, Medical Sciences Campus, University of Puerto Rico.

Health professionals who serve Hispanic children with disabilities need normative data on which to base their clinical decisions. There are few Puerto Rico studies on typical island preschoolers' motor, speech-language, social, cognitive, hearing and self-help skills. This interferes with pediatricians, psychologists, speech-language pathologists, occupational therapists, and physical therapists in service to determine the functional level of children referred for evaluation and therapy. This data scarcity hinders assessments of what is and what is not normal in these children. To address this informational lag we conducted a study of eight normal middle SES Puerto Rican children (4 girls, 4 boys), 2;9 to 3;0 years of age. Their normality level was determined by observational assessments by their day care personnel, and graduate students in communicative disorders. Subjects were being raised at home and at an early childhood services center. Children were brought individually to a testing room where they interacted with the examiners and responded to verbal and non-verbal stimuli following a standard script to elicit developmental skills with motor and linguistic responses. All subject reactions were audio or videotaped for posterior analyses. Subjects spent 40 to 55 minutes in this elicitation. Re-

sults indicate that, among other accomplishments, these preschoolers can imitate sounds, walk on a straight line, blow soap bubbles, wash and dry their hands without assistance, pair and use objects correctly, express 2-5 words phrases, and participate alone in simple activities with strangers.

- A-51 The PBL Case as a Behavioral Stimulus.** Philip C. Specht, Ph.D., Pharmacology Dept., UPR School of Medicine, San Juan, PR.

Problem-based learning (PBL) is an instructional methodology founded on the principles of learning psychology. The use of small discussion groups, the guidelines of student responsibilities, and the role of the faculty facilitator are all based on the premise that this structure will facilitate the learning, retention, and recall of content relevant to the profession. In addition, the structure is also intended to develop effective learning and thinking patterns, as well as skills involved in interpersonal relations. In this context, the case, which embodies the problem, can be considered to be a psychological stimulus intended by the faculty to evoke learning behaviors in the students. This perspective can be used in the process of case writing, to guide the selection of material to be presented, and to shape the order and pace of the presentation. It can be argued that the commonly observed neglect of basic science in the PBL discussions can be traced to the difficulty of finding effective stimuli to evoke interest in basic science issues.

- A-52 Systems Changes in Assistive Technology.** M. Miranda, President's Office, University of Puerto Rico.

The Puerto Rico Assistive Technology Project is an organization designed to change the systems for the provision of assistive technology for persons with disabilities in Puerto Rico. This presentation will focus on various approaches towards systems changes, which strategies have been successful and which have not. The approach for systems change takes into con-



sideration collaboration with different agencies and organizations, legislative action, the implementation of an information web and technology-oriented efforts. Outcomes obtained from this effort range from changes in local laws to the establishment of collaborative initiatives with government agencies, universities and organizations in the United States. Different approaches and the influence of the Project in the decision making process at all levels have proven to be important elements to take into consideration when attempting to change the service provision system for persons with disabilities in Puerto Rico.

**A-53 Team Work Among an Elementary Public School** A. Nuñez, M. Torres, C. Dávila, C. Román, L. Pérez Interdisciplinary Project of Excellence in Services, College of Health Related Professions

The Interdisciplinary Project of Excellence in Services is working with the Puerto Rico Department of Education, Special Education Program to develop a collaborative service model among health providers, special education teachers and families students with disabilities in a public school. In this model of the therapists are using different strategies to work with the students to develop functional skills. Clinicians are working together in co-therapies, group therapies or individual therapies according to students needs. They are also conducting natural environmental interventions. After six months of work in this Project the clinicians have developed administrative processes to facilitate student's referrals to the services and establish communication with children families.

**A-54 A Program for the Development of Basic Work skills for School Youth with Special Needs** C. Carle, M. Aponte, A. Font Interdisciplinary Project of Excellence in Services, CHRP

The Interdisciplinary Project of Excellence in Services provides multidisciplinary services for the special education population. These are referred by Puerto Rico Special Education Pro-

gram. One of the Project's component is group therapies for students 12 to 18 years old. The purpose is to strengthen basic skills for work. In this modality an occupational therapist and a speech-language pathologist guide the students through the process of developing, planning and undertaking functional activities related to money administration, decision making and organization skills. Students have improved leaderships skills and demonstrate better management of money during activities. Also, their social communication skills have improved during their daily activities.

**A-55 Impacto del Adiestramiento a Profesionales que Enseñan/ Trabajan con Niños y Jóvenes con Autismo** A. Ortiz, y Y. Jiménez / Proyecto Autismo Infantil CPRS - RCM

A través de los años ha quedado demostrado que los servicios y los profesionales que trabajan con la población con autismo en Puerto Rico son escasos. Muchos profesionales carecen de conocimiento actualizado sobre las nuevas tendencias y las filosofías educativas para esta población. Esta situación se agrava cuando pensamos que cada día más niños son diagnosticados con este desorden. Preocupados por este hecho, el Proyecto de Autismo Infantil, junto al Departamento de Educación de Puerto Rico, ha ofrecido talleres a través de la isla para orientar a profesionales. Los maestros adiestrados en estos talleres expresan que no ha habido un aumento significativo en la prestación de servicios y/o programas educativos a esta población. Se investigó las causas de esta situación. Se encontró que, entre otras cosas, la falta de materiales es una de las causas.

**A-56 Recomendaciones de Dentistas y Pediatras sobre el Cuidado de la Salud Bucodental en Pre-escolares** K. Crespo, J. Giusti, J. Rodríguez, G. Vélez (Escuela de Odontología, Universidad de Puerto Rico)

Aún cuando se ha logrado incrementar el uso de los servicios y mejorado la condición de salud oral de la población, es de preocupación que no se estimule una

mayor utilización de servicios entre los niños en edad pre-escolar. El propósito de esta investigación fue conocer el tipo de orientación que brindan los pediatras y dentistas de Puerto Rico sobre la necesidad de que los niños reciban cuidado dental en edades pre-escolares. Se envió un cuestionario a todos los dentistas generalistas y pediátricos, así como a los pediatras en Puerto Rico (N=1,946), para recopilar información acerca del tipo de orientación que éstos ofrecen a los padres de los niños pre-escolares, sobre el cuidado de la salud oral. Además, se deseaba conocer la edad recomendada para que el infante acuda por primera vez a un examen dental. Se obtuvo un 30 % de respuestas de los dentistas generalistas (n=315), 53% de los odontopediatras (n=33) y un 28% de los médicos pediatras (n=238). Se encontró que el 88% de los pediatras, 44% de los dentistas generalistas y 79% de los dentistas pediátricos encuestados, recomiendan algún complemento de flúor a sus pacientes pre-escolares. Cerca del 80% de los pediatras ofrecen información sobre los hábitos alimentarios que pueden afectar la salud bucal, hábitos de higiene oral y otros que pueden afectar la dentición. Más de un 80% de los dentistas generalistas pediátricos ofrecen este tipo de información a los niños(as) pre-escolares. El 28% de los dentistas generalistas y 24% de los odontopediatras recomiendan sellantes de fisura en dientes deciduos, mientras que un 60% y un 85%, respectivamente, recomiendan los mismos para los dientes permanentes. Se encontró que más pediatras recomiendan la primera visita al dentista entre 1 y 2 años de edad que los dentistas. Se recomienda que los dentistas pongan más énfasis en las protecciones específicas, tales como complementos de fluor y sellantes de fosas y fisuras. Además, se recomienda una comunicación más efectiva entre los dentistas y pediatras sobre el cuidado de la salud bucal de los pre-escolares.

**A-57**

**Prevalence of Caries and Dental Fluorosis in Twelve Year Olds in Puerto Rico.** A. Elías, K. Crespo, C. Gierbolini, J. Giusti, C. Toro. UPR School of Dentistry. (CIDIC)

Dental Fluorosis is defined as a hypoplasia of the enamel or dentin, classified according to its intensity. It has been shown that the ingestion of fluoride from different sources may cause dental Fluorosis. Fluoride has also been attributed with a 20 to 40 percent reduction in caries. The aims of this study were to assess the prevalence of dental caries and Fluorosis of twelve year old children in Puerto Rico. A probabilistic sample of schools stratified by eleven health regions, type of schools and grade profile, economic index and urban and rural location was de-

signed. In each region we planned to select with probability proportional to enrollment 5 public and 1 private, elementary and intermediate schools. In each school 25 twelve year old children were recruited. Clinical evaluation was conducted following National Institute of Dental Research methodology and criteria for DMFT and Dean Index for Fluorosis. A total of 1,443 subjects were evaluated from April to December 1997. The DMFT obtained was 3.74 (SD 3.45), considered moderate by World Health Organization (WHO) This index ranged from a high in the Mountain Region (5.0) to moderate in the west (3.06), San Juan (3.42), and Metropolitan Regions (3.43). These values are higher than the DMFT= 1.73 reported for USA in 1987. Of the total subjects evaluated 51.8 percent presented some type of Fluorosis. Of these subjects 48% were very mild, 3% moderate and 0.8% severe. The Community Fluorosis Index of 0.39 is not considered by NIDR as of public health significance. The teeth most affected by the condition were the maxillary first molars and the first bicuspid. A fluoridation program is recommended in order to reduce dental caries, however the prevalence of Fluorosis should be closely monitored, clinically and chemically. If a fluoride program is established, health professionals should be alerted towards the correct dosage if prescribing fluoride supplements.

**A-58**

**Developing Alliances for Interinstitutional Research, Education and Training: The ISIR Experience.** N. LINARES. Initiatives on Interdisciplinary (Re)habilitation Services, President's Office, University of Puerto Rico.

The complexity of current community socioeconomic and health problems calls for augmented interdisciplinary and interinstitutional collaboration in addressing minority needs. Latino, African-American, and Native-American individuals share many life conditions and have also unique difficulties. These situations require university professors, investigators, clinicians, and students to engage in coordinated actions with other higher education institutions and public and community services organizations to investigate, and propose new management, intervention, and assistance paradigms that are cost-effective in dealing with social, educational, rehabilitation, and health issues. The University of Puerto Rico's President Office is engaging in collaborative research and education ini-

tiatives with the National Institutes of Health, the REALAN alliance, and the Puerto Rico departments of Health, of Correction and Rehabilitation, of The Family, and of Education in improving the research capabilities of US minority individuals, in providing better health and rehabilitation services, and in preventing violent behaviors in children and youth from neighborhoods at-risk, To succeed in these endeavors we have developed new skills in team

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work, have learned to function as a virtual organization, have acquired linguistic and cultural abilities to compete in the US environment, have established community needs assessments methods, and have increased our expertise in obtaining external funds for related projects. An outcomes-oriented success evaluation will provide us data on what works in these alliances.

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**POSTER  
PRESENTATIONS**



**A-59 Long bone fractures in very low birth weight infants at birth: obstetrical considerations.** I. García MD\*, I García MD FAAP, A de la Vega MD FACOG. Departments of Pediatrics and Obstetrics and Gynecology, UPR School of Medicine, Medical Sciences Campus, San Juan, Puerto Rico

Fetal fractures of the long bones during obstetrical procedures are not common in the absence of fetal bone disease. The reported incidence is less than 1%. When obstetrical trauma is the etiology, the most common finding is difficult breech vaginal delivery. However, fractures have been reported in cephalic deliveries and cesarean sections (C/S). Although macrosomia has been implicated in birth trauma, we have identified fractures in very low birth weight infants (VLBW) born by C/S. A vertical midline incision for C/S is the preferred method for easing extraction of these infants in breech position in order to provide ample space for atraumatic delivery. However, many obstetricians may perform low transverse incisions due to ease of repair, less bleeding, and lower risk of future uterine rupture. We report four cases of VLBW infants presenting long bone fractures at birth. **Methods:** Medical records and x-rays from four infants admitted to our institution during a one year period who presented fractures at birth were reviewed. Possible risk factors including type of delivery, uterine incision for C/S, presence of oligohydramnios, fetal presentation, fetal distress, APGAR score, birth weight, and presence of metabolic or bone disease were evaluated. **Results:** There were three patients with femoral fractures born at our institution by C/S in breech position among 750 C/S performed during that year. Another patient presenting a humeral fracture was born at another institution by a traumatic abdominal delivery. Birth weights ranged from 850-970 grams. All patients had low APGAR scores at 1 and 5 minutes of birth. In two patients there was history of oligohydramnios and in three cases fetal distress was identified. All had serum calcium, phosphorus, and alkaline phosphatase levels in the normal range. The x-rays were reviewed and all fractures were considered acute although in one case there was the possibility of an intrauterine origin. **Conclusions:** Fractures

of the long bones occurring at birth are usually associated to maneuvers employed during difficult breech vaginal deliveries. This maneuvers are the same for abdominal extractions. The common finding of a low transverse incision in our series points towards a possible association. The lower uterine segment may be underdeveloped and small in preterm pregnancies and therefore not provide ample space for atraumatic delivery. A vertical incision is indicated in almost all of these cases.

**A-60 Congenital Diaphragmatic Hernia and Endocardial Fibroelastosis in a Newborn.** A González\*, C Concepción, A Espinosa, A de la Vega, H Martínez. Department of Pediatrics: Neonatology and Cardiology Section. Department of Obstetrics and Gynecology. Department of Pathology. University of Puerto Rico, School of Medicine.

Congenital Diaphragmatic Hernia ( CDH ) is a common major anomaly frequently diagnosed during the prenatal period. Patients with CDH have an increased incidence ( 40% - 57%) of others malformations. Associated anomalies like ventricular septal defect, coarctation of the aorta, trisomy (13, 18, 21), myelomeningocele, and hydrocephalus have been well documented. Reports of CDH associated to endocardial fibroelastosis (EFE) has not been found in the literature review. EFE is characterized by abnormal thickening of the endocardium of one or both ventricles. Is a rare condition with an incidence of 1 out of 5000 - 6000 births and is usually diagnosed at autopsy. We report a term male infant born to a 21 y/o G1POAO female after 39 1/7 weeks gestation with a prenatal sonogram, 2 days prior to delivery, showing left ventricle enlargement with decreased contractility and thickened ventricles walls. He was born via vaginal delivery, required orotracheal intubation and was transferred to the neonatal intensive care unit. The Apgar score was 1/0/3 at 1 min, 5 min and 10 min respectively and birth weight 3500 grams. Physical examination was significant for an scaphoid abdomen, no dysmorphic features were noticed. Chest x ray was compatible with left diaphragmatic hernia and mild cardiomegaly. An

echocardiogram was done and revealed severe left ventricular dysfunction, severe mitral regurgitation, and pulmonary hypertension. Patient's condition continued deteriorating, developing cardiorespiratory arrest at 48 hours of age with no response to advanced CPR. Autopsy revealed left diaphragmatic hernia with pulmonary hypoplasia and endocardial fibroelastosis. In summary, we present a newborn with two major malformations not previously associated, both with a high mortality rate. The clinical implication is serious due to poor prognosis and complicated management. Careful prenatal heart evaluation is recommended in patients with the diagnosis of CDH.

- A-61 Increased Incidence of Fungal Infections in Premature Infants less than 1,750 grams.** J Rivera MD\*, I García MD, M Valcarcel MD. Department of Pediatrics, Neonatology Section, University of Puerto Rico, Medical Sciences Campus.

**Background:** Premature newborn patients are at risk of disseminated fungal infections due to prolonged hospitalizations, weak immune defenses, treatment with broad spectrum antimicrobials, use of intralipids and central catheters while admitted to an intensive care unit. Bayley et al, reported an overall 3.8% incidence of systemic fungal infection with mean age of infection being 33 days, in very low birth weight infants admitted to an intensive care unit. Preliminary data from 1996 in the University Pediatric Hospital revealed a higher than expected incidence of early-onset disseminated fungal infection in premature newborns admitted to the neonatal units, consistent with reports in literature. **Methods:** Retrospective review of all positive cultures for fungi (reported by Infectious Diseases Service) during 1996 was done. No surface cultures were included. Approximately 200 premature newborns 1,750 grams or less are admitted per year to the University Pediatric Hospital neonatal services. Time of onset, site of sampling, reported organism and birth weight were assessed. **Results:** A total of 46 positive cultures for fungi were obtained from newborn infants with birth weight of 1,750 grams or less representing at least 23% (46

cultures/200 admissions) positive fungal cultures in the overall 1,750 grams population. Mean age detection was 29 days. In this population, at least 6.5% (13/200) of all premature newborns less than 1,750 grams had a positive culture related to disease detected during the first 15 days of hospitalization. Organisms isolated included *Candida parapsilosis*, *Candida albicans* and *Candida tropicalis*. **Conclusion:** A higher than expected incidence of early-onset fungal infections in premature newborns was found in our population. In view of increasing incidence of this condition, it is important to assess the incidence of intrapartum candidiasis and identify predisposing factors that can make a premature fetus or newborn prone to congenital candidiasis or early-onset disease.

- A-62 Epitopes Cross-Reactivity between DP and BT Detected by Rabbit Polyclonal IgG.** C. Mora\*, F. Montealegre, A.M. Díaz, School of Medicine, UPR, Ponce School of Medicine.

Cross-reactivity between mites is a subject of great importance for diagnosis of allergy and hyposensitization. In order to define shared and unique allergens from the mites *Dermatophagoides pteronyssinus* (DP) and *Blomia tropicalis* (BT), rabbits were immunized with body extracts of each mite using a long term immunization schedule. The antisera titers were monitored by ELISA, showing cross-reactivity. The IgG fraction from each serum was purified by affinity chromatography, and the reactivity was tested by Western blot. Three antigens of cross-reactivity were detected. The presence of these antigens can be responsible of cross-sensitization between house dust mites and storage mites.

- A-63 Development of Dengue Envelope Expression Vector for Dna Immunization.** Colón.C., Martínez, I., Vorndam. V., Beltrán, M., Vergne, E., and Kraiselburd, E. Department of Microbiology & Medical Zoology, School of Medicine, P.O. Box 365067. Public Health Service, Center for Disease



Control and Prevention, Division of Vector-Borne Infectious Diseases, Dengue Branch San Juan, Puerto Rico 00921-3200.

Dengue viruses belong to the flavivirus genus of the family Flaviviridae. These viruses consist of four antigenically different serotypes, known as Den 1-4. The primary vector of transmission is the mosquito *Aedes aegypti*. Dengue is the major cause of epidemics throughout the tropical and subtropical regions of the world. It has been estimated that annually over one hundred million cases of Dengue fever occur. The dengue fever is a debilitating illness that could result in a hemorrhagic fever and shock syndrome. Despite many years of research effort, vaccine development still remains at the experimental stage. DNA vaccination has come to be the preferred means of eliciting antigen-specific immunity against various pathogens. The effort of this work is to develop a DNA vaccine against all four dengue serotypes, and specifically against type-2 virus. Toward this aim the envelope (E), and PreM genes of New Guinea Dengue 2 were PCR amplified and cloned into the plasmid expression vector (pJW4304). The resulting plasmid construct (Vec D-2) was transfected into Cos cells by electroporation and tested for envelope expression *in vitro*. The E protein was expressed by Vec D-2 as evidenced by Immunofluorescence (IFA) using dengue-specific monoclonal antibodies. To test the *in vivo* expression of Vec D-2, a pilot experiment was performed using a group of 8 Balb/c mice. Three six weeks olds mice were inoculated intramuscularly with 100µg of Vec D-2 (group 1). Two mice received a combination of Vec D-2 and a plasmid expressing GMCSF (group 2). Controls consisted of mice inoculated either with pJW4304 (n=1), with PBS (n=1) or with the GMCSF plasmid alone (n=2). Mice were primed on day 0, and boosted on weeks 6,12,14. Blood samples were obtained through the retro orbital plexus route before each inoculation. Serum samples were serially diluted and examined for Den antibodies by indirect IFA, performed using mosquitoes cells (C636 cells) infected with Den-2. Positive sera were also assayed for Den-2 virus neutralizing antibody in a virus plaque reduction neutralization test (PRNT). All animals from

group 2 and 1/3 group 1 mice developed dengue-2 antibodies detected by IFA. The neutralizing antibody titers developed by group 2 ranged from 1:80 to 1:160. These results showed that administration of Vec D-2 plus GMCSF expression vectors resulted in the induction of dengue-specific neutralizing antibodies in inoculated mice. Thus, Vec D-2 was immunogenic and was able to elicit protective immune responses against dengue-2 virus.

**Geographical Distribution of *Angiostrongylus cantonensis* in Puerto Rico**  
A64 R. Wiscovitch, C. Maldonado and W. J. Kozek. Department of Microbiology & Medical Zoology, School of Medicine and Department of Environmental Health, School of Public Health, Medical Sciences Campus, U.P.R., San Juan, P.R. Rec Juan, P.R.

Recent studies have indicated that *Angiostrongylus cantonensis*, a zoonotic parasitic nematode which was believed to be restricted to South East Asia, the Pacific region, and Africa, has been detected in several Caribbean islands, including Cuba, Bahamas, Hispaniola, Puerto Rico, and in the U.S. in Louisiana. To determine the geographical distribution of this nematode and other zoonotic helminths in Puerto Rico, a total of 162 rats were trapped in different areas of the Island and examined for *A. cantonensis* and other helminths. Rats captured in the following areas harbored *A. cantonensis*: San Juan, Río Piedras, Guaynabo, Toa Baja, Arecibo, Mayagüez, Ponce, Fajardo, Trujillo Alto, Humacao, Cayey, Adjuntas, Maricao and Peñuelas, but those from Caguas, Cayo Santiago and Peñuelas did not harbor this parasite. Highest rates of infection with *A. cantonensis* were found in rats from Humacao. Other helminths detected which have zoonotic potential include *Capillaria hepatica*, *Hymenolepis diminuta* and *Moniliformis moniliformis*. Although the number of rats captured at each site was relatively small, the results obtained indicate that *A. cantonensis* is enzootic or only in the coastal areas of Puerto Rico, but is also well established in inland areas. Its apparent absence from Puerto Rico in 1960s suggests that it may have been introduced into the Caribbean relatively recently, and the local environmental factors favor its rapid extension throughout this region. Since *A. Cantonensis* and the other zoonotic helminths harbored by rats pose a health hazard to humans, further studies will be conducted to define the enzooticity and the geographical extension of these organisms in the Caribbean.

- A-65 Examination of Nematode Larvae by Soft X-ray Microscopy** W. J. Kozek, J. Brown, W. Meyer-Ilse, C. Larabell and M. Moronne, Department of Microbiology and Medical Zoology, Medical Sciences Campus, U.P.R. San Juan; Center for X Ray Optics, Advanced Light Source, and Innovative Microscopies Group, Life Sciences Division, E.O. Lawrence Berkeley National Laboratory, Berkeley, CA.

Application of soft X-ray microscopy to study protozoa prompts us to test the efficacy of this technique on small metazoa. Glutaraldehyde-fixed microfilariae of *Dirofilaria immitis*, the dog heartworm, and the newborn larvae of *Trichinella spiralis*, were examined with the XM-1 X-ray microscope at the ALS Berkeley National Laboratory, and by transmission and scanning electron microscopy. X-ray microscopy allowed visualization of the general structure and internal organization of both larvae, clearly showing the cuticle and body wall, nerve ring areas, components of the digestive tract, and special structures such as the excretory vesicle and cell of the microfilaria, and the genital primordium in *T. spiralis* larvae. In addition, it depicted the general shape of cells, their arrangement and interconnections, which is difficult to demonstrate, in very small metazoa, by other microscopy techniques. These preliminary results indicate that X-ray microscopy is a very useful technique to examine not only study of other types of biological specimens. Further refinement of this technique will make it extremely effective in providing new information about the structure and function of biological organisms, especially when used in conjunction with other electron microscopy techniques. (Supported by the Director, Office of Energy Research, OBES, MSD, US DOE contract No. DE-AC03-76SF0098, and by 'RCMI' Award RR-03051, from NCRR, NIH.)

- A-66 Localization of FMRFamide peptide and Neuropeptide Y in the anterior nervous system of *Dirofilaria immitis* (Nematoda, Filarioidea)** Deborah Nieves-Mendez\* and Wieslaw Kozek Microbiology and Medical Zoology Department, Medical Sciences Campus, University of P.R.

Neuropeptides identified to date in intestine-dwelling nematodes, e.g. *Ascaris* spp., include FMRFamide peptide and Neuropeptide Y (NPY). Since little is known about the peptidergic com-

pounds which occur in the nervous system of tissue-dwelling parasitic nematodes, this study was conducted to demonstrate the presence of FMRFamide and NPY peptides in the anterior main ganglia of *Dirofilaria immitis*, the heartworm of domestic dogs. Indirect immunofluorescence technique in conjunction with confocal scanning laser microscopy was used to identify FMRFamide and NPY-3 immunoreactive neurons in the anterior nerve system of *D. immitis* whole mount preparations previously fixed in Zamboni solution. Substitution of the primary antibody by Super Block solution was used as a control in all experiments. Strong immunofluorescence reaction to FMRFamide antiserum was observed in the region of the nerve ring and in nerve cells, which appear to be a part of the amphidial and ventral ganglia. Positive reaction to NPY-3 antiserum was detected in the lateral pharyngeal nerves, and in one of the minor pharyngeal commissures. These results suggest that the neurochemical components of the *D. immitis* nerve system include neuromodulators which belong to the FMRFamide and NPY families. This study is being continued to detect the presence of serotonin in the anterior nervous system of *D. immitis*.

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- A-67 COMPARISON OF THREE DIAGNOSTIC METHODS FOR MALARIA IN BUENAVENTURA, COLOMBIA** Gervais G<sup>3</sup>, Carrasquilla G<sup>1</sup>, Banguero M<sup>2</sup>, Sanchez P<sup>2</sup>, Algarin E<sup>3</sup>, Barker RH<sup>4</sup>, Serrano, AE<sup>3</sup> <sup>1</sup>FES Foundation, Cali Colombia; <sup>2</sup>Instituto de Salud del Pacifico (INSALPA), Buenaventura, Colombia; <sup>3</sup>UPR Medical School, Department of Microbiology and Medical Zoology, San Juan, PR; <sup>4</sup>Worcester Foundation, MA
- Buenaventura, located in the main port of the Colombian Pacific, is one of the few endemic

zones where urban malaria is reported. Surveys during the past few years have consistently shown malaria prevalence between 3.6% to 8.2% in urban and periurban areas, as measured by thick smear or DNA hybridization. The traditional method for diagnosis has been thick smears prepared from blood samples. Quantification of Buffy Coat (QBC) and the Polymerase Chain Reaction (PCR) have recently been introduced as alternate methods in malaria diagnosis. The purpose of this study was to compare the three methods in terms of sensitivity and ease of use given the facilities available in Buenaventura. The results show that prevalence of malaria was similar by QBC and thick smear, but it was significantly higher by PCR, although disagreements were found at low level parasitemias. Our study confirms previous studies that showed higher sensitivity for the PCR analysis method, which appears to be a method with convenient applications for the surveillance of malaria in the city of Buenaventura.

**A-68 Identification of *Leishmaniasis* transmission in Puerto Rico.** Algarin E<sup>1</sup>, Almodovar PI<sup>2</sup>, Ortiz B<sup>4</sup>, Duckett CN<sup>3</sup>, Pedreros F<sup>3</sup>, Gervais G<sup>1</sup>, and Serrano AE<sup>1</sup>. <sup>1</sup>UPR School of Medicine, Department of Microbiology and Medical Zoology; <sup>2</sup>UPR School of Medicine, Department of Dermatology; <sup>3</sup>UPR Department of Biology and <sup>4</sup>Caguas Veterinary Hospital.

This is the first report of *Leishmania* transmission in Puerto Rico, which is a non-endemic zone for this disease. A thorough review of the literature shows that, although imported cases have occurred occasionally, local transmission of veterinary leishmaniasis has not been reported. Biopsies taken from two equines which have never been out of Puerto Rico show numerous intracellular organisms with kinetoplastids, consistent with *Leishmania sp.* Parasites were successfully isolated from lesions in the equines, and are currently being maintained in culture. PCR analyses using *Leishmania*-specific primers give a positive reaction, which has been confirmed by Southern Blotting. Further work will pursue identi-

fication of the infecting parasite species by species-specific PCR-Southern Blot analyses, and by sequencing of kinetoplastid DNA, as well as carrying out an entomological survey of the area to identify possible vectors.

**A-69 Promoting Regeneration of Peripheral Nerves Across Long Gaps In Vitro.** A. Rivalta, F. Quiles & D.P. Kuffler. Inst. of Neurobiology and Dept. of Physiology, Medical Sciences Campus, UPR, 201 Blvd. del Valle, San Juan, Puerto Rico.

Peripheral axons are capable of regenerating across short gaps in their pathways. Various methods have been developed in attempts to improve this regeneration *in vivo*. In general it has been assumed that the maximum distance across which a nerve could grow in a silicon chamber connecting the central and distal nerve stumps was 1 cm. In our experiments we have been testing the influence of gelatin, and extracellular matrix components inside the chambers on axon regeneration. In our best case, after 6 weeks the axons regenerated 15 mm across a gap to reach the distal nerve stump. However, for clinical cases, much longer regeneration is required. The present experiments were designed to determine whether we could get adult rat sciatic nerve axons to regenerate across long of more than 2 cm. A 1 cm piece of sciatic nerve was removed from a rat, the cells dissociated, and some of the dissociated cells injected into silicone chambers filled with gelatin. The proximal and distal nerve stumps of the sciatic nerve were sutured to the ends of the silicone chamber leaving gaps of up to 29 mm. After only 4 weeks a fibrin bridge extended the entire 2cm across a gap, and regenerating axons had extended across the whole gap and into the distal nerve stump. These results indicate that this technique promotes more successful and rapid axon regeneration across long gaps than seen in other experimental techniques. Experiments are under way to determine whether given more time, the axons can regenerate across longer gaps between the central and distal nerve stumps.

These results hold good promise for improving axon regeneration *in vivo* in a clinical environment.

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**A-70 Biochemical Analysis of Peripheral Nerve Released Factors.** A. Morales, S. García-Castiñeiras, & D.P. Kuffler, Inst. of Neurobiol. and Dept. of Physiol., Med. Sciences Campus, UPR, 201 Blvd. del Valle, San Juan

The purpose of the present experiments is to determine which neurotrophic factors released from a denervated peripheral nerve are physiologically responsible for promoting axon regeneration, and whether they act singly or in combinations. It is important to ascertain the specific roles of these molecules to determine their suitability for the treatment of degenerative diseases and injury. Peripheral nerve conditioned medium (CM) was separated into three molecular weight fractions (0-100, 100-300 and >300kD) using molecular weight cutoffs filters. Each fraction had a slightly different neurotrophic influence on process length and morphology. When the three molecular weight fractions of CM were combined, the processes were significantly longer than those of control neurons, but not than those of neurons exposed to the original CM. However, the combined CM fractions induced process morphology that was almost identical to that induced by the complete CM. Although increasing the concentration of the CM fractions increased process length, the morphologies of the processes remained unchanged. These results show that: (1) the peripheral nerve releases at least three different neurotrophic factors; (2) each factor exerts a different neurotrophic influence, (3) the factors work together synergistically in promoting process outgrowth, and (4) the morphology of the processes seen in the complete CM results from the interactions of at least three factors. We have also carried on SDS-PAGE analysis which indicates that the molecular weight of some proteins in the CM is within the range of that of the known neurotrophins. Further experiments include Western Blots, and bioassays using fractions from a size exclusion column. Supported by: ARO DAAL03-90-G-0189,

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**A-71 PGE<sub>1</sub> Inhibits Activity of the Alpha-3 Isoform of Na,K-ATPase in the Neuroblastoma Cell Line SH-SY5Y.** K. Woolcock and S.C. Corey. Department of Pharmacology and Institute of Neurobiology, UPR-RCM, San Juan, PR.

The effect of prostaglandin E<sub>1</sub> (PGE<sub>1</sub>) on Na,K-ATPase (Na Pump) activity has been studied in retinoic acid-differentiated SH-SY5Y neuroblastoma cells. The cell line expresses three types of opioid receptors, mu, delta, and kappa, all of which are negatively coupled to adenylyl cyclase. PGE<sub>1</sub> stimulates adenylyl cyclase in SH-SY5Y cells that have been differentiated for 6 days with 10 μM retinoic acid. Western blot analysis of cell membranes revealed that the cells express only the 3 isoform of the catalytic subunit of Na,K-ATPase. Enzymatic activity was determined by Pi liberation from ATP in intact, alamethacin-permeabilized cells incubated with and without 1 mM ouabain. After exposure to PGE<sub>1</sub> for 10 min, Na,K-ATPase activity was inhibited by 38%. The inhibitory effect began to wane with 15 min pre-incubation. The mu agonist DAMGO, 10<sup>-6</sup> M to 10<sup>-8</sup> M, reversed the inhibition caused by PGE<sub>1</sub>. The most probable explanation is that PGE<sub>1</sub> increased intracellular concentration of cAMP, activating protein kinase A, and causing phosphorylation of Na,K-ATPase, which inhibits its activity. DAMGO inhibits adenylyl cyclase, reversing the effect of PGE<sub>1</sub>. The data demonstrate the cell line is useful for investigating opioid modulation of Na pump activity. [Supported by a NIH-MARC faculty pre-doctoral fellowship, by the NIH- MBRS programs, and by funds from Interamerican University.]

**A-72 Cross-Signaling between T-Tubule Calcium Channel and the Sarcoplasmic Reticulum Calcium Release Channel in Crustacean Muscle Fibers.** Monterrubio, J., Lizardi, L. and Zuazaga, C. Inst. of Neurobiology, U.P.R. Med. Sci. Campus.

The tonic flexor muscle fibers of the crustacean *Atya lanipes* do not normally generate Ca<sup>2+</sup>

action potentials or inward  $\text{Ca}^{2+}$  current. However, when exposed to tetracaine (0.3 mM), a blocker of the sarcoplasmic reticulum (SR)  $\text{Ca}^{2+}$  release channel,  $\text{Ca}^{2+}$  action potentials and inward  $\text{Ca}^{2+}$  current are observed (Monterrubio et al., Biophys J. 68:A175, 1995). We proposed that tubular  $\text{Ca}^{2+}$  channels are inactivated at rest by a  $\text{Ca}^{2+}$ -dependent mechanism. We have now tested this hypothesis by measuring intracellular free- $\text{Ca}^{2+}$  levels,  $[\text{Ca}^{2+}]_i$ , at rest and during E-C coupling using the  $\text{Ca}^{2+}$  indicator Fura-2. The basal  $[\text{Ca}^{2+}]_i$  was found to be between 150 to 190nM. Tension and the  $[\text{Ca}^{2+}]_i$  transient increased in amplitude depending on the strength of the depolarizing stimulus, with  $[\text{Ca}^{2+}]_i$  reaching a maximum between 600 to 800nM. The  $\text{Ca}^{2+}$  necessary for contraction mainly comes from the SR, because 0.3mM tetracaine almost completely blocked tension and the  $[\text{Ca}^{2+}]_i$  transient. BAPTA injection reduced  $[\text{Ca}^{2+}]_i$  below 100nM and elicited large  $\text{Ca}^{2+}$  action potentials. These  $\text{Ca}^{2+}$  spikes were inactivated by the  $\text{Ca}^{2+}$  released from the SR by 0.5mM caffeine. Our data demonstrate that tubular  $\text{Ca}^{2+}$  channel activity is controlled by the  $\text{Ca}^{2+}$  released from SR. These results further support the existence of cross-signaling between the T-tubule  $\text{Ca}^{2+}$  channel and the SR  $\text{Ca}^{2+}$  release channel that is facilitated by the microarchitecture between them. (Supported by NIH grants NS-07464, RR-03051, GM-08224 and NSF HRD9353130).

**A-73 Distribution of Myomodulin-like Immunoreactivity in the Central Nervous System of *Aplysia*** - G. Maynard-Salgado, B. Ramos, S. Soto, M.W. Miller<sup>1</sup>, and C. Zuazaga<sup>2</sup>. Inst. of Neurobiology, and Depts. of Anatomy<sup>1</sup> and Physiology<sup>2</sup>, UPR

The myomodulin-related peptides were originally isolated and characterized in the mollusc *Aplysia*. Recent investigations suggest that this family of peptides is present in additional phyla. In this study, wholemount immunohistochemical techniques were used to assess the distribution of the peptide in the central nervous systems of three freshwater tropical shrimps of the genus *Aplysia*. The most prominent system of immunoreactive cell bodies, located in the subesophageal ganglion, consists of four

ventral midline cells and two larger lateral cells. Each of these six cells projects a single fiber into the ipsilateral circumesophageal connective. The fibers originating from the two lateral cells decussate in the esophageal commissure. Rostral to the commissure, each decussating fiber fuses with the non-crossing pair to form a single ascending bundle that projects to the brain and exits via the ipsilateral antenna nerve. Additional cell bodies are located in the fifth thoracic ganglion and in each abdominal ganglion with the exception of the sixth. One or two fibers are present in each of the thoracic and abdominal connectives and all of the thoracic and abdominal ganglia contain widespread fine neuropilar systems. Supported by RCMi G12RR-03051 (NIH), NS-07464 (NIH), MBRS GM-08224 (NIH) and Puerto Rico EPSCoR (NSF).

**A74 Basic Fibroblast Growth Factor Applied to the Optic Nerve After Injury Increases Cell Survival in the Retina.** A. López-Roca, I. Soto, J. Soto and R.E. Blanco, Institute of Neurobiology and Dept. of Anatomy, UPR-RCM, Dept. of Biology, University of Puerto Rico, San Juan, P.R.

The frog retina is a good system in which to investigate the reactions of retinal ganglion cells (RGCs) to axotomy, without the complicating effects of an inhibitory nerve environment. Optic nerves of *Rana pipiens* were cut between eye-ball and chiasm and Nissl stains of flat-mounted retinas were prepared at 2 week intervals after the operation. Basic fibroblast growth factor (bFGF) was applied to the optic nerve stump after the operation. Retinas 6 weeks after axotomy contained dying cells, the nuclei of which were mostly pyknotic. Some apoptotic nuclei were also detected. The density of cells had declined on average by 13% compared to controls. Addition of bFGF to the optic nerve stump eliminated this decrease. Fewer than 50% of the ganglion cells were still present 26 weeks after the nerve was cut. A second application of bFGF six weeks after the injury rescued an additional 15% of neurons in the ganglion cell layer. Frog RGCs undergo significant cell death after axotomy, some of which is due to apoptosis, and can be tempo-

rarily rescued by repeated applications of bFGF to the nerve.

Supported by NIH S06 08224 and G12RR-03051.

A-75

**The *shaking-b<sup>2</sup>* Mutation Eliminates Electrical, Not Chemical, Synapses in the Giant Fiber System of *Drosophila melanogaster*.** J. M. Blagburn, H. Alexopoulos, J. A. Davies and J. P. Bacon, Institute of Neurobiology and Dept. of Physiology, UPR-RCM, San Juan, P.R., and Sussex Centre for Neuroscience, School of Biological Sciences, University of Sussex, Brighton, UK.

Mutations in *Drosophila shaking-B* perturb transmission and dye coupling in the giant fiber escape system. The objective of this study was to determine the structural and ultrastructural effects of the *shaking-B<sup>2</sup>* (*shak-B<sup>2</sup>*) mutation. Antibody staining showed that Shaking-B protein is concentrated in plaque-like structures in the regions of neuropil where the giant fiber (GF) contacts the peripherally synapsing interneuron (PSI) and the tergotrochanteral motoneuron (TTM). Electron microscopy of these contacts revealed regions of close membrane apposition ( $3.25 \pm 0.12$  nm) with faint cross-striations. These contacts had a single row of large presynaptic vesicles, similar to those at rectifying electrical synapses in the crayfish GF system. The GF-TTM contacts also exhibited many sites of chemical synaptic transmission. In *shak-B<sup>2</sup>* mutants the electrical synapses were eliminated but there was a greater proportion of chemical contacts at the GF-TTM synapse. Gap junctions ( $1.41 \pm 0.08$  nm) were abundant between peripheral glial processes; these were unaffected in the mutants. We conclude that (1) the GF-TTM connection is a mixed electrical and chemical synapse, (2) the *shak-B<sup>2</sup>* mutation selectively eliminates electrical synapses, not chemical synapses, and (3) conventional gap junctions between glia are not affected by the mutation.

Supported by NIH NS07464 and BBSRC, UK.

**Establishment of Whole Embryo Cul**

A-76

**tures to Study Synapse Formation in the Cercal Sensory System of the Cockroach.** M.A. Sosa<sup>1,3</sup> and J.M. Blagburn<sup>2,3</sup>. <sup>1</sup>Depts. of Anatomy & <sup>2</sup>Physiology and <sup>3</sup>Inst. of Neurobiology, UPR MSC

The cercal sensory system of the cockroach (*Periplaneta americana*) is well suited for studying synapse formation, due to the high degree of specificity of the connections formed between sensory afferents and their target giant interneurons (GIs). We have previously described the development of synapses between the first instar's two cercal sensory afferents and GI2 and GI3. To further characterize the mechanisms involved in target selection among these neurons, we are devising a culture preparation that will allow us to perturb the system experimentally as the embryo develops. After sterilizing the ootheca, embryos are isolated in medium and transferred to the culture chamber. CNS neurons are exposed by opening the embryo and/or its cerci along the dorsal midline using fine forceps. Chambers are sealed with a sterilized coverslip. Following our protocol, embryos as young as 15 days (~50% development) can be kept alive for at least 2 days at 30°C. The sensory afferents and GIs have normal membrane potentials, and the afferents can generate action potentials. Embryos kept for longer periods of time seem to develop at a slower pace than embryos kept in their own oothecae. Also, embryos that are older at the time of culture have a better chance of survival in the culture chambers. The technique for establishing cockroach whole embryo cultures holds promise, as it could lay the groundwork for a wide array of additional approaches to study synapse formation and development. Support: NIH NS07464, RR03051, NSF Minority Postdoctoral Fellowship and CIDIC.

A-77

**Distribution of Myomodulin and Serotonin in the Terminal Ganglion of the First Instar Cockroach, *Periplaneta americana*.** E. S. Hill and J. M. Blagburn, Institute of Neurobiology and Dept. of Biology, UPR, and Dept. of Physiology, UPR-RCM, San Juan, P.R.

Experiments were carried out to determine the presence of serotonin and the neuropeptide

myomodulin in the terminal ganglion of the first instar cockroach using immunocytochemical methods. The terminal ganglion of the cockroach contains 7 pairs of wind-sensitive interneurons, known as giant interneurons (GIs), which receive input from cercal sensory neurons and project to thoracic interneurons. The GIs represent the first level of processing of wind information in the complex escape circuit. Immunocytochemical staining shows that in the terminal ganglion, myomodulin-like immunoreactivity is observed in a pair of neurons and in many processes of the neuropil, and that serotonin-like immunoreactivity is observed in many pairs of neurons and throughout the neuropil. Additionally, double-labeling experiments with Lucifer Yellow and processing for immunocytochemistry using a fluorescent secondary antibody have shown the identifiability of the immunopositive neurons. Cobalt fills have been used to examine the dendritic fields and axonal projections of the immunopositive neurons. In conclusion, both serotonin and myomodulin are present in the terminal ganglion of the first instar cockroach and the morphologies of some of the immunopositive neurons have been characterized.

Supported by RCM, NSF EPSCoR and NIH NS07464.

**A-78 The Structure of Cardiac Muscle Thick Filaments.** Robert W. Kensler. Department of Anatomy, Univ. of Puerto Rico Med. Sch., San Juan, PR 00936

Although cardiac muscle has been extensively studied, very little information is available on the detailed macromolecular structure of the cardiac muscle thick filament. To elucidate the structure of these filaments, we have developed a procedure that allows the isolation of the cardiac thick filaments for study by electron microscopy and computer image analysis. As we have previously reported (Biophys. J. 74:A363), the negatively-stained isolated filaments appear periodic with a helical repeat every third crossbridge level (43 nm). Computed Fourier transforms of the filaments are detailed, showing a strong set of layer lines corresponding to a 43 nm near-helical repeat out to the 6th layer line, with additional meridional reflections ex-

tending to at least the 12th layer line in averaged transforms of the filaments. Analysis of the phase differences for the primary reflections on the 1st layer line of transforms from 15 filaments showed an average difference of 170°, close to the value of 180° expected for an odd-stranded structure. Computer-filtered images of the isolated thick filaments and comparison with models unequivocally demonstrate a three-stranded arrangement of the crossbridges on the filaments. This investigation was supported by a 'Research Centers in Minority Institutions' award RR-03051, from the National Center for Research Resources (NIH) and a Grant-in-Aid Award from the American Heart Association of Puerto Rico.

**A-79 Stimulatory Effect of Maitotoxin on the Calcium-Dependent Contractile Activity of Gastric Smooth Muscle.** M. Rubio-Dávila, R. Lugo-López, and G. Escalona de Motta. Univ. of Puerto Rico, Dept. of Biology and Institute of Neurobiology, 201 Blvd. del Valle, San Juan, P.R. 00901.

Maitotoxin (MTX) is a water-soluble toxin produced by the marine dinoflagellate *Gambierdiscus toxicus* and found in the body of various herbivorous reef fish. This toxin appears to act as a calcium channel activator and its biological activities are dependent on extracellular Ca<sup>++</sup> levels. The fact that the contractility of gastric smooth muscle is critically dependent on the influx of Ca<sup>++</sup> from the extracellular medium makes this tissue an ideal experimental model to evaluate the mechanism of action of MTX. We have studied the action of MTX on the spontaneous contractions generated by muscle strips of the cardiac region of the frog (*Rana pipiens*) stomach. Isometric recordings of the contractile activity were done in the presence of various extracellular calcium concentrations showing that these responses have strict dependence on transmembrane calcium influx. Application of picomolar concentrations of MTX resulted in an increase in the phasic contractions that progressed to a near tetanus. This stimulatory effect was blocked in the presence of verapamil, an L-type calcium channel blocker but not in the presence of  $\omega$ -conotoxin, an N-type channel antagonist sug-

gesting the presence of L-type calcium channels in this preparation. Supported by NIH Grants GM08102 and MH48190 and by an ATLANTEA Project funded by the University of Puerto Rico.

**A-80 Delta Opioid Receptors in Amphibian Smooth Muscle: Contractility and Enkephalin-Like Immunoreactivity.** M. Rubio-Dávila<sup>1,3</sup>, I. Cruz-Jové<sup>1,3</sup>, A. Rodríguez-Centeno<sup>1,3</sup>, R.E. Blanco<sup>2,3</sup> and G. Escalona de Motta<sup>1,3</sup>. Univ. of Puerto Rico, Depts. of <sup>1</sup>Biology and <sup>2</sup>Anatomy, and <sup>3</sup>Inst. of Neurobiology, 201 Blvd. del Valle, San Juan, P.R. 00901.

Opiates are known as modulators of contractility in mammalian enteric muscles. In frog (*Rana pipiens*) stomach, phasic contractions have both, a cholinergic and an adrenergic modulation. This work examines the influence of enkephalins on the contractions of the frog gastric *muscularis externa* muscle strips. Met-Enk was the most potent of delta opiate receptors agonists used, increasing by more than 50% the force of the contractions while reducing their frequency to half the control value. Leu-ENK produced similar effects, but of lower intensity, particularly on the force. Histochemical observations revealed Enk-like immunoreactivities in frozen muscle sections. Varicose fibers showing Met-Enk-like immunoreactivity were seen running along the circular layer of the *muscularis externa*. These pharmacologic and histologic observations point to a modulatory role for delta receptor opioids in the contractile activity of amphibian gastric smooth muscle. (Supported by NIH grants GM08102 and MH48190 to G.E.M.; in part by NIH grants GM08224 and NSF07464 to R.E.B.; and by DoD Instrumentation Grant N00014-93-1380 (ONR)). BNTX was provided by RBI as part of the Chemical Synthesis Program of the NIMH Contract N01MH003

**A-81 Effect of Cardiac Glycosides and Amiloride Derivatives on the Action of Palytoxin in Frog Skeletal Muscle** J. Rivera\*, S.E. Figueroa, J.V. Pérez and G. Escalona de Motta. Univ. of Puerto Rico, \*Cayey Campus and Inst.

of Neurobiology, 201 Blvd. del Valle, San Juan, P.R. 00901.

Palytoxin (PTX) is one of the most powerful marine toxins known by mankind. The main target of the PTX is the Na<sup>+</sup>/K<sup>+</sup> ATPase, however, several evidences point to the possibility of acting on the Na<sup>+</sup>/H<sup>+</sup> exchanger and the Na<sup>+</sup>/Ca<sup>2+</sup> exchanger. To examine this possibility, we studied the effect of two amiloride derivatives: benzamil, which is an Na<sup>+</sup>/Ca<sup>2+</sup> inhibitor, and 5 N,N ethylisopropyl- amiloride (EIPA), which is an Na<sup>+</sup>/H<sup>+</sup> inhibitor in frog skeletal muscle. Cymarin, a potent cardiac glycoside and a Na<sup>+</sup>/K<sup>+</sup> ATPase inhibitor, was also evaluated. Using a mechanoelectrical transducer, we recorded contractile responses of *Rana pipiens rectus abdominis* muscles. Addition of PTX caused sustained contractions under control conditions. These contractions were indicative of a depolarization in the muscle membrane. Incubation with benzamil showed small potentiation of the contractile action of PTX. In muscles treated with EIPA, the PTX response was reduced to 66% when compared to the control. When cymarin was added to the EIPA treatment, the PTX induced response was reduced to 48%. Cymarin alone, reduced the PTX response to 51%. These observations suggest that the primary targets of palytoxin in frog skeletal muscles are both the Na<sup>+</sup>/K<sup>+</sup> ATPase and the Na<sup>+</sup>/H<sup>+</sup> exchanger. (Supported by NIH Grants GM08102 and MH48190)

**A-82 K<sup>+</sup> Channel Density Increases Selectively in the Endfoot of Retinal Glial Cells During Bullfrog Development.** Legier Rojas\* and Richard K. Orkand\*. Universidad Central del Caribe, Bayamón, PR\* and Institute of Neurobiology, UPR, RCM, San Juan PR\*.

The radial glial (Müller) cells that span the retina, have a remarkable distribution of ion channels in adult amphibia that mediate extracellular K<sup>+</sup> spatial buffering. 94% of the membrane conductance of these cells resides in K<sup>+</sup> channels in the endfoot processes apposed to the vitreous humour. We now report that this regional specialization is found in cells isolated from adult (>120 day old) bullfrogs but not in those from 10-20 day old tadpoles (stages



34-36). Using the cell attached patch-clamp technique we found, in agreement with previous studies in salamanders, that the endfoot of adult cells had  $19.1 \pm 2.4$  (mean  $\pm$  S.E.,  $n=81$ ) channels/patch whereas the soma had  $1.81 \pm 0.32$  ( $n=21$ ) channels/patch. In the tadpole, the respective values were  $4.29 \pm 0.27$  ( $n=79$ ) for the endfoot and  $2.25 \pm 0.25$  ( $n=27$ ) for the soma. The conductance of the inward rectifier  $K^+$  channel, channel kinetics and the resting membrane potential were similar at both the endfoot and soma of both adults and embryos. Supported by NSF (EPSCoR); NIH (NINDS).

**A-83 Properties of Müller (Glial) Cells from Frog Retinal Center and Periphery.**

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Müller (glial) cells were obtained from the retinal center and periphery of adult frogs (*Rana pipiens*). Membrane currents (I) were recorded using the whole-cell voltage-clamp technique.  $[K^+]_o$  was changed from 3 mM to 1 mM for outward I ( $I_{KO}$ ) and from 3 mM to 10 mM for inward I ( $I_{KIN}$ ). In cells from retinal center, the  $I_{KIN}/I_{KO}$  ratio was 4.5 at the endfoot and 1.8 at the soma. In cells from retinal periphery, the  $I_{KIN}/I_{KO}$  ratios were higher, 6.1 at the endfoot and 3.0 at the soma; there is less inward rectification in the somatic than in the endfoot membrane. Currents spread readily from endfoot to soma in the peripheral cells whereas soma and endfoot appeared to be electrotonically isolated in central cells. The relative decrement of large inward currents was stronger than that of small outward currents; this difference („artificial rectification“) is explained by a model. In the retinal periphery,  $K^+$  currents through Müller cell stalk processes may allow for siphoning of excess  $K^+$  from both plexiform layers into the vitreous body. In the central retina, however, such currents may redistribute  $[K^+]_o$  changes only within a short (interlaminar) range, at best. Supported by NIH (NINDS, RCMI), NSF (EPSCoR).

**A-84 Angiotensin Converting Enzyme Activity and Impairment of Impulse Propagation in the Failing Heart.**

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Congestive heart failure (CHF) is a major cardiovascular problem worldwide. Neurohumoral, vascular and cardiac alterations have been postulated to be involved in the etiology of this condition, yet the precise mechanisms remain undefined. To evaluate the electrical properties of heart in CHF, electrophysiologic studies were performed on the right ventricle of cardiomyopathic hamsters (TO-2) at late stage of the disease (11-months old). Age matched normal hamsters were used as controls. Our results indicate severe abnormalities in the electrical parameters of cardiomyopathic hamsters. These abnormalities include: a low resting potential ( $-67.8 \pm 0.83$  mV in TO-2 vs.  $-78.5 \pm 1$  mV in controls,  $p < 0.05$ ,  $n=15$ ), a reduced amplitude of the action potential ( $68.2 \pm 4.1$  mV in TO-2 vs  $83.8 \pm 3$  mV in control animals,  $p < 0.05$ ,  $n=18$ ) and a 15% decrease in conduction velocity ( $P < 0.05$ ). To investigate the relationship between these electrical abnormalities and the activation of the cardiac renin-angiotensin system (RAS), the angiotensin converting enzyme (ACE) activity was determined in the ventricles of 11-months old cardiomyopathic animals. ACE activity was 2-fold higher in cardiomyopathic than in control hamsters ( $0.563 \pm 0.027$  vs  $0.244 \pm 0.016$  nmol/mg.min,  $p < 0.0001$ ,  $n=20$ ). By contrast, in 2-months old hamsters, ACE activity values ( $0.244 \pm 0.04$  nmol/mg.min), was not different from age-matched controls ( $p > 0.05$ ). The increased ACE activity observed in heart tissue, might indicate an enhanced cardiac angiotensin II synthesis in 11-months old TO-2 hamsters. These results suggest a strong correlation between the activation of the cardiac RAS and the severe impairment of impulse propagation leading to the generation of cardiac arrhythmias in the failing heart. (Supported by American Heart Association and Merck, Sharp and Dohme)

**A-85** **A self-adjusting myotendinous organ in the spine ligament of the sea-urchin *Eucidaris tribuloides*.** N.L. Pérez Acevedo<sup>1</sup>, D.S. Smith<sup>2</sup>, J. T. del Castillo<sup>1</sup>. <sup>1</sup>Institute of Neurobiology, UPR-MSU, San Juan, Puerto Rico, and <sup>2</sup>University Museum, Oxford University, Oxford, U.K.

The ligament has the shape of a hollow truncated cone at the spine-test articulation. Any movement imparted to the spine will stretch half of the ligament, while compressing the other half, forming short-lived wrinkles. However, at rest the ligament appears smooth and shiny whatever the inclination of the spine. We have found that drugs that act on muscle fibers modified the duration and the appearance of wrinkles. In ligaments exposed to cholinergic agonists (such as ACh and CCh, 1mM) the narrowest wrinkles appeared, always perpendicular to the collagen columns at angles of 45° to 90°. They also remained at the surface of the ligament. However, in presence of compounds that block muscle contraction the width of wrinkles increases, and they disappear faster than controls. We have found that individual wrinkles decay with a time constant that can be as short as one second. Pharmacological and physiological experiments suggest a direct role for the slender muscle fibers that cross-link the collagen cylinders. When muscle fibers contract, they pull the collagen elements tightly across the calcareous microbeams within the stereom insertion regions, increasing friction between them. The fact that wrinkles disappear when the ligament is in a relaxed state suggests a flow of collagen over the struts. Thus, the ligament of the echinoid *Eucidaris tribuloides* behaves as an energy dissipating myotendinous organ. In this way, *Eucidaris* combines the intraligament muscle fibers with collagen type I to produce a variable length tendon with self-adjusting properties.

#### **Western Blot Analysis of Proteins**

**A-86** **in Frog and Toad Retinas which React with Anti-keyhole Limpet Hemocyanin.** E. Kicliter<sup>1</sup>, S. C. Specht<sup>1\*</sup>, N. Lugo<sup>1</sup> and D.L. Meyer<sup>2</sup>. Institute of Neurobiology, Univ. of Puerto Rico, Med. Sci. Campus, San Juan, PR 00901<sup>1</sup> and Dept. of Neuroanatomy. Sch. of Med., Univ. of Göttingen, Germany<sup>2</sup>.

In some species of anuran amphibians retinal horizontal cells as well as ganglion cells react with an antibody to keyhole limpet hemocyanin (anti-KLH). In other species anti-KLH reactivity is noted only in ganglion cells. We sought to determine the molecular size(s) of the reactive proteins. We used one species whose horizontal cells are reactive (*Bufo marinus*) and one whose horizontal cells are not (*Rana pipiens*). Retinal proteins were separated by electrophoresis in an SDS-polyacrylamide gel (SDS-PAGE) and then transferred to nitrocellulose (Western blot). Following overnight incubation with anti-KLH, protein bands that reacted positively with anti-KLH were detected with the ABC technique. In the *Bufo* material we observed some 16 immunopositive bands with indicated molecular weights ranging from 250 KDa to less than 100 KDa. In comparable *Rana* material several of the higher molecular weight bands were not present. These findings suggest that reactivity to anti-KLH in ganglion cells has a different basis from the reactivity in horizontal cells. (Supported, in part by NIH grants MH-48190 and NS-07464 and the German Science Foundation).

**A-87** **Neuroactive Substances in the Suprachiasmatic Nucleus of a Hibernator.** J. López<sup>1</sup>, D.I. Lugo<sup>2</sup> and N. Lugo<sup>1</sup>. Institute of Neurobiology, Univ. of Puerto Rico, Med. Sci. Campus., San Juan, PR, 00901<sup>1</sup>, Dept. of Biology, Univ. of Puerto Rico Río Piedras Campus, San Juan, PR, 00931<sup>2</sup>.

A circadian pacemaker in the mammalian suprachiasmatic nucleus (SCN) imposes a temporal organization on physiological processes and behavior. The SCN receives afferents from many sources, particularly the retina, and has been shown to contain a number of neuroac-

tive substances in rats and hamsters. Since the SCN is involved in circadian rhythms, it would be interesting to understand the rhythms of expression of neuroactive substances in hibernators. We have studied neuroactive substances in the suprachiasmatic nucleus of a seasonal hibernator, the thirteen-lined ground squirrel (*Spermophilus tridecemlineatus*) during a 12h light/12h dark cycle. Immunofluorescent techniques and the avidin-biotin (ABC) method were used to assess the presence of peptides and modified amino acids in the SCN. Substance P-like immunoreactivity was seen in axonal terminals throughout the SCN; some terminals of this field extended into other hypothalamic sectors. Serotonin-like immunoreactivity was also found in axonal terminals distributed throughout the SCN, but they were confined to the SCN. GABA-like immunoreactive neurons were also found. The distribution of GABAergic neurons will be examined using *in situ* hybridization (antisense GAD 65 and GAD 67 oligonucleotides). Our results suggest that neuroactive substances previously described in rats and hamsters are also present in the ground squirrel SCN. Supported by NIH Grants MH-48190 and G12RR-03051.

**A-88 Selection and Use of Habitat by the Introduced Rhesus and Patas Monkey Populations in Southwestern Puerto Rico.** Janis González Martínez, Caribbean Primate Research Center, University of Puerto Rico, Medical Sciences Campus

Introduced populations of rhesus macaques (*Macaca mulatta*), and patas monkeys (*Erythrocebus patas*) have been free-ranging on the mainland of southwestern Puerto Rico since the 1960s. Much concern has been publicly expressed regarding the health status of these primate populations and the transmission of infectious agents between these animals and humans. In order to assess the potential hazard to the community it was necessary to delimit and identify the areas used by these populations. This paper addresses the extent of contact between these free-ranging populations and humans. A three-year study (1990-1993) combined radio-telemetry with visual observations to collect information on the size of the

populations, the composition of social groups, diet, their daily movements, and habitat use. Habitat selection was considered at two levels: (1) selection of an overall home range area for the entire patas and rhesus monkey populations vs. total study area, and (2) for each individual group, proportional habitat use vs. actual home range habitat composition. Habitat use by a group was estimated by the proportion of scores within each habitat type in each group's home range. Interactions with humans were minimal. The patas and rhesus monkey populations were not using habitat in a random fashion and they exhibited preference and/or avoidance towards several habitat types and avoided contact with humans.

**Sex Differences in Preproenkephalin mRNA Levels in the Caudate Nucleus of Adult Rats.** M. Febo<sup>1</sup>, J.A. Angulo<sup>2</sup>, M. Rivera<sup>1</sup> and A.C. Segarra<sup>1</sup>. <sup>1</sup>School of Medicine, Univ. of Puerto Rico, MSC, and <sup>2</sup>Hunter College, CUNY, NY.

Opioid expression has been associated to addiction. Increased levels of opioids has been observed to enhance addictive behaviors whereas administration of opioid antagonists are used to curtail drug and alcohol craving. In humans, there is a higher percentage of men addicted to drugs of abuse when compared to females. In this study we decided to investigate if there is a sex difference in estrogenic regulation of an endogenous opioid system in brain areas that are involved in drug seeking behavior. Cryostat sections of the caudate nucleus of control and estrogen treated male and female rats were hybridized to a <sup>35</sup>S preproenkephalin oligonucleotide probe and quantified with the assistance of an image analysis system. Sex differences were observed between castrated oil treated animals, males exhibiting higher preproenkephalin levels and smaller cell soma size than females. Estrogen treatment increased preproenkephalin levels in females and decreased cell soma size to levels comparable to those found in the male. In conclusion, these results indicate a gender difference in preproenkephalin levels in the caudate nucleus which are independent of circulating sex steroid levels. It appears that only

female enkephalinergic caudate neurons are capable of responding to estrogenic induction of gene expression. It is possible that the increased preproenkephalin levels observed in the male population may be correlated to the higher addictive behaviors observed in the male. This study was supported in part by the Associate Deanship of Biomedical Sciences and by RCMC Grant RR-03051.

**A-90 Sex Differences in Estrogenic Regulation of Preproenkephalin mRNA Levels in the Preoptic Area of Prepubertal Rats.**

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Opioids have been implicated in sexual differentiation of the brain and in the regulation of reproductive behavior in adult rats. Previous studies have indicated that estrogen administration in adults regulates preproenkephalin mRNA levels in several hypothalamic brain nuclei. We have determined preproenkephalin mRNA levels in estrogen-treated juvenile male and female rats to investigate the developmental pattern of estrogenic regulation of enkephalinergic neurons in the preoptic area. Rats were treated with estradiol benzoate (40 µg/kg) or oil from day 21-23. Sections of the preoptic area (POA) were studied by *in situ* hybridization histochemistry at the single cell level and quantified with the assistance of an image analysis system. Our data indicates that males contain higher levels of preproenkephalin mRNA per neuron than females. However, if this results are analyzed in terms of preproenkephalin mRNA levels per unit of neuronal cell area, this difference disappears. In addition, our results indicate that estrogen causes an upward shift in the amount of mRNA expressed per cell, females demonstrating a greater response to estrogen than males. Taken together, these results indicate a sex difference in total preproenkephalin levels and in estrogenic regulation of preproenkephalin mRNA in the POA of juvenile rats. This study was supported in part by the Associate Deanship of Biomedical Sciences and by RCMC Grant RR-03051.

**A-91**

**Determination and Quantitation of Environmental Organic Pollutants in Edible Fish Tissue by GC-MS Ion Trap** R.D. Ramírez<sup>1,2</sup>, J. Bloom<sup>1,2</sup>, J.F. Rodríguez<sup>1,3</sup>, C.J. Rodríguez<sup>1,4</sup>, B. Jiménez<sup>1,2</sup> <sup>1</sup>Center for Environmental and Toxicological Research; <sup>2</sup>School of Pharmacy; <sup>3</sup>Biochemistry Department; <sup>4</sup>School of Public Health Medical Sciences Campus University of Puerto Rico, San Juan, Puerto Rico 00935

Tissue from sardines and moharra fish captured at the San José Lagoon in San Juan, Puerto Rico, were analyzed to determine levels of organic pollutants. The U.S. EPA designated this Lagoon a National Estuary. Polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs) and organochlorinated pesticides were determined to measure the probable exposure levels of these contaminants in humans resulting from fish consumption. Analyses were performed using a GC-MS and GC-MS-MS Ion Trap systems. Preliminary data from fish tissues revealed a total PCB concentration range between 15 - 30 ng/g. Most of the PCBs detected were tetra-, penta-, and hexachlorinated biphenyls. DDE, a metabolite of DDT, was the only organochlorinated pesticide detected with a concentration range between 0.4 - 2.7 ng/g. PAHs such as naphthalene, fluorene and acenaphthene were also detected. A simple clean-up procedure has been developed in which recovery percentages for PCBs were over 95%, and for most organochlorinated pesticides were over 90%. However, work is being done to optimize recovery percentages for the PAHs and dieldrin. Soxhlet extraction and Microwave Assisted Extraction (MAE) were performed for comparison purposes; no significant differences were found in terms of recovery. Even though, MAE can be an alternative extraction method due to its quickness and the amount of solvent required.

**A-92**

**Aportación de un Curso de Ciencias Básicas a la Educación Orientada al Cuidado Farmacéutico** E. Vega, L. González y M. Martínez, Escuela de Farmacia, UPR

Objetivos: 1) Rediseñar el curso Anatomía-

Fisiología (AF) para contribuir al desarrollo de competencias que capaciten al estudiante para brindar cuidado farmacéutico, 2) Auscultar la opinión de los estudiantes en relación con la aportación del curso rediseñado a su crecimiento profesional.

**Metodología:** Rediseño para el desarrollo de destrezas de solución de problemas, toma de decisiones y pensamiento crítico (esenciales para brindar cuidado farmacéutico). Se usan estrategias y metodologías de enseñanza y evaluación que propician el desarrollo de conceptos, destrezas y actitudes. Se revisó el contenido del curso para incluir el concepto de cuidado farmacéutico y se incorporaron casos clínicos. Se diseñaron dos (2) cuestionarios para conocer la opinión de los estudiantes sobre la aportación del curso a su desarrollo profesional. Se preguntó su opinión sobre el logro de los objetivos del curso, el desarrollo de competencias y la contribución de metodologías de enseñanza y evaluación al logro de estos, y su percepción sobre el ambiente en la sala de clases y la efectividad de la maestra como facilitadora de su aprendizaje.

**Resultados:** El uso de metodologías de enseñanza y evaluación planificadas resultó en mayor participación del estudiante propiciando un aprendizaje activo. Las experiencias educativas contribuyeron al logro de los objetivos trazados y al desarrollo de destrezas como solución de problemas, toma de decisiones y pensamiento crítico. Se familiarizaron con el concepto de cuidado farmacéutico y se amplió su visión sobre la profesión. Señalaron que en el ambiente de la sala de clases predominaron factores emocionales positivos como interés y entusiasmo y que estos contribuyeron más a su aprendizaje.

**Conclusión:** El curso AF rediseñado, según los estudiantes, logró desarrollar competencias para brindar cuidado farmacéutico. Esto evidencia que un curso de ciencias básicas contribuye al desarrollo de competencias profesionales.

A-93

**Chemical Analysis of Organic Pollutants in Sediments from a Tropical Estuary Lagoon in the Caribbean.** Roberto Marrero-Ortiz, J. Bloom, J.F. Rodríguez, B. Jimenez and C.J. Rodríguez, Center for Envi-

ronmental and Toxicological Research, Medical Sciences Campus, University of Puerto Rico, San Juan, Puerto Rico.

The San Juan Bay Estuary System (SJBES) located in Puerto Rico, was designated in 1993 an Estuary of National Importance, by the United States-Environmental Protection Agency (US-EPA). The San José Lagoon is the largest (1,400 hectares) of the estuary system. Centuries of intensive use and development has left the SJBES stressed and contaminated (USGS, 1996). The identification of organic pollutants in sediments from the San José Lagoon are being characterized to determine the extent of contamination in this system. Sediment samples were Soxhlet extracted with hexane/methylene chloride (3:2). The extract was concentrated and eluted through a multilayer clean-up separation column packed with sodium sulphate, activated copper, florisil, silica and neutral alumina. Analysis of chlorinated hydrocarbons (pesticide and PCBs) and polycyclic aromatic hydrocarbons (PAHs) were performed by GC/MS/Ion Trap system and GC/MS/MS using Selected Ion Monitoring. Preliminary results show the presence of biphenyls (trichloro-, tetrachloro-, pentachloro-, and hexachloro-), and phenanthrene. Soxhlet extraction will be compared to microwave extraction in terms of time, percent of recovery, extraction and efficiency. Toxicological characterization of sediment extracts will be performed in order to evaluate toxicity levels throughout this system.

A-94

**Detección Temprana de Cáncer de Mama: Planificación de un Modelo de Promoción de Salud para Mujeres de Edad Mayor.** Oliver Vázquez M., Sánchez Ayéndez M., Suárez Pérez E., Vélez Almodóvar H., Arroyo Calderón Y. Escuela Graduada de Salud Pública, UPR, P.O.Box 365067, San Juan, 00936-5067

Los hallazgos de la investigación "Conocimientos y creencias sobre el cáncer de mama en mujeres de 65 años o más en P.R." fueron utilizados para planificar un programa modelo de promoción de la salud dirigido a aumentar

el cumplimiento de las mujeres de edad mayor con las medidas recomendadas para la detección temprana del cáncer de mama. El programa tiene como objetivos el que las mujeres tomen conciencia sobre el cáncer de mama como problema de salud en la edad avanzada y sobre la importancia de su detección temprana, cambien sus actitudes negativas; y desarrollen destrezas para el autoexamen y para comunicarse efectivamente con los profesionales de la salud. El modelo es uno abarcador que contempla la integración de los siguientes componentes: educación en salud a las mujeres, adiestramiento a los profesionales de salud que las atienden y coordinación de los recursos necesarios para llevar a cabo la mamografía y el examen clínico. Esta dirigido a minimizar las barreras personales y áquellas relacionadas con el sistema de servicios de salud que predisponen, refuerzan o facilitan las conductas de riesgo para el cáncer de mama en las mujeres de edad mayor. Al minimizar las barreras se espera un aumento en el cumplimiento de estas mujeres con las medidas de detección temprana. El alcance del programa se evaluará a corto y a largo plazo con un diseño experimental que medirá la efectividad de las acciones tomadas para alcanzar la meta propuesta.

**A-95**

**Barriers Affecting Breast Cancer Screening among Elderly Puerto Rican Women.** M. Sánchez Ayéndez, E. Suárez Pérez, M. Oliver Vázquez, H. Vélez Almodóvar, R. Rosario Rosado, C.M. Nazario Delgado. School Of Public Health, UPR, POBox 365067, San Juan, 00936-5067

Most studies on breast cancer in elderly women focus on epidemiology and the effect of screening modalities. Few address equally important issues related to how cultural value orientations, beliefs and barriers pertaining to the health care system affect screening practices. This study looks at personal and external barriers affecting breast cancer early detection practices among women 65+ in Puerto Rico. A questionnaire was constructed and validated for the population under consideration. A sample of 500 elderly Puerto Rican women stratified by area of residence and socioeconomic status participated in the study. Thir-

teen per cent of women perform a monthly BSE, 45% have undergone a CBE during last year, and 25% have a mammography performed every two years. Two-fifths have never had a mammogram. The principal reason cited by women for not having a mammogram were: not having symptoms, no referral from a physician, not perceiving themselves as susceptible to the disease and forgetfulness or careless. Fifty per cent of women indicated that no physician had recommended a mammography during the last 5 years. Forty-six percent of the women did not indicate gender preference in terms of the attending physician. The same proportion stated they prefer a female physician. Education was the most important variable accounting for differences among the participants. Lack of referral from a physician and receiving information about breast cancer were the most significant factors for compliance with a mammogram.

**A-96**

**Knowledge and Beliefs of Breast Cancer among Elderly Puerto Rican Women: Procedures to Validate Scales.** E. Suárez Pérez, M. Sánchez Ayéndez, M. Oliver Vázquez, H. Vélez Almodóvar, R. Rosario Rosado, C.M. Nazario-Delgado, School of Public Health, UPR, POBox 365067, San Juan, P.R. 00936-5067

This paper presents the results of the validation procedures to measure personal and external barriers affecting breast cancer early detection practices among Puerto Rican elderly women. An initial questionnaire was designed based on the scientific literature review and focus group experiences. To determine its reliability and validity, 50 elderly women, stratified by type of profession and place of residence, were interviewed twice. Consistency of the questionnaire was analyzed using binomial test, matched test, Pearson correlation, and Chronbach coefficient effect. Factor analysis (FA) was the statistical technique used to analyze the grouping of the knowledge and beliefs statements. FA indicated that three scale for knowledge and one for beliefs could be constructed. The purpose of scales was to identify differences among groups, according to breast-cancer early detection practices. Construct va-

lidity was performed to determine the number of statistical associations between the scales and these practices. The results showed that the beliefs scale were associated with having a of mammogram ( $p < 0.05$ ), and visit to the gynecologist ( $p < 0.05$ ) while the knowledge scale for early-detection was associated with the clinical breast examination ( $p < 0.05$ ). The beliefs scale had better consistency than the knowledge scale. The Chronbach's alpha was 0.75 for the beliefs scale and 0.30, 0.41 and 0.43 for each defined knowledge scale, respectively.

A-97

**Incorporation of Preventive Practices in the Care of Diabetes Mellitus Type 2 Patients in a Community Health Center in Puerto Rico.** AI Moscoso<sup>1</sup>, EV La Cruz<sup>2</sup>, NL Arce<sup>3</sup>, D Quiles<sup>4</sup>. <sup>1</sup>Centro Mujer y Salud, UPR, Med Sc Campus, PO Box 365067, San Juan; <sup>2</sup> San Francisco Hospital, San Juan; <sup>3</sup>Asociación Empleados ELA, San Juan; <sup>4</sup>La Concepción Hospital, San Germán, P.R.

**OBJECTIVES:** Type 2 Diabetes Mellitus (DM), a chronic condition that affects more than 500,000 Puerto Ricans, is the third cause of death, the main cause of blindness and amputations. Guidelines for preventive care are available. Their use can prevent costly and devastating complications especially in a managed care environment. The study evaluates the incorporation of those practices in the care of patients in a community health center by family medicine physicians. **METHODS:** Type 2 diabetic patients outpatient records (N=161) were evaluated and data was collected about the characteristics of preventive measures incorporated in their care were during a complete year and compared with recommended preventive care guidelines. **RESULTS:** Documentation reflected low levels of compliance with recommended guidelines of care in the year under study. Average number of visits was 3 per year. Risk factors do not tend to be assessed as part of the patient history. A physical exam was included as part of most visits but specific exams components related to prevention of DM complications were not frequently recorded: funduscopy (28.6%), neurological (19.8%), vascular (11.3%). Most frequent referrals were done

to Ophthalmologists (28.6%) and very few to other specialists. Compliance with diagnostic tests varied. Only 22.4% of cases had a glycosylated hemoglobin which is the best indicator of level of glucose control. **CONCLUSIONS:** State of the art preventive care of DM, while available in Puerto Rico, is not documented as being incorporated as part of the regular care of the average patient. Even where protocols for proper care exist, the patient is not benefitting from their use as they are adjusted in response to economic resources. A combined effort of all concerned agencies is necessary to address this Public Health concern.

A-98

**Conocimientos de Nutrición que Poseen los Maestros de la Escuela Lincoln en San Juan.** J. Centeno, I. Anglero, M. Rodriguez. Programa de Internado en Dietética, Dept. Graduado, Colegio de Profesiones Relacionadas con la Salud, Recinto de Ciencias Médicas

La educación en nutrición en las escuelas es una estrategia para mejorar la salud. Uno de los objetivos para el Año 2000 es mejorar la educación en nutrición en las escuelas. Esta provee un ambiente ideal para aumentar los conocimientos, moldear actitudes y desarrollar destrezas en nutrición. Para lograr esto los maestros deben tener conocimiento en nutrición. El objetivo de esta investigación fue el de evaluar los conocimientos de nutrición que tienen los maestros y empleados del Comedor Escolar de la Escuela Elemental Abraham Lincoln en San Juan. Para esto se diseñó un formulario que incluía preguntas sobre nutrición. El formulario se administró a 14 maestros y 4 empleados de comedores escolares en marzo de 1997. Luego de analizar los datos obtenidos se encontró que los maestros tenían dificultad en los conocimientos de nutrición especialmente en relación a la pirámide alimentaria - colesterol y cantidad de alimentos a consumir específicamente el grupo de la leche. Los empleados del comedor tenían buen conocimiento sobre nutrición. En febrero de 1998 se ofreció una clase sobre nutrición a maestros y empleados de comedores que habían participado en la investigación de 1997. Luego de la clase se administró el

formulario nuevamente y se encontró que los conocimientos de los maestros habían mejorado en relación con la prueba inicial.

**A-99 “Evaluación de Satisfacción con Servicios Ofrecidos a los Pacientes de una Unidad Psiquiátrica Hospitalaria”** Jessica R. Rodríguez-Camacho, B.A., c.M.S. Investigación Evaluativa de Sistemas de Salud, Escuela Graduada de Salud Pública, Recinto de Ciencias Médicas, Universidad de Puerto Rico.

La evaluación de satisfacción con servicios de salud es uno de los ámbitos de calidad dirigido a recoger la opinión del paciente sobre los servicios que recibe y cuya meta final es satisfacer las necesidades de los usuarios, dentro de parámetros de alta calidad, cumpliendo así con los propósitos institucionales. Un estudio de satisfacción fue realizado en una unidad psiquiátrica hospitalaria con la finalidad de evaluar los servicios y determinar los factores que están asociados al nivel de satisfacción expresado por los participantes. Utilizando la versión en español del Cuestionario de Satisfacción de Clientes (CSQ-8 por sus siglas en inglés), se entrevistó un total de 80 pacientes hospitalizados entre los meses de abril y septiembre de 1997 en el Hospital Universitario de Carolina, obteniendo un 98.7 por ciento de respuesta. Los resultados de los análisis descriptivos demuestran que, en general, los participantes expresaron estar altamente satisfechos con los servicios recibidos (78.5%). Al realizar los análisis de regresión múltiple, la percepción comparada de su salud mental y la persona que decide el alta fueron las variables que resultaron estar significativamente asociadas al nivel de satisfacción general ( $p < 0.05$ ). Mientras mejor percibe el paciente su salud mental, en comparación a otras personas de su edad, más satisfecho tiende a estar con los servicios. De igual forma, el paciente que es dado de alta por acuerdo mutuo entre él y su médico, tiende a estar más satisfecho con los servicios recibidos. Estos resultados nos llevan a concluir que la percepción de su estado de salud y la participación en su cuidado son factores que influyen en el grado de satisfacción del paciente con los servicios psiquiátricos hospitalarios.

**A-100**

**Emergency Medical Services Program for Children and Families in Puerto Rico.** Martin, M.; González, R. ; Rivera, L. UPR School of Medicine, Department of Pediatrics PO Box 365067, San Juan, P.R. 00936-5067

This 2 years project seeks to improve the Puerto Rico Emergency Medical Services System to address children's particular needs. The goals and objectives of the project are: first build up the state EMS system in order to provide adequate service for children, second establish an adequate pediatric data base for outcome measurement and surveillance, third increase the level of knowledge, skills and resources available for first responders and hospital staff to care for children during the pre-hospital and ER phase of an emergency, and fourth to provide support to established injury prevention initiatives. A broad advisory committee will be appointed to oversee EMS for children. Rough data collected by different agencies will be linked and assessed by a software instrument designed to analyze statewide pediatric emergency database. A curriculum on pediatric emergencies and resuscitation will be developed and implemented to train first responders and ER personnel. A resource center will be developed in the program facilities and some activities will be carried out to support injury prevention initiatives in the island. Results after first quarter of this two years project are the establishment of the Advisory Committee, the development and adaptation of the curriculum, creation of a newsletter for first responders, identification and collaboration with a data collection and software designer specialist to develop a proposal for the operational project on new database system implementation project and finally the active participation and support to government, private and community initiatives such as Safe Kids-P.R., American Academy of Pediatrics-P.R. chapter, Puerto Rico Highway Safety Agency etc. Funding is provided by the Mother & Child Health Bureau (grant 724002-01) in collaboration with the UPR Medical Sciences Campus. More time is needed to collect a preliminary data to formulate conclusions and measure changes in the emergency medical service delivered to our children.



**A-101 Health Information Gateway for the Caribbean (HIGC)** Corrada-Del Rio, F; Collazo, ML; González-Pérez, M. University of Puerto Rico Medical Sciences Campus Library

HIGC is an outreach project funded by the National Library of Medicine through a contract with the University of Maryland, Southeastern/Atlantic Region of the National Network of Libraries of Medicine. The project seeks to strengthen the library's computer and communications infrastructure in order to better serve the campus community, area hospitals, and other health institutions in the Caribbean by delivering up to date and timely information where it's needed. Central to the project is the remote sites' access to the library's server in which medical and biomedical information databases are installed (MEDLINE, AIDSLINE, IPA, HEALTHSTAR, MICROMEDEX, CINAHL, ERIC, POPLINE, AND CCINFO). Activities at selected sites include computer infrastructure enhancement, user training, professional development of librarians, and faculty training in medical informatics. Services have been extended to Medical Sciences Campus clinical sites in Carolina, Canóvanas, and Gurabo, and to the library of the School of Medicine and Pharmacy of Haiti. The information service model at the campus clinical sites is designed to be replicated at other area hospitals and health institutions. The Haiti model represents the development of a state-of-the-art small medical library in an underserved and underdeveloped area. It has infrastructure enhancement, collection development, and staff training components.

**A-102 Theophylline Tablets Prepared by Using Water Soluble Diluents and Direct Compression.** D. Aldonado, S. Bosques E.S. Ghaly: School of Pharmacy, M.S.C, U.P.R.

The design and development of theophylline tablets using two different water soluble diluents was studied and compared. Based on the hypothesis that the release of theophylline may depend on the type of diluent used, the main objective of this study was to examine the physical properties of different blends prepared by using different water soluble diluents

and compacted tablets and also to test the invitro drug release. Blends containing 10% theophylline as drug model, 10% Starch 1500 as disintegrant, 1% magnesium stearate as lubricant and 79% lactose, granular mannitol or granular mannitol 2080 as water soluble diluent were used to prepare tablets by direct compression. The target tablet weight was 400 mg + 5% and the target hardness was 7-9 Kp. The data showed that granular mannitol formulations appear to be dense, with better flowability, dissolution rate and resulted in tablets of better mechanical strength. The dissolution of drug from granular mannitol was complete at 240 minutes, from granular mannitol 2080 was 95.9% and only 78.5% from lactose formulation. This study demonstrated that the use of granular mannitol as water soluble diluent has potential in pharmaceutical formulation and can offer the formulator control over both mechanical strength of the tablets and dissolution rate of the drug.

**A-103 Sustained Release Spheres Prepared by Cross Linking Technique.** S. Peca and E.S. Ghaly: School of Pharmacy, Medical Sciences Campus, University of Puerto Rico.

The use of sustained release formulations is convenient and beneficial to the patient due to cost effectiveness, patient compliance, reducing side effects of drugs among others. In this study sustained release theophylline spheres were prepared by cross linking technique using Carrageenan and different cross linking agents. The effects of different levels of polymers (2%, 3 and 4%) and different cross linking agent (potassium chloride, calcium chloride and aluminum sulfate) on the physical properties of the spheres and on drug release were investigated.

As the polymer level was increased in the formula, the size of the spheres was decreased, the bulk density increased and the disintegration time increased. The invitro dissolution data showed that the drug release was decreased as the percent of polymer was increased from 2% to 4%.

The percent drug release from spheres containing 2% polymer was 92.3% while the percent drug release from spheres containing 4% polymer was 100.9% at two hours of testing disso-

lution. Additionally as the percent cross linking solution used was increased, the drug release from spheres was increased. The percent drug release from spheres containing 2% calcium chloride as cross linking agent was 50.2% while the percent drug release from spheres prepared with 4% calcium chloride was 75.9% at 2 hours of testing dissolution. The cross linking of the polymer with cation produces a tridimensional network that may offer the formulator control over drug release.

**A-104 Polyethylene-Chlorpheniramine Maleate Sustained Release Matrix.** Y.S. Alvarez E.S. Ghaly: School of Pharmacy, Medical Sciences Campus, U.P.R.

There are many pharmaceutical products in the market today that provide some type of prolonged release of active drug. The objective of this study is to investigate the effects of using different levels of hydrophilic polymer such as Sentry Polyox WSR NF-303 on chlorpheniramine maleate release. Also the effects of drug level, polymer level and diluent type on drug release were evaluated. Formulations containing 10% drug, polymer (20, 30 and 40%) and diluent (lactose fast flo, Avicel PH-101 and Emcompress were mixed by geometrical dilution in a V blender and compacted into tablets. The invitro drug release was tested using USP Apparatus I and the swelling was evaluated by examining the weight of the tablets at different time intervals for 12 hours. The results obtained showed that formulation containing 30% polymer and lactose fast flow was easy to process and was the best compared to other formulation. The invitro drug release from formulations containing different levels of drug showed that as the level of drug was increased, the drug release was decreased. The drug release from formulation containing 4% drug was 78.8% while formulation containing 10% drug gave 64.9% drug release after 6 hours of testing dissolution. The use of Sentry Polyox WSR NF-303 provides new alternative for formulation of sustained release matrix of antihistaminic drug.

**A-105 Development and Characterization of a Topical Nystatin Gel.** D.

Quiñones and E.S. Ghaly: School of Pharmacy, Medical Sciences Campus, University of Puerto Rico.

Recently, plastic/polymeric films have evolved as novel drug delivery systems for their therapeutic and formulation advantages. The objective of this study was to evaluate the effects of combination of different ratios of polymers on the diffusion rate of nystatin from the gel. Also to investigate the rheological characteristics of different formulations. The drug level was held constant at 1.72% w/w, the level of propylene glycol which was used as cosolvent and penetration enhancer was also held constant at 2% w/w. The ratios of Carbopol 934: Methocel polymers were varied as: 10:0, 0:10, 6:4, 4:6 and 5:5. The diffusion study was performed using a donor-receptor diffusion cell and cellulose ester as a membrane. Samples were withdrawn from the receptor at different time intervals up to 24 hours. The data showed that formulation containing Carbopol: Methocel (1:0.5) gave higher percent drug diffusion (11.9% at 6 hours) while formulation containing Carbopol: Methocel (1.5:0) gave lower percent drug diffusion (5.5% at 6 hours). The rheological data showed that nystatin gel containing Carbopol: Methocel equal percent (0.75% w/w) was of high viscosity (164 -  $2.4 \times 10^3$  centipoise) while formulation containing only 1.5% Methocel was of low viscosity (400 - 600 centipoise). A successful design of topical gels with suitable plastic viscosity can be achieved by combination of suitable ratio of different polymers to obtain preparation with suitable consistency, viscosity, release of ingredients and other desired properties.

**A-106 Limited-Access Alcohol Drinking in Rats: Morphine Reduces Intake of a Preferred Concentration.** B. Hine. School of Pharmacy, Univ. of Puerto Rico

Previous work with high alcohol-sensitive (HAS) rats from the University of Colorado indicated that they drink very little 10% ethanol (ET) in 2-bottle preference tests (PT). Treatments that have been reported to increase ET drinking in other strains, such as sucrose-substitution and low opiate doses, have been ineffective in these

animals. In an earlier continuous-access PT, HAS females reacted to 1.5 mg/kg morphine sulfate (MS) doses by a reduction in forced and free-choice 10% ET intake over a 3-week period. The present study used a 2-hr limited-access test to assess the reliability of this inhibitory effect of MS, using a previously-identified preferred ET concentration and a lower acute MS dose.

Female HAS rats (n=24), 90-103 days old, were singly-caged and given access to water and a 5% (v/v) ET solution in a 2-bottle choice test for 2 hours/day (food always available) for 4 weeks with daily alternation of solution positions. During Week 3, bodyweight, water, and ET intake data were recorded, and rats were divided into 2 groups, matched on the basis of daily ET intake over the last 3 days (0.6-2.0 g/kg/day). A single 0.9% NaCl (SAL) or 0.75 mg/kg MS s.c. injection given 15 min before fluid access produced a 4.1% (SAL) versus a 12.8% (MS) reduction in ET intake ( $p > 0.5$ ) from pre-injection 3-day means. The ET:total fluid ratio increased by 6.3% in SAL rats, versus a 19.1% reduction in MS rats ( $p = 0.02$ ). These results confirm and extend previous findings of the high sensitivity of HAS rats to the depressant effects of MS on ET drinking, to a limited-access procedure using a preferred ET concentration. In future work, effects of MS on intake of ET solutions varying in palatability will be assessed.

**A-107 Prolonged Release Compacts Prepared by Using Hydrophilic Polymer.** W.M. Collazo, E.G. Class and E.S. Ghaly: School of Pharmacy, Medical Sciences Campus, U.P.R.

The controlled release of drugs has been an objective of pharmaceutical formulators in the past twenty years. One of the objective of this study is to prepare sustained release theophylline tablets using hydrophilic polymer (Avicel RC 581). The drug level was held constant at 10% , the polymer level was varied between 10%, 30 and 40% , and the remaining was Lactose Fast Flo. Magnesium stearate was used as lubricant at level of 1%. The different formulations were blended by geometrical dilution in V - Blender and were compacted by direct compression, the tablet weight was 450 mg + 5% and the target hardness was 7-9 Kp.

Tablets were evaluated for physical properties and were tested for invitro drug release using USP basket rotational method for 6 hours. The dissolution data showed that as the percent of polymer increased from 10% to 40%, the drug release decreased. Tablets prepared with lactose fast flo and 40% release 67.6% drug while tablets prepared with 10% polymer released 94.5% drug after 3 hours of testing dissolution. Additionally the drug release from compacts containing 40% polymer was found to follow the diffusion controlled model for inert matrixes up to 4 hours. The use of different levels of hydrophilic polymer such as Avicel RC-581 can modify the drug release from tablets prepared by direct compression.

**A-108**

**Modelo de Prestación de Atención Farmacéutica en Psiquiatría a través de una Pasantía Clínica.** L. González, M. Martínez Y E. Vega, Escuela de Farmacia, Universidad de Puerto Rico

**Objetivos:** Proveer atención farmacéutica a pacientes con problemas de salud mental y exponer a los estudiantes a un modelo de práctica de farmacia en el área de psiquiatría ambulatoria.

**Metodología:** Se rediseñó una pasantía en el área de psiquiatría ambulatoria con el propósito de atemperar esta a la meta de la Escuela de Farmacia de preparar profesionales con la capacidad de proveer atención farmacéutica. La actividad principal de los estudiantes es prevenir, identificar, resolver y documentar problemas relacionados con medicamentos utilizando un proceso sistemático. Este proceso incluye desde la selección de pacientes y la identificación de problemas hasta la documentación de sus recomendaciones y la evaluación de los resultados. Se trabaja en equipo y hay amplia oportunidad para interactuar con médicos y otros integrantes del equipo de salud. Además hay análisis de artículos de la literatura farmacéutica y estudios de casos. Al finalizar la experiencia práctica se administró un cuestionario para determinar la opinión de los estudiantes sobre la aportación de la pasantía a su crecimiento profesional.

**Resultados:** Diez y siete estudiantes partici-

paron de esta pasantía de 72 horas. Se evaluaron 68 expedientes médicos y se identificaron 185 problemas relacionados con medicamentos, la mayor parte de estos relacionados a la falta de pruebas de laboratorio para proveer seguimiento a los medicamentos. A través de las intervenciones de los estudiantes se proveyó atención farmacéutica a 44 pacientes .

Todos los estudiantes indicaron que consideran que la pasantía les ayudó mucho o bastante a brindar atención farmacéutica, formular un plan de intervención, documentar

sus intervenciones, evaluar la literatura, trabajar en equipo y promover la salud pública. Conclusiones: Debido al rediseño de la pasantía se logró proveer atención farmacéutica a un grupo de pacientes. Además se expuso a los estudiantes a un modelo de práctica que estos pueden implantar. Este nuevo enfoque requiere mayor esfuerzo de parte del profesor y apoyo administrativo de las instituciones involucradas. No obstante, los beneficios que resultan para el estudiante, el paciente y al avance de la profesión merecen este esfuerzo.