



ABSTRACTS

RESEARCH AND EDUCATION FORUM



ORAL PRESENTATIONS

A-1 Características de Familias en Programas de Estimulación Pediátrica Temprana.

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Se discutirán los hallazgos con un cuestionario que se le administró a una muestra tomada al azar de las familias que reciben los servicios de estimulación en los Centros Pediátricos de Puerto Rico.

El cuestionario recoge datos sociodemográficos para determinar el perfil de los usuarios de estos servicios. Además solicita identificación de las áreas en que les hace falta recibir adiestramientos.

Fue administrado por el personal que labora en los Centros Pediátricos, cuando las familias asistieron a recibir los servicios durante los meses de octubre y noviembre de 1998.

Los resultados preliminares señalan que el 90.63% de las personas que llevan a los niños a las terapias son féminas, el 76.04% se identifican como las madres, el 6.25% como padres, el 5.21% como los abuelos(as), 2.08% los encargados y el 1.04 otros.

En el área de escolaridad los resultados indican lo siguiente 3.12% tienen 6to grado o menos, 20.83 completaron el 9no grado, 30.21% completaron 4to año, 17.71% tienen un grado asociado, 17.71% tienen bachillerato, 1.04% maestría, 7.29% señalan tener otros estudios.

Esto es un proyecto desarrollado en colaboración con la Oficina de la Ley 51, el Departamento de Salud y el Proyecto Interdisciplinario de Excelencia en Servicios.



A-2 Differential Characteristics in High and Low Functioning Autism.

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Autism is a developmental disability with an underlying neurological basis. The condition has three major areas of impairment: (1) social interaction, (2) language and communication, and (3) cognitive abilities evidenced as a restricted range of activities and interests. The differential diagnosis of autism is reached through careful clinical observations made by professionals trained in this area. A child has to meet a certain number of criteria from each of the three categories as they appear in the DSM-IV. There are various behavioral conditions associated to autism that are shared by other disorders,

which complicates the diagnostic process. Nevertheless, the DSM-IV's criteria recognize that symptoms in each category can range in severity from severe to milder. Most of the DSM-IV criteria statements begin with the words "absence or difficulty" in a certain ability. This has resulted in most professionals working in the diagnosis to informally use the term of High Functioning Autism (HFA) or Low Functioning Autism (LFA). Traditionally, these terms have been used to refer to the functional outcome of adults diagnosed with autism during childhood. Furthermore, HFA should not be confused with another associated disorder, Asperger disorder. The Childhood Autism Project (PAI) is working towards refining the diagnostic process. The goal is to develop a functional as well as a differential diagnosis, based on specific empirically determined criteria. This will be a useful method in determining the child's specific treatment and educational needs. A study is being conducted to determine specific characteristics that help distinguish children with HFA or LFA among those children served by PAI. Results that have been analyzed indicate that, among other traits, the single most distinguishable characteristic is the level of social functioning, specifically social communication.




A-3 Proyecto para Aumentar Autoestima en Niños de 4to y 5to Grado.

A. B. Morales,

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Estudio pre-experimental piloto para determinar el efecto de intervenciones estructuradas para aumentar el autoestima de niños de 4to y 5to grado. La autoestima es importante para la salud mental (Bednar, 1992; Clark & Clemens, 1998). Los problemas sociales de Puerto Rico son atribuibles a poca salud mental (Parrilla, 1997). Guevara (1997) recomienda la prevención de enfermedades mentales desde la niñez. La muestra consistió de 30 estudiantes de escuela elemental seleccionados por sus maestros por problemas de conducta y por bajo aprovechamiento escolar e higiene personal pobre. Se reunieron por 2 semestres en los que se le administró una escala tipo likert de 16 reactivos para medir auto estima antes y después de las intervenciones. Se dividieron en 2 grupos de 15 cada uno, y se reunían 1 vez en semana por 2 horas. En las sesiones se realizaron diversas intervenciones, con el propósito de aumentar el autoestima de los participantes. Las edades eran de 9 a 13 años, 37% eran del sexo femenino y 63% del sexo masculino, 87% de cuarto grado y 13% de quinto grado.

El nivel de autoestima antes fluctuó entre 9 a 23, con una media =17 y el nivel después estaba entre 18 a 30, media=25. La prueba *t* arrojó que existía diferencia significativa entre el nivel de autoestima antes y después ($t = -12.148, 23gl, p = .000$). Los niños presentaron un nivel de autoestima pobre al comenzar el proyecto y al finalizar había aumentado su autoestima a un nivel moderado. La conducta de estos niños mejoró notablemente. El programa para aumentar el autoestima con niños de escuela elemental permitió mejorar el comportamiento de este grupo. Se recomienda repetir el proyecto con niños más pequeños, con sus padres o tutor y sus maestros. Limitaciones se incluye que la muestra fue por conveniencia, 6 sujetos no terminaron por deserción escolar, mudanza, cambio de escuela y un niño no continuó debido a que la estudiante de enfermería con la cual había establecido confianza se gradúo.



Manejo Colaborativo en Terapia Ocupacional y Patología del Habla en el Ambiente Escolar de un Cliente con Diagnóstico de Epilepsia y Digénesis Cerebral.

A-4

A. Nuñez.

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Se presentará trabajo colaborativo en Terapia Ocupacional y Terapia del habla con un niño con epilepsia y digénesis cerebral. Como técnica terapéutica se utilizan terapia sensorial integrativa, mientras de forma simultánea y estructurada se trabaja con el uso adecuado de objetos, función pragmática de pedir, entre otros. Los resultados preliminares indican que el niño ha logrado permanecer sentado trabajando en diferentes actividades entre 5 a 20 minutos en terapia. En el salón de clases se ha observado que puede permanecer sentado en su pupitre por períodos de tiempo que anteriormente no toleraba. La maestra de educación especial ha expresado su satisfacción por la ejecución del niño desde que está recibiendo los servicios



Cognitive and Language Skills of Puerto Rican Juvenile Offenders.


A-5

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There is a strong relationship among juvenile delinquency, dropping out of school, communication disorders, and at-risk social environment. This calls for

investigations that assist health professionals in understanding these Puerto Rican juvenile offenders and their characteristics in order to address those factors that trigger delinquency acts against their communities and schools. A literature research suggests a link between juvenile violence and language disabilities. With this in consideration, we investigated the profiles of juvenile offenders in order to investigate their alcohol/drug consumption, firearm use, and language/cognitive behaviors. A sample of 9 juvenile offenders between 12;6 and 14;0 years of age was evaluated. These youngsters were evaluated to assess their language skills and their cognitive abilities. For these assessments we used the Woodcock Language Proficiency Battery and the Test of Non-Verbal Intelligence. A questionnaire on their life histories was administered to these youngsters to obtain information on their delinquency background, school performance, attitudes, and family environment. Each youngster was evaluated during 120 minutes. Results indicate that 77% of them had moderate – to – severe language and cognitive disabilities characterized by difficulties in inferring rules, remembering information, and expressive vocabulary.



Cognitive-Behavioral Processes That Affect Substance-Dependent Schizophrenic African-American Males' Completion of Inpatient Treatment,

A-6

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Cognitive-behavioral factors that affect inpatient treatment completion were identified and described in a sample of 139 male veterans, using the Relapse Prevention Model (Marlatt & Gordon, 1985) as the theoretical framework. A comparison of the completion group with the noncompletion group revealed which variables are significantly associated with completion of dual diagnosis inpatient programs. The majority of the participants were African-American, had completed at least a high school education, averaged 39 years of age, and were homeless, although they averaged a net pension of \$646 per month. All patients had a history of severe mental illness and long-term dependence on cocaine, with a mean of nine previous psychiatric hospitalizations. More than half did not complete inpatient treatment, with a peak noncompletion occurring during the first three-day interval at the beginning of the month, shortly after the arrival of their disability payment. Among noncompleters, more thought it would be difficult to complete treatment, although their goal was total abstinence. Cocaine relapse

was found more among noncompleters who did not have a case manager assigned. Among those patients who relapsed while in the hospital, more gave self-medication of schizophrenic symptoms as the explanation for cocaine use. Noncompleters were found to have more positive symptoms of schizophrenia, particularly thought disorganization and tension clusters. Recommendations were provided in terms of significance of findings for the improvement of nursing care in serving this challenging psychiatric population. (Supported by educational grants from EMFP and SAMHSA, American Nurses Association).



Differential Diagnosis in Autism.

A-7

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Autism is a neurobiological disorder that affects almost all areas of development, with the social and communication delay and interest stereotyped behaviours the most prominent features of the disorder. The disorder is first apparent when the child is between 1 ½ to 3 years of age and has some specific behaviours that need to be present in order to be diagnosed. There are some developmental disorders that can be wrongly diagnosed as autism because they have some common traits. Some of them can be ADHD, sensory modulation disorders, mental retardation, language disorders, and genetic syndromes. The social aspect of the autism disorder seems to be the most important one for the differential diagnosis. Some children who have been referred to the Diagnostic Clinic at the Infantile Autism Project are being wrongly referred to as autistic. During year 1998-99 the clinic has received some of these misdiagnosed cases. We are conducting an investigation to determine those traits that distinguish autism from these other disorders. Preliminary findings indicate that social-emotional aspects and developmental history constitute the most distinct characteristics of this condition.



Perfiles del MMPI-A de Adolescentes con Trastornos Emocionales y Normales.

A-8

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Estudio descriptivo comparativo con el propósito de determinar la adecuación del MMPI-A con adolescentes entre 14 a 18 años. Este instrumento se está usando en

Puerto Rico y sin embargo solo ha sido estudiado por García (1995) y en este estudio. Es necesario continuar validando esta herramienta en nuestro país. Se escogieron 52 sujetos diagnosticados con psicopatología que recibían servicios en diversas clínicas ambulatorias. El segundo grupo consistió de 61 adolescentes sin historial de psicopatología. Los resultados reflejan un aumento significativo en las escalas que miden el fenómeno de depresión tanto en el grupo "normal" como el que sufre psicopatología. Este estudio permite evidenciar que el MMPI-A ayuda a discriminar depresión entre grupos de adolescentes "normales" y con psicopatología. Los participantes obtuvieron puntuaciones significativamente altas en las escalas que medían depresión tanto en los que tenían diagnóstico como en las adolescentes sin diagnóstico.



Expressive Language Skills of Typical Puerto Rican Toddlers.

A-9

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Within the child development realm there is an urgent need to develop normative scales on which speech-language pathologists, physical therapists, psychologists and other health care professionals can base their evaluation, assessment and treatment decisions. There are few studies with very young Puerto Rican children. We conducted a study of eight normal Puerto Rican children (4 girls-4 boys) between the ages of 2;0-2;6; to determine the normal parameters of language expression. Their normality level was determined through individual observational assessments by graduate students in communicative disorders. The testing was conducted in clinical rooms, and took between 40 to 55 minutes. Through the elicitation of spontaneous speech and language from the children with the use of a standard script, results indicate that the toddlers produced more than 50 utterances and showed a highly variable vocabulary with different semantic categories within a time frame of 30 to 40 minutes. The mean length in words of their utterance was nearly 3 words.



Modelo de Enseñanza Estructurada e Inclusión a la Inversa para Niños con Autismo.

A-10

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Cuando se buscan métodos de intervención para niños con la condición de autismo, casi siempre se habla de sistemas segregados. Ante la preocupación de que una de las áreas de mayor dificultad en esta condición es el área social y de interacción, se estableció un grupo piloto de inclusión a la inversa (mayoría de niños con autismo – 3, minoría de niños típicos – 2). En el mismo se pretendía exponer a los niños a lo que es la condición de autismo y a lo que es el desarrollo típico. Se esperaba ver cambios en áreas como social, verbal e imitación. Para los niños típicos se esperaba que aprendieran a compartir con niños especiales, además del progreso en destrezas típico del niño al cual se le trabaja un aprendizaje dirigido. Al evaluar un período de 4 meses (agosto a diciembre), se evidenció que el progreso no fue tan dramático como esperábamos para los niños con autismo. Por ejemplo en el área verbal hubo dominio de hasta 4 destrezas en los niños típicos versus 1 destreza en los niños con autismo. Asimismo, en el área social hubo dominio de hasta 10 destrezas en los niños típicos versus 4 en los niños con autismo. En el área de imitación el que mejor progreso tuvo se igualó a los típicos, pero en los otros dos niños el progreso fue mucho menor. A pesar de estos resultados, cuando se entrevistó a los clínicos estos reportaron cambios significativos a nivel social, imitativo y de interacción. Se llegó a la conclusión que estos cambios no se podían medir a través de los protocolos utilizados. Por lo tanto, se desarrolló un formulario que mida estas conductas y el mismo se está utilizando para evaluar el cambio.



A-11 Slow Pressor Response of Angiotensin II and Oxidative Stress.

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It has been shown that chronic infusions of sub pressor doses of angiotensin II (AII) produces a progressive and significant increase in blood pressure. Since large doses of AII stimulates oxidative stress (OS) it has been thought that his effect could be responsible for potentiating the pressor responses of AII. This study was undertaken to examine if the development of slow responses of AII is accompanied by increased OS. This effect was estimated by measuring the concentration of 8-epi Iso prostaglandins

F-2alpha (isoprostanes), which is a sensitive marker of super oxidation. The study was conducted in 14 male Sprague-Dawley rats (250-350 GM) which blood pressure (SBS) was measured daily by a tail cuff method. After a control period of seven days the animals were divided in two groups. The experimental group (EG) (n=7) was implanted with the Alzet mini pumps that deliver an infusion of AII at the rate of 5 ng/kg/min for two weeks. The control group (CG) (n=7) received the vehicle (0.95 saline) during the same period. The last day of the experiment the rats were bled by heard puncture under anesthesia for determination of plasma levels of isoprostanes and plasma renin activity (PRA). It was found that SBP of the EG exhibited a significant increase from 114+3.6 to 145+3.1 mmHg. In contrast the SBP of the CG remained unchanged (116+3.5 to 124+2.3 mmHg). The hypertension of the EG was not accompanied by changes in free isoprostanes when compared to control group (207+48, 194+52). In addition, no differences were found in PRA between both groups (14.82 vs 15.12 ng/ml/hr). These results were interpreted as indicating that the development of hypertension due to slow responses of AII may not be accompanied by an increase of OS.



A-12 Mi Hijo/a y Yo en la Escuela: Un Modelo Colaborativo para el Desarrollo de Iniciativas Comunitarias.

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La Clínica de Pediatría Primaria ubicada en el Centro de Salud Familiar de Cataño, promueve la participación activa del Residente de Pediatría y demás personal profesional de apoyo en iniciativas comunitarias como lo es el proyecto Mi Hijo/a y Yo en la Escuela. Este proyecto es una iniciativa de la Clínica y ha sido coordinado en colaboración con el Distrito Escolar de Cataño y Bacardi Corporation. El mismo se llevó a cabo por cinco miércoles consecutivos durante los meses de agosto y septiembre de 1997. Los objetivos específicos del proyecto fueron fomentar y capacitar a los padres y madres de las siete escuelas elementales del Distrito Escolar de Cataño que por primera vez tienen niños/as en la escuela, en cada una de las siguientes áreas: a) participación plena en la comunidad escolar de sus hijos/as; b) cómo facilitar el desarrollo integral del niño/a utilizando el conocimiento sobre aspectos preventivos de salud y aspectos psicopedagógicos; y c) utilización de modelos y estrategias de crianza y disciplina que estimulen el desarrollo

educativo del niño/a y que a su vez propendan el desarrollo de un ciudadano ejemplar. Estos objetivos fueron atendidos a través de 8 charlas o talleres de los cuales 6 fueron ofrecidos por residentes y personal de apoyo de la Clínica de Pediatría Primaria. La participación de padres durante las cinco fechas fluctuó entre un máximo de 67 participantes a un mínimo de 44. Los participantes evaluaron como primordialmente sobresaliente la preparación de los conferenciantes y la manera en que comunicaron la información. A su vez, evaluaron la información ofrecida como de mucha utilidad indicando que su conocimiento sobre el tema aumentó mucho. Se llevó a cabo una evaluación Post-Proyecto un año más tarde (septiembre del 1998). Las evaluaciones de los participantes sugieren que los objetivos del proyecto fueron logrados. Además, éste proveyó el ambiente y experiencias adecuadas para facilitar y promover la participación activa del residente de pediatría y personal de apoyo en actividades que promuevan el desarrollo y mejoramiento de la comunidad.



A-13 Neurodevelopmental Outcome in Infants with Antenatal Exposure to Thyroxine and Glucocorticoid.

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Respiratory distress syndrome (RDS) is a common and serious complication of the premature infant. Antenatal glucocorticoid and intra-amniotic thyroxine have been used to accelerate lung maturation. Few follow-up studies addressing the long term effects of the antenatal administration of these drugs have been reported in the literature. The Australian collaborative trial reported detrimental effects at 12 months follow-up of infants exposed to antenatal thyrotropin-releasing-hormone and glucocorticoid. In view of these findings, we evaluated the neurodevelopmental outcome of infants who received either intra-amniotic thyroxine combined with glucocorticoid versus steroids alone. **Methods:** Patients were assigned to a double-blind randomized controlled trial using the two drugs. Neurodevelopmental evaluation was performed using the Bayley Scales of Infant Development and the Child Behavior Checklist. Other studied variables included medical complications, growth parameters, and sociodemographic status. Wilcoxon rank test or t-test was used for the analysis. **Results:** Forty-five infants were evaluated at 20 months of age. No statistical difference in neurodevelopmental outcome was found between the two groups.

| Treatment Group | MDI | PDI | Behavior | LDA | CDA |
|---------------------|------|------|----------|-------|-------|
| Steroids n=23 | 77.3 | 82.4 | 71.6 | 13.25 | 16.5 |
| Thyrox/steroid n=25 | 79.6 | 83.1 | 81 | 14.97 | 17.63 |
| p value | 0.61 | 0.76 | 0.18 | 0.14 | 0.54 |

MDI=Mental Developmental Index, PDI=Psychomotor developmental Index, CDA=Cognitive Developmental Age, LDA=Language Developmental Age) **Conclusions:** The preliminary data showed no significant differences in neurodevelopmental outcome between the two treatment groups at 20 months age. No detrimental effects were found with the two drug regimens. (Funded by RCMI-Clinical Research Center).



A-14 Two Years Experience with Universal Hearing Screening of Newborn Infants in the University Pediatric Hospital.

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Background: Reduced hearing acuity during infancy and early childhood interferes with the development of speech and verbal language skills. The prevalence of newborn and infant hearing loss is estimated to range from 1.5 to 6.0 per 1,000 live births. The most important period for language and speech development is the first 3 years of life. In United States the average age of hearing loss identification is around age of 3. The National Institutes of Health Consensus Development Conference Statement of March 1993 recommended the implementation of universal newborn hearing screening to promote the early identification of hearing impaired infants. The Neonatal Intensive Care Unit of the University Pediatric Hospital in San Juan, Puerto Rico has been offering hearing screening to its high risk population since 15 years ago, but in the last 2 years this screening was extended to all the infants prior to discharge from its units. **Methods:** Study population consisted of 884 infants discharged from nurseries during a two year period (1997-1998). Hearing screening using clinical auditory brainstem response (ABR) was done during 1997 and automated auditory brainstem response was started during the 1998 to all the infants prior to discharge and with more than 34 weeks post conceptional age. Exclusion criteria included patients with tracheotomies, oxygen dependency, intravenous fluids on scalp, and patients on incubators. Infants who failed the screening test were followed up in 1 month with a clinical ABR. **Results:** Screening was achieved for 76%

of the infants in 1997 and 87% in 1998. Successful screening was achieved in 85% of the patients in 1997 and 97% of the patients in 1998. Six patients were identified to have bilateral moderate to profound hearing impairment for an incidence of 1.8% and 1.5% for each year, respectively. **Conclusions:** The incidence of moderate to profound hearing impairment (sensorineural and conductive) was low when compared to other programs. A larger sample is needed to establish the real incidence since a yearly variation is reported. Screening of well baby nursery is also needed to determine the exact incidence of hearing impairment in our population. (Funded by the Council Development Deficiencies Contract #961321).

A-15 Use of Home Remedies in Two Primary Care Pediatric Centers.

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A clear definition agreed by investigators of what *alternative medicine* means, is not well established. In spite of the lack of a clear definition of the term, the truth is that the prevalent use of this type of alternative therapies is high in the general population (Eisenberg *et al.* 1993). Most articles in the literature, although reported, have not included data on the prevalence of use of alternative medicine for the pediatric population. The only article in the US focused on children was done in 1994 exploring hispanic mothers' beliefs and practices regarding children's health problems. Home remedies often constitute the first line of attack against illnesses in the hispanic population (Mikhail, 1994). Nevertheless, there is no scientific data that confirms this statement. Are home remedies a significant aspect of children health care? The purpose of this study is to determine and compare the frequency in the use of home remedies in a hispanic group of children attending a public primary center and a group attending a private practice. *Home remedies* are defined in this study as common home-made products used for health care, such as herbal teas, juices or plant extracts; its use usually based on cultural and traditional beliefs. The study seeks also to explore how mothers define a home remedy; patterns and circumstances in which they use these remedies on children; and reasons that motivate their choice of use. A descriptive cross sectional study was done with a sample which consisted of parents of children attending a primary pediatric clinic, both at a public and a private center. A questionnaire explored several variables related to the patterns and circumstances of use of home remedies. Eighty-two percent of the

surveyed population know about home remedies and seventy-one percent use these remedies in the pediatric population. An effort to avoid secondary effects of medications was found to be the principal motivation to use home remedies. Home remedies are mostly used to treat conditions that affect the gastrointestinal tract, respiratory system, skin and headaches. It would be very useful to study in detail home remedies with its effects in order to give adequate health care and orientation to parents based on customs, beliefs and traditions.

A-16 The Use of the Standardized Patient Methodology in the Teaching of Skills for First Response Personnel.

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In the past years, funds have been allocated for the development of Emergency Medical Services for Children Programs throughout the nation. A common core element in these projects is the offering of training activities for first responders and hospital personnel. However, it still remains a challenge how to train adults with different areas of expertise, to deal with crisis and emotional situations. The Standardized Patient Methodology has proven to be an excellent educational strategy for adults in developing skills to deal with emotional crisis. The trainees of the PR-EMSC Project Course on Pediatric Emergencies are exposed to two standardized situations with the following objectives: learn to manage difficult patients, learn effective ways to deal with own emotions, demonstrate skills to calm dysfunctional patients, learn to inspire hope and trust, and demonstrate adequate use of communication skills. One hundred fifty trainees have experienced the situations including among them, physicians, nurses, emergency medical technicians, paramedics, policemen, firefighters, social workers, and teachers. They have rated the experience as extremely valuable and realistic, and recommend it to be applied to all training programs available in this area.

A-17 Ocurrencia de Eventos Trascendentales en la Embarazada y los Efectos Adversos en Nacidos Vivos - Hospital Municipal de San Juan: 1995-96.

Rivera-Tubéns H, Ramos G, y Díaz N.

Diseño retrospectivo, de casos y controles. Se cuantificaron las variables presentes en cada participante como posibles factores de riesgo mediante un índice. Se comparó el índice de cada grupo: el grupo de casos que

fueron las mujeres con bebés pretérmino, y/o bajo peso al nacer (n=532), y el grupo de controles que fue una muestra aleatoria de mujeres con bebés a término, y de más de 2,500 g. (n=1194).

Existe una diferencia estadísticamente significativa en la edad promedio de ambos grupos, siendo la edad materna de los casos mayor que la de los controles. La proporción de intentos e ideas suicidas fue dos veces mayor en los casos que en los controles ($p < 0.01$). Se encontró relación entre tener un bebé de bajo peso al nacer o pretérmino y haber experimentado dos o más eventos trascendentales durante el embarazo. ($p < 0.05$). No se evidenció una relación estadísticamente significativa de otros eventos de forma individual.

En la atención de la mujer embarazada se deben identificar, atender y prevenir los factores de riesgo social. La prevención de estos eventos trascendentales durante el embarazo promoverá bebés más saludables.



A-18 Assessing Diets of Children Either Exposed or Not Exposed to Environmental Tobacco Smoke.

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Twenty-four hour dietary recalls (24h) and food frequency questionnaires (FFQ) are two commonly employed instruments to assess diets. It is known that dietary intake can depend on many factors including age, gender, ethnicity and environmental factors such as exposure to environmental tobacco smoke (ETS). We have assessed dietary intake using 24h and FFQ in children either exposed or not exposed to ETS focusing on 2 nutrients: energy (E), a macronutrient and vitamin C (vit. C), a micronutrient important as an antioxidant. Comparisons were made on the basis of age, gender and body mass index (BMI) in 513 children ages 2-12 routinely visiting the Cataño Health Center. Mothers of the children completed both dietary questionnaires as well as one on exposure to ETS. Results show that 24h values fall below FFQ estimates being about 70% for E and 55% for Vit. C, respectively. Data from the 24h suggests that vit. C consumption in children exposed to ETS is below that of non-exposed children and that boys consume more vit. C than girls. Comparisons of FFQs and 24h of children in the upper vs. the lower quadrants of BMI indicate that lighter weight children tend to overestimate their intake of E and vit. C while heavier children tend to

underestimate these nutrients. Overall conclusions are that FFQ and 24h provide similar information about E and vit. C albeit with actual values for 24h being below FFQ estimates. It also appears children exposed to ETS consume less vit. C than non-exposed children, a fact that could have implication for increased disease risk. Supported by USDA NRI Grants Progr. #94-37200-0602.



A-19 Hyperinsulinemic Hypoglycemia of the Fetus Disrupts the Development of Langerhans Islands of the Pancreas.

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The processes needed for normal development of Langerhans islands of the pancreas during the prenatal period are not known. Early in embryogenesis the alpha and beta cells are dispersed throughout the parenchymal tissues, only later to migrate together to form the structural units essential in carbohydrate metabolism. We are proposing that at least periodic hyperglycemia in fetal plasma is needed to motivate the alpha and beta cells to aggregate. The contemporary management of patients with diabetes mellitus in pregnancy emphasizes strict control of glucose levels in maternal plasma. Because of the large glucose gradient between maternal and fetal plasma, it is possible that the desirable glucose concentration in maternal blood would produce too low a concentration of glucose in fetal blood for optimal development of the Langerhans islands. To test this hypothesis we examined the pancreas of fetal Rhesus monkeys made hypoglycemic by implantation of insulin ejecting micropumps between 113 and 120 days of gestation (term=167 days). The pumps remained implanted for a period of 2 weeks until the time of delivery by hysterotomy. The fetal glucose level at delivery in the hyperinsulinemic group was 20.8 mg (range 12.0-30.0) and 35.0 mg (range 25.0 to 50.0) in the age matched control. The hyperinsulinemic group exhibited the expected changes in organ size and weight excluding that of the brain. The results regarding fetal macrosomia have been reported previously (Diabetes 33:7, 656-670 1984). Histologic examination of the pancreas of the hypoglycemic and hyperinsulinemic fetus revealed a complete absence of Langerhans islands with the beta cells dispersed throughout the parenchymal tissue. In contrast, the control animals showed completely formed

Langerhans islands. We conclude that fetal hypoglycemia disrupts the processes needed to form Langerhans islands in the primate. *presently at Dept. of OBGYN, Harvard University, Boston, MA, USA.



A-20 **Pregnancy Outcome of Patients with Antiphospholipid Syndrome Treated with Heparin/Low Dose Aspirin And Intra-Amniotic Thyroxine.**

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Antiphospholipid syndrome (APS) is associated with a high incidence of perinatal morbidity and mortality. Since most complications affecting the fetus-neonate are related to acute and chronic malfunction of the placental, the risk increases throughout pregnancy. Treatment with heparin and low dose aspirin, and close fetal monitoring, with intervention as needed has been relatively effective in treating this condition, but the success has been limited by complications of prematurity associated with early delivery. We present our experience with acceleration of fetal maturity with intra-amniotic thyroxine in these patients. A total of 20 patients with APS were studied. Prior to this study, a total of 44 APS complicated pregnancies were analyzed. Among these, there were 14 abortions (31.8%), 9 second trimester fetal deaths (20.4%), 6 third trimester fetal deaths (13.6%) 7 neonatal deaths (15.9%) and 8 live births (18.2%). Average GA at delivery was 27.7 weeks (range 20–41). In the present series all patients were treated with combination of heparin 10,000 units sc bid, and aspirin 81 mg orally each day started during the first trimester. Intra-amniotic thyroxine 500 Fg weekly was given starting at 28 weeks of GA until an L/S ratio of 2.0 or greater was documented. If not occurring spontaneously, delivery was accomplished electively at 35 weeks. Among this group there was 1 abortion in association with an amniotic band syndrome. A total of 19 live pregnancies were achieved with no documented perinatal mortality. Average GA at delivery was 34.7 weeks. Eight patients (18%) delivered prior to 34 weeks with no associated perinatal morbidity.

Conclusion: treatment with heparin, low dose aspirin, and intraamniotic T4 was effective in improving perinatal outcome in these patients. Acceleration of fetal maturation eliminated the complications associated with preterm birth, and allowed an earlier (35wk) termination of pregnancy.

A-21 **Development of a Cultural-Sensitive Breast Cancer Early Detection Brochure for Low Income Women in Puerto Rico.**

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Breast cancer is the major cause of death women older than 50 years in Puerto Rico. Most of these cancers are diagnosed at a late stage. In the last ten years several organizations had implemented early detection programs islandwide, including educational materials related to clinical breast exam, breast self-exam, and mammography. We hypothesized that the present educational materials are not sensitive to the needs of the low-income women in Puerto Rico. To test this hypothesis we conducted several focused group sessions with low-income puertorrican women attending a breast clinic. The characteristics as well as the capacity to transmit the basic concepts of an early detection program were assessed. The questionnaire was given to 235 women with a mean age of 57 ± 4 years. Most of the participants (95.8%) were puertorrican. Seventy five percent of these women participated in the focused groups. Most women (51.7%) had an educational level below high school. Only 51.1% read the newspaper. Most women (87.2%) had received some kind of educational materials related to early breast cancer detection. All of the agree the information had been helpful. Most of them (95.7%) will like more information in Spanish. Only 10.6% will like to receive information in English. Evaluation of the brochures showed that this group of women prefer: large letters, simple sentences, no graphs, photos or diagrams, medium size brochures, and information about community organizations they can call. Based on these results we developed an early detection brochure for low-income puertorrican women in Spanish. This brochure is available to physicians as well as community organizations.



A-22 **Breast Cancer Screening: Knowledge, Beliefs and Practices Among Older Puerto Rican Women.**

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This presentation describes older Puerto Rican women's knowledge and beliefs of breast cancer and screening tests, and the relationship between them and reported screening practices. It focuses on the knowledge and misconceptions that the women have of breast cancer and screening

methods and the extent to which they follow recommended guidelines or are concerned about breast cancer.

Data were gathered through interviews with 500 Puerto Rican women 65 and older. Analysis of the data indicate that clinical breast examination was the most often used early detection practice, followed by the mammogram; with self examination a distant third. Most of the respondents know about the ways to detect breast cancer and of some symptoms related to it, and also that the possibility of breast cancer increases with age. In terms of beliefs, most of the respondents thought that hitting, bruising or hurting the breast can cause breast cancer. Many also believed that women who had never had intercourse were not at risk of developing the disease. Education and age were determining factors for differences in knowledge and beliefs. Analysis also focused on relationship between a specific knowledge or belief item and detection practices. Research on minority older women should focus on detection and recognition of personal factors that hinder following recommended screening.



A-23 Breast Cancer Detection Programs did not Increased the Diagnosis of Early-Stage Breast Cancer.

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Breast cancer is the most common cancer in puertorrican women. In addition, it is the major cause of death in women after 50 years. Several early detection programs have been instituted in the island over the last decade. We hypothesize that these programs have increased the detection of early stages of breast cancer in this population. To test this hypothesis, we merged the cancer registries databases from two major hospitals in Puerto Rico: the University District Hospital and the I. Gonzalez Martinez Oncologic Hospital. The data was analyzed using the Access Program. A total of 8,025 patients were accrued from 1990 to 1995. Breast cancer accounted for 1357 cases (17%). The distribution of cases by stage were: stage I-40(2.9%), stage II- 137 (10.1%), stage III-616 (45.5%), and stage IV 564 (41.6%). The most common pathologic diagnosis were infiltrating duct cell carcinoma (77.1%), comedocarcinoma (13.9%), medullary (12.9%), lobular (2.8%), inflammatory (2.6%),

and others (10.6%). Surprisingly, 52% of cases presented metastatic disease at the time of diagnosis. The most common site of metastatic diagnosis were: lymph nodes (77.5%), bone (11.4%), lung (4.0%), brain (4.2%), liver (1.5%), skin (0.4%), and other (1.0%). Based on these findings, we conclude that contrary to our hypothesis, in our population most women are diagnosed at a late stage of breast cancer. These results demonstrate: 1) the urgent need of up-to date population-based statistics, and 2) the need of prospective studies to analyze the cultural, economical, and physical barriers to efficient early detection programs in our population.



A-24 Bcl-x1 is a potent Inhibitor of Apoptosis in Bone-Metastatic Breast Carcinoma Cells.

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Apoptosis or programmed cell death is one of the regulatory mechanisms by which the cancer cell survives in a hostile environment. This phenomenon is regulated by different groups of proteins with different intracellular targets. The bcl-2 family and the caspases family are the most widely studied. The former is located adjacent to the mitochondrial membranes, while the latter is present mostly in the cytosol. The bcl-2 family consists of a group of apoptosis inhibitors (ex. bcl-2, bcl-x1), apoptosis promoters (ex. bax, bak), and survival antagonists (ex. bcl-xs). At present, the role of these proteins in the apoptosis mechanisms of bone-metastatic breast carcinoma cells is unknown. Due to the widespread distribution of bcl-2 in malignant tumors, we hypothesize that bcl-2 is one of the major proteins involved in the apoptosis mechanism of bone-metastatic breast carcinoma cells. To test this hypothesis, we determine the expression of bcl-2, bax, bcl-x1, bak, and bcl-xs in parental as well as bone-metastatic breast carcinoma cells. Denatured (SDS-treated) cellular extracts were run in Western blots, transfer to nitrocellulose membranes, exposed to the appropriate antibodies, and detected by the horseradish peroxidase method. A total of 80 mg of protein was loaded in each lane. Molecular weight standards were used in all experiments. The Excel program was used to estimate the molecular weight of the unknown bands. Cellular extracts from doxorubicin-treated MCF-7 cells were used as positive control. There was no difference in the expression of bcl-2, bax, or bcl-xs between the parental and bone-metastatic breast carcinoma cells. None of the cell lines expressed bak. Only bcl-x1, a potent inhibitor of cell death, was significantly overexpressed in the bone-

metastatic breast carcinoma cells compared to the parenteral cells. Bcl-x1 inhibits apoptosis in bone-metastatic breast carcinoma cells. Bcl-x1 has been shown to play a role in the cell's response to oxidants, and resistance to chemotherapeutic agents and radiation therapy. Further studies will elucidate whether it might be a good target for the development of new treatment modalities.



A-25 **Dramatic Impairment of Dehydrogenase Activity in a Chronically Infected HIV-1 Cell Clone Treated with Dexamethasone or SIN a Nitric Oxide Donor.**

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Pathogenic mechanisms in HIV infection involve a cellular imbalance in oxidative/reductive processes, resulting in the formation of higher than normal levels of toxic substances such as nitric oxide, and in the depletion of precursors for the synthesis of molecules such as DNA. In this sense dehydrogenases are key molecules involved in such imbalances, as they participate in crucial steps of metabolic pathways such as glycolysis or the Krebs cycle. Thus, their functional integrity is required for cell survival. We wanted to examine the dehydrogenase activity of cells induced into apoptotic death by two different pathways. For this, we measured the activity of lactate (LDH), glucose 6-phosphate (G6PDH), NADH (NADDH), isocitrate (ICDH), glycerol 3-phosphate (G3PDH) and glutamate (GIDH) dehydrogenases in 48 and 72 hour cultures of HIV-infected cells treated with dexamethasone and SIN-1, a nitric oxide donor, using a CTC tetrazolium reduction assay and spectrophotometry. Our results indicate that both agents reduce the cellular activity of all dehydrogenases measured; LDH activity in particular, is almost totally abolished while for the other dehydrogenases at least 50% of their activity is lost upon treatment. This interference of both types of treatment with crucial metabolic pathways is reflected in the loss of cell viability measured by trypan blue and propidium iodide staining, or in the loss of mitochondrial membrane potential, which are characteristic of apoptotic death. This experimental model promises to be very useful in future studies of the role of Dehydrogenases during the apoptosis - driven depletion of lymphoid cells during HIV infection. Supported by: RCM1- RR03051, RCM1-Clinical Research Center Grant RR11126-04, MBRS - S06-GM08224 and Deutscher Akademischer Austauschdienst.

Barriers to Enrollment of Minority Populations in Clinical Trials: Role of the Primary Physician.

A-26

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Accrual of breast cancer patients to clinical trials in minority populations is very difficult. In our experience, less than 5% of breast cancer patients are evaluated to participate in clinical trials. Educational activities to promote patient participation in clinical trials usually focused on patient information. This practice has had little impact in the accrual of patients in minority populations. To assess the role of primary physicians in the accrual of patients to clinical trials in our community we developed a questionnaire to evaluate: 1) the knowledge, 2) attitudes, and 3) patient referral patterns for clinical trials. A total of 40 primary physicians from the island were evaluated. The mean age was 47.5 ± 12.5 years. Twenty-six (65%) were males and fourteen (35%) were females. Most physicians (87.5%) knew that there were clinical trials for cancer prevention, diagnosis, screening, and treatment. They also recognized (82.5%) that these studies are available in the island. Most of them (85%) considered that patients that participate in clinical trials are not "guinea pigs". All of them agree that informed consent is a critical aspect in the execution of clinical trials. A 62.7% consider that they will not lost a patient if they participate in clinical trials in other institution. Interestingly, only 25% offer clinical trials as an alternative to their patients. Most of them recognized that the most important limiting factor is their core knowledge about clinical trials. Based on these results we conclude that any campaign to increased accrual of minority populations to clinical trials should also targeted the primary physicians in the community.



A-27 **cAMP Protects Endothelial Cells from Tunicamycin-induced Apoptosis *in vitro*.**

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We have reported earlier that the N-glycosylation inhibitor tunicamycin (TM) blocked capillary endothelial cell proliferation in a dose and time-dependent manner by inducing apoptosis. Accumulation of immunopositive Factor VIII:C suggested that under-/non-glycosylated cellular glycoprotein might serve as an endogenous inducer of apoptosis. In addition, we have observed that

cAMP enhanced Glc₃Man₉GlcNAc₂-PP-Dol biosynthesis as well as Factor VIII:C N-glycosylation and consequently potentiated the endothelial cell proliferation. We have, therefore, asked if cAMP could protect the cells from the TM action. In our study apoptosis was induced in a synchronous culture by exposing the cells to TM (1 ig/ml) for 32 hours. The apoptotic response was not dependent on additional protein synthesis, since cycloheximide (1 ig/ml) had no effect. Addition of 8Br-cAMP (2 mM), forskolin (1 iM) and IBMX (50 iM) however, abolished the apoptotic response in an order 8Br-cAMP (66%)>forskolin (46%)>IBMX (42%). Under the current experimental condition, cAMP protection was significant for 72 hours. In conclusion, activation of Dol-P-Man synthase by cAMP-dependent protein phosphorylation either allosterically activates the GlcNAc-1P transferase or upregulates its gene expression thereby neutralizing the TM effect. Supported by CIDIC funds from the University of Puerto Rico Medical Sciences Campus.



The Effect of Dietary Vitamin E on Breast Cancer Risk Aurora Vassos*

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A case-control study was conducted to evaluate the association between tocopherol (dietary vitamin E) and breast cancer. Cases and controls were similar ($p > 0.05$) in demographic and socioeconomic characteristics as well as in dietary intake in addition to other breast cancer risk factors such as oral contraceptives, hormone replacement therapy and family history of breast cancer in relatives. Participants completed a Food Frequency Questionnaire to collect dietary information to test the study hypothesis as well as to evaluate the effect of dietary confounders. The crude odds ratio for breast cancer and α -tocopherol intake (≈ 8 mg equiv) was 0.77 but this protective effect was not statistically significant (95 % CI = 0.31 - 1.90). Total kilocalories, polyunsaturated fat and percent calories from fat were distributed equally among cases and controls. Cases and controls were statistically different regarding total fat, saturated fat, monounsaturated fat and cholesterol. These variables were potential confounders and produced statistical interactions. This study showed a non statistically significant 23 % reduction in risk for breast cancer when the dietary intake of tocopherol in cases and controls was compared, probably due to similar intake of dietary fat, the main source of vitamin E.

A-29 **A New Sonographic Sign for the Detection of Congenital Cleft Palate.**

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The diagnosis of cleft palate is usually a difficult task. This is mostly due to the fact that this is a domed tri-dimensional structure that is difficult to demonstrate within the two-dimensional constraints of sonographic imaging even when normal. In most cases, diagnosis of cleft lip leads to the detection of a cleft palate.

Normally, with the mouth closed, the palate comes in contact with the tongue making visualization of this structure impossible. However, in cases of a cleft palate, fluid is present in this open space creating an acoustic window that allows clear identification of the tongue surface. By visualizing the face in profile (mid-sagittal view) these findings are evident.

We present 3 cases of cleft lip detected in utero in which the presence of this sign allowed correct identification of a cleft palate in 2 cases and a normal palate in the third.

In the future we hope to evaluate if this sign permits diagnosis of isolated cleft palate (without associated cleft lip).



Propranolol Eliminates Epinephrine Mediated Interference of Labor in Teenage Patients.

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Young primigravidas have been known to have shorter labors than their counterpart age 20-25 (Bull, John Hopkins Hosp 33:2, 1922). Since it is unlikely that the teenager is less anxious, than the older parturient, we explained this phenomenon by the paucity of myometrial beta adrenergic receptors in this age group. To test this hypothesis we randomized a sample of 42 primigravidas age 12 to 19 in two groups. Group A received a beta adrenergic blockade with propranolol (2 mg iv) at the time of admission after she was judged eligible for vaginal delivery, while group B served as control. The use of analgesics, sedatives, and oxytocin was left at the discretion of the obstetric team. The mean duration of labor in the control group was 5.8 hrs (range 3.0-11.0), which is less than half of the duration of labor in primigravidas in general. The group that had received propranolol had a mean duration of labor of 3.9 hrs (range 1.2-6.0). As expected the duration of labor was less of patients who had received propranolol at the outset of

uterine contractions. It is of note that neither the frequency, nor the intensity of uterine contractions changed significantly after administration of propranolol. There were also no differences in 5 minutes Apgar scores, which had a mean of 9.2 (range 8-10).

Conclusion: the relatively short labor of teenagers seems to be due to the incompletely developed adrenergic beta-receptors of the myometrium. Beta adrenergic blockade, nevertheless reduces the length of labor. The capacity to attenuate labor by endogenously released epinephrine might be a function of menarche to conception interval of the teenage patient.



A-31 Steroids vs Steroids and Thyroxin to Accelerate Fetal Maturation of Patients in Arrested Preterm Labor.

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Various phospholipids in amniotic fluid correlate well with the functional maturity of the lung and other organs of the preterm infant. The purpose of this prospective, double blinded, randomized study was to compare the effect of dexamethasone (DM) administration to the mother (6 mg q12 hrs x 4), considered by many to be an effective accelerator of fetal maturation, with that of DM combined with intraamniotic administration of 500 Fg of thyroxin. The L-S ratio was chosen as the indicator of maturity. We report the results of 159 patients enrolled in the study. Arrested preterm labor was the indication for acceleration of fetal maturation in both groups. Fetal age at the initiation of the treatment ranged between 24 to 32 weeks. The mean of initial L-S ratio for patients receiving DM and T4 was 1.211, and for patients receiving only DM it was 1.078, giving a difference of 0.133 (p value = 0.3327). The mean of increase in L-S ratio for each category after the first week of treatment (L/S2 - L/S1) was 0.477 for the DM and T4 group, and 0.326 for the DM group, resulting in a difference of 0.152 (p value = 0.0352). The mean increase in L-S ratio for each category after the second week of treatment (L/S3 - L/S2) was 0.478 in the DM and T4 group and 0.333 in the DM, group giving a difference of 0.145 (p value = 0.473). This difference was statistically not significant.

Conclusion: patients in preterm labor accelerate maturation of the fetus by endogenous release of TRH, which makes the administration of exogenous materials to further accelerate maturation relatively inconsequential, as long as the pregnancy is prolonged for more than 3 days.

A-32 Removing the Entire Trophoblast Population by Hysterectomy Rescues a Moribund Patient with Preeclampsia.

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We have proposed that preeclampsia is due to some yet to be identified substance(s) released by the trophoblast when the oxygen tension of that tissue falls below 30 torr (Amer J Perinatology, 1989; 6:133). The substance(s) stimulates the contractile proteins of the myometrium to initiate labor, but earlier in gestation when the myometrium is non-responsive, the stimulation affects the contractile proteins of the circulatory system and the CNS. This results in numerous disturbances, the most important of which is increase the inotropic effect of the myocardium, giving a rise in the filtration pressure in the capillaries with resultant edema, hypovolemia, hemoconcentration, and ultimately a reduced perfusion of most tissues. The cure of this potentially life threatening condition, therefore, should be either the normalization of PO₂ of the trophoblast, or when it is not possible, the removal of the trophoblast population. At or near term this is achieved by the delivery of placenta, and earlier in gestation, when trophoblasts have invaded the myometrium, by hysterectomy.

The following case supports such a hypothesis. A 16 year old primigravida was admitted at 22 weeks of gestation with edema, proteinuria, BP 140/90 and pulse of 140/min. Her main problem, however, was pulmonary congestion. In spite of increasing plasma oncotic pressure with albumin, and reducing with propranolol the inotropic effect of myocardium, and the epinephrine mediated arteriolar dilatation in pulmonary circulation, she required mechanical ventilation with increasing pressures. Her condition, nevertheless, worsened with PO₂ decreasing to 50 torr. She became hypotensive with BP 80/50 torr while receiving Intropin at maximal doses. She was considered moribund by the Medical Intensive Care Unit. The patient was then taken to the operating room by our staff where supracervical hysterectomy was performed. In one hour her arterial PO₂ had risen to 108 torr, and her lung edema had began to clear. She was extubated 9 days later, and was discharged home in good condition on postoperative day 11.



A-33 Prophylactic Adrenergic Beta Receptor Blockade during Labor Virtually Eliminates Cesarean Sections for Failure of Labor to Progress in Primigravidas,

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Failure of labor to progress has become in the USA the most frequent indication for delivery by cesarean section. We propose that dysfunctional labor in homo sapiens is the result of the inhibitory effect of endogenously released epinephrine upon the contractile proteins of the myometrium. Primates give birth in an unprotected environment. Thus nature has endowed them with rich beta adrenergic receptors in the myometrium to enable primates to inhibit labor when birth would endanger the life of the mother or that of the newborn. This epinephrine mediated inhibition is expected to be greater in primigravidas than in multiparas because of the greater anxiety of the former. Fifty seven primigravid patients admitted in early active labor were randomized in two groups. Group A received propranolol 2mg iv (a beta adrenergic receptor blocking agent), while patients in Group B served as controls. Inclusion criteria were absence of clinically determined CPD, malpresentation or malposition. Treatment with propranolol decreased the CS rate from 21.0 per cent in the control group to 6.2 per cent in the propranolol treated group. No patient in this group required delivery by CS who received propranolol early in labor. Based on this experience we have initiated routine use of propranolol in all our primigravidas admitted in early labor.

A-34 Knowledge Level Among Professional Nurses Managing Conscious Sedation Procedures in Out-Patient Settings

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Registered nurse anesthetists (RNA) are the only qualified nurse providers to administer intravenous conscious sedation (CS). However, over the past 15 years, increasing demands for CS in out-patient settings, created a responsibility shift from the RNA to a trained registered nurse (RN). The purpose of this descriptive-correlational study is to assess the CS knowledge level (KL) of RNs in hospital out-patient settings.

A sample of 20 RNs ($M=38.8$ years) was selected from 3 hospital out-patient settings in Puerto Rico. The conscious sedation knowledge assessment test (CSKAT), which is composed of 5 demographic items and 24 CS knowledge multiple choice questions, was utilized to assess the KL of the 5 male and 15 female RNs.

Satisfactory CS KL was achieved with a ³ 80% score. It was found that only 25% of the sample achieved a passing score of ³ 80%. 40% obtained a score of 70-79% (moderate KL), and 35% obtained a score < 70% (low KL). Inferential statistics was utilized to correlate KL with the demographic data. No significant correlations were found between KL and gender ($t = 0.073$, $df = 1$, $p > 0.05$), and KL and academic preparation ($T = .353$, $p = .103$). Significant bivariate correlation was found between KL and age ($T = -.402$, $p = .030$), KL and experience ($T = -.559$, $p = .007$), and KL and types of training obtained ($T = -.649$, $p = .002$). Based on this study, the author concluded that enhanced training could improve CS knowledge of the RN. Also, further investigation is needed to address the unsatisfactory KL of the senior, more experienced RN.

A-35 Oxytocin Induced Uterine Contractions as Powerful Accelerator of Fetal Maturation of Preterm Macrosomic Fetus.

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We have reported previously that preterm labor, if suppressed with diazoxide (Hyperstat) for more than 72 hrs, greatly reduces the relative frequency of RDS among newborns even with birthweights as low as 800 g to 1300 g. We explained this phenomenon by the action of the fetal TRH, TSH, T3 and T4, released in response to the period fluctuations in fetal P02 caused by uterine contractions. Our department has a policy to accelerate fetal maturation with intraamniotic T4 of patients with gestational diabetes mellitus to reduce the adverse effects of fetal hyperinsulinemia, and to deliver the fetus before the 36 week. When a patient refused administration of T4, we proposed to her to try to accelerate fetal maturation with oxytocin induced uterine contractions, which she accepted. The patient was a 36 y, g4 p3 (all by CS) at her 34th week of gestation by LMP and early sonogram. The fetus was macrosomic for age. Because the L-S ratio was only 1.17, she was admitted and given daily infusions of oxytocin (6 hrs) for 4 days. Administration of oxytocin produced regular uterine contractions but no cervical changes. After two days a second amniocentesis was performed yielding an L-S ratio of 2.20. After 2 more days of the same treatment the L-S ratio was 3.25. The decision was then reached to deliver her by CS. The 3,400 g fetus received an Apgar score of 9, and was transferred to regular nursery. The newborn had an entirely uneventful in-hospital course.

Re-interpretation of Friedman's Curve.

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Friedman's Curve was the first graphical analysis of the progression of labor, which was based on a study of 500 primagravidas. It related the dilatation of cervix against time, and subdivided labor in two phases: the latent phase and the active phase. The active phase was further subdivided the phase of acceleration, maximum slope, and deceleration phase. We define labor as uterine contractions in which the postcontraction length of the myometrial fiber is less than the precontractual length leading to a progressive reduction in uterine volume, and thus expulsion of the fetus. Therefore the changes in cervical diameter did not seem to be a relevant indicator of the changes occurring in the myometrium. The main problem, however, from using cervical diameter as a measure of progression labor arises from the fact that the cervix is retracted over a sphere or an approximation of a sphere, by virtue of which a constant rate of the cervical retraction will result in a progressively decreasing changes in diameter of the cervix. Therefore, the "Deceleration phase" in the Friedman's Curve does not in reality represents a decrease in expulsive efforts by the uterus, but the inevitable reduction in the changes of diameter of the cervix as the equatorial plane of the presenting part is approached. If Friedman's Curve is transposed on a circular line representing the fetal head, it is evident that in the so called "Deceleration phase" there is actually an increase in the rate of cervical retraction over the presenting part. This is consistent with the observation that uterine contractions usually tend to increase in frequency and intensity as labor advances. We recommend that changes in cervical diameter are not used as indicators of progression of labor without given consideration to geometric considerations what happens to the rate of retraction of cervix over a spherical presenting part when this process is recorded as a projection on a line.

Duration of Labor: Comparison of Primiparas with Multiparas ,

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There is no valid explanation of the large discrepancy of the duration of labor between primigravidas and multiparas. Based on reports, each covering more than 10,000 term deliveries, the mean duration of labor in primigravidas is about 5 to 6 hours longer than that of

multiparas. This discrepancy is attributed to the rigidity of the nulliparous cervix. Considering the greater thickness of the cervical wall, and the higher collagen content of the cervix of the multipara, the above explanation is difficult to accept. We have proposed a hypothesis (www.obgyn.today.org) that the long duration of labor in man in contrast to other primates (in videotaped rhesus monkeys it is only 37 minutes) is due to the inhibitory action of epinephrine on the myometrium, which prevents the required postcontraction shortening of the myometrial fibre. Thus the difference in the length of labor between primigravida and multipara would be explained by the lesser release of epinephrine by the multipara. Our ongoing study with primiparas involves blocking the beta adrenergic receptors of the myometrium with propranolol (Inderal) 2 mg iv. which is given shortly after the admission. Patients with cx dilatation more than 5 cm on admission were excluded. The mean duration of labor among our first 44 primigravida patients was 5.2 hrs. which is about 3 hrs. less than in multiparas. The duration of labor, however, was much shorter of patients who received propranolol at the time of initiation of labor. Two such teenage primigravidas delivered within one hour after propranolol. Mean apgar score at 5 minutes for the 44 primigravida patients was 9.1.

Conclusion: The long labor in homo sapiens is due to epinephrine mediated inhibition of the uterine muscle. After beta adrenergic blockade with propranolol the duration of labor in primigravidas is substantially shorter than that in multiparas without such blockade.

Intraamniotic Thyroxin to Accelerate Fetal Maturation of Diabetic Patients with Fetal Macrosomia.

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The relative frequency of fetal macrosomia, has remained unchanged over the last decade among patients with gestational diabetes mellitus in spite of improved morphometry of the fetus, and a more intensive monitoring of blood sugar levels of the pregnant individual. The failure to eliminate fetal macrosomia is likely due to the fact that even short episodes of fetal hyperglycemia result in a prolonged release of insulin from fetal pancreas. Fetal macrosomia is not only of consequence at the time of delivery, but it is likely to lead to permanent sequelae such as an excessive quantity of fat cells, and structural changes in organs such as the liver

and the myocardium resulting from fetal hyperinsulinemia. We are proposing that a more satisfactory approach to eliminate hyperinsulinemic macrosomia is to accelerate the maturation of the fetus, and to deliver it before the 36th week of gestation. Our previous work has established that acceleration of fetal maturation by intraamniotic thyroxin for a period of two to three weeks creates sufficient functional maturity as early as at the 31st week of gestation as not to require intensive care of the neonate. Patients with gestational diabetes were offered a treatment schedule which consisted of weekly administration of 500 µg of T4 intraamniotically starting at the 31st or 32nd week of gestation, and delivering the fetus once the L-S ratio of amniotic fluid exceeded 2.5, and PG was detectable, which in this group of patients occurred between 34 to 35 weeks. Our ongoing study presently contains 16 patients. Ten have had uneventful deliveries either by the vaginal route or by cesarean section for other indications other than macrosomia. The largest fetus weighed 3523 g of a patient who by fetal morphometry early in gestation was only 36 weeks pregnant. No newborn required admission to the intensive care unit, and no newborn suffered from episodes of neonatal hypoglycemia.

Conclusion: Based on our more than 15 years experience with acceleration of fetal maturation with intraamniotic thyroxin, we recommend that patients with gestational diabetes mellitus be managed by accelerating fetal maturation, and by delivering the fetus before the 36 week, thus eliminating admissions prior to delivery, eliminating intrapartum complications caused by fetal macrosomia, and minimizing permanent sequelae caused by fetal hyperinsulinemia.

Probably the Most Anemic Patient ever Undergoing Hysterectomy.

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There is no universal agreement of what is the minimal RBC concentration a patient should have before undergoing elective major abdominal operation. Most anesthesiologists consider 10g of Hgb per 100ml to be acceptable. We are presenting a case where a patient with Hgb of 3g / 100ml tolerated abdominal hysterectomy without complications. The patient was a 50 y/o, gravida 0 with a uterine bleeding of 3 years duration secondary to large leiomyomata uteri. She also had history of thrombasthenia of Glassman. Because she was Jehovah witness, and therefore refused blood transfusions and

hysterectomy, she was treated with hormones and iron. Patient reported to our Emergency Room complaining of weakness. Blood pressure was 90/50, pulse 140, respiration 22, temperature 37.1°. Hemoglobin concentration was 3.3g/100 ml. Patient was told that she had a life threatening medical condition, and was urged to have a hysterectomy preceded by blood transfusions. She refused treatment because she had decided to die. She was treated with intravenous fluids, 3000 U Epogen and Premarin. Vaginal bleeding continued, and 24 hrs later Hgb had declined to 3.0 gm with Hct of 9.3. At that time patient gave consent for surgical intervention, but denied blood transfusion. She was made aware that she might die during the operative procedure. Under general anesthesia a supracervical hysterectomy and bilateral salpingo-oophorectomy was performed. Estimated blood loss was about 100 cc. The operation was performed in less than 30 minutes. Postoperative Hgb was 2.7g/100ml, and Hct was 8.5, heart rate fluctuated between 140 and 160 min. She was discharged in 10 days, and two months later she returned for follow up with hemoglobin of 14 gm.

Conclusion: Normovolemic chronic anemia, even of extreme degree, is not a strong contraindication for hysterectomy.

A-40 Decompression of Fetal Ascites in a Patient with Rh Incompatibility Resolves Developing Preeclampsia.

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There is no scientifically valid explanation in the literature for the development of preeclampsia in patients with Rh-factor incompatibility. According to our proposed hypothesis (Amer J Perinat Med 6:2, 1989) preeclampsia is caused by some yet to be identified substances released by the trophoblast when the PO₂ of that tissue falls below 30 torr. Compression of the IVS by edema of chorionic villi is one of such conditions causing hypoxia of the trophoblast. The high filtration pressure in the villous capillaries arises from the elevated resistance of the erythropoietic fetal liver to blood returning from the umbilical vein. Increase in intraabdominal pressure of the fetus by ascites further increases the resistance to venous return from the placenta. Thus suppressing hepatic erythropoiesis by RBC transfusion, and removing the ascitic fluid by paracentesis, should reduce the compression of the IVS, and thus cure preeclampsia. The following case supports our theory. 29y, Rh neg, g6, p4,

at 27 gw was admitted because of fetal hydrops. BP was 140/90 and she had 2+ proteinuria. Paracentesis of the fetus revealed an intraabdominal pressure of 210 mm H₂O. After removal of 150 ml of ascitic fluid, the pressure fell to 80 mm H₂O, which was equal to that of intraamniotic pressure; 25 ml of PRBC was infused into the umbilical vein. Fetus tolerated the procedure well. Twelve hours later was BP 120/80 torr, and 24 hours later it was 110/70. Proteinuria had cleared.



A-41 Temporary Arrest of Uterine Contractions Resolves Arrest of Labor Refractory to Oxytocin and Propranolol.

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We have reported to the Society of Gynecologic Investigation that blockade of myometrial beta receptors with propranolol (Inderal) resulted in vaginal deliveries of 67% of patients scheduled for cesarean section because of arrest of labor of 2 to 5 hours duration at a cervical dilatation of 5cm to 8cm. One of the factors for failure to achieve vaginal delivery was later identified as OP position of the fetus. The rest of the refractory cases remained unexplained. We propose now that the cause was the failure of the exhausted myometrial cell to undergo the required progressive postcontraction shortening, which is a requisite for cervical dilatation. Therefore, we decided to stop, or at least to attenuate, uterine contractions for a brief period (2 hours) to determine whether such change in myometrial activity will affect the postcontraction length of the myometrial cell, and hence the rate of cervical dilatation. Our pilot study contains 8 cases, 3 were primigravidas and 5 were multiparas. Cervical dilatation at arrest of labor ranged between 5cm to 7cm, and the station between -3 and -1. Maximal progression of cervical dilatation after propranolol was only 1cm. Uterine contractions were attenuated with MgSO₄ (2g/h) since diazoxide (Hyperstat) was not available. After 2 hours magnesium infusion was stopped, and resumption of labor was awaited. In most cases, oxytocin was not used for augmentation of contractions. All 8 patients had spontaneous deliveries. One patient delivered in 30 minutes after cessation of magnesium infusion from a previous cervical dilatation of 5cm at -3 station. None had postpartum uterine atony.

Conclusion: Temporary tocolysis of patients in arrested labor, and refractory to propranolol, resulted in spontaneous deliveries in all of the 8 cases which otherwise would have required CS

A-42 Bystander CPR in Two Pittsburgh Communities.

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Despite recent reduction in mortality, coronary heart disease continues to be the leading cause of death in the United States. Most of these deaths occur suddenly and out of hospitals. There is strong evidence that cardiopulmonary resuscitation provided by a bystander at the scene of an arrest doubles the chances of survival for a victim.

It is clear that the death rate for coronary heart disease is higher among blacks than among whites yet black individuals are less likely to receive bystander CPR. Research evidence as to exactly why this problem exists is not clear.

The purpose of this study was to refine and test a questionnaire, which will identify the number of individuals trained to do CPR, their willingness to perform CPR and Potential barriers to performance. Following pilot testing, a mailed questionnaire was administered to a random sample of adults residing in two Pittsburgh communities where demographics indicate a high proportion of African American residents at risk for heart disease. A systematic random sampling design was used to select adult residents of the target communities. Completion and return of the questionnaires indicated the subject's willingness to participate in the study. Data analyses used were descriptive statistics to delineate: (a) demographic characteristics of the sample; (b) experience with CPR; (c) willingness to learn CPR and (d) perceived barriers. Inferential statistics will be used to ascertain differences in willingness to learn CPR and perceived barriers according to selected demographic characteristics. The questionnaires were analyzed for qualitative themes. This study which fills gaps in the literature on CPR among minority populations will contribute to the decrease mortality among African American citizens who are at great risk for sudden death due to cardiac etiology.



A-43 Relación entre la Educación que las Madres Reciben, su Actitud hacia la Lactancia y el Tiempo que Lactan a su Bebe.

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Este estudio de tipo descriptivo— correlacional, con una muestra de 30 madres del municipio de Cataño entre las

edades de 21 – 30 años de edad, con hijos entre 1 a 3 años de edad. El 17% eran casadas, el 12% con educación Universitaria, 41% con dos hijos y el 43% proveniente de familias con varios hijos, el 30% lactó hasta 6 meses y solo el 3% hasta 12 meses. Se utilizó un cuestionario de 27 reactivos sobre conocimiento de los beneficios de la lactancia materna, tiempo de lactancia, actitud hacia la lactancia materna y su experiencia con la lactancia. Los datos demuestran que la mayoría de las madres posee conocimiento promedio sobre los beneficios de la lactancia. El medio más frecuente para obtener conocimiento fue el hospital, el tiempo promedio fue de 4.6 meses. La correlación positiva entre el conocimiento sobre los beneficios de la lactancia y el tiempo que la madre lactó al bebe ($\rho = .987, p < .05$). Además hubo una correlación positiva entre actitud de la madre hacia la lactancia y el tiempo que permanecieron lactando ($\rho = .9929, p < .05$). La actitud de estas madres estuvo por debajo del 70 % de la puntuación máxima posible, lo cual indica que es necesario aumentar los esfuerzos para dar orientación y motivar a las madres en edad fértil. Se requiere incluir mas educación a las enfermeras y otros profesionales para ayudar a comenzar el proceso de lactancia. Fomentar la lactancia materna para que sea reconocida. Se recomienda realizar un estudio cuasi experimental donde la variable independiente sea la educación.



A-44 Molecular Characterization of 'endosymbionts' of *Dirofilaria Immitis*.

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Previous studies have identified intracellular bacteria in several species of filariae. These organisms appear to be organotropic, being detected to date only in the hypodermis, rachis, and oocytes, and seem to be vertically transmitted from one generation of filariae to another. The association between these organisms and their filarial host is unknown, but may be similar to that of insects and their endosymbionts. Since these organisms may represent a new taxonomic group, this study was conducted to determine their taxonomic position by the use of molecular biology techniques. *Dirofilaria immitis* adults obtained from naturally infected dogs were dissected to obtain organs which contain the 'endosymbionts' (uterus and ovaries); testes, organs without the 'endosymbionts', served as controls. DNA was extracted from the filariae and the 'endosymbionts'. The 16S rRNA gene of these

'endosymbionts' was amplified by PCR with primers that amplified most eubacterial 16S rDNA's, and the data was used to determine phylogenetic relationship between the organisms and the *Rickettsiales*. The PCR products from ovaries and uteri, which produced a band of approximately 1.5 kb in agarose gel electrophoresis, were purified and sent for sequencing to Robert Wood Johnson Medical School DNA Synthesis and Sequencing Laboratory, Piscataway, New Jersey. A 1495 base sequence of the 16S rRNA gene, obtained by alignment of the partial sequences, was submitted in the Gene Bank, and can be accessioned by number AF088187. The 3.5 version of the PHYLIP software package was used to infer the phylogenetic relationship of these 'endosymbionts' by constructing a phylogenetic tree of the 16S rRNA gene and those of other Proteobacteria. The results indicate that the organisms of *D. immitis* may be endosymbionts belonging to the alpha subdivision of the Proteobacteria, are most closely related to the *Rickettsiaceae*, and apparently to belong to the *Wolbachia* group. Supported, in part by the RCMI award RR-03051 from the division of Research of Resources, NIH.



A-45 Effects of Spouse Abuse in Puerto Rican Women in the Island and in the Mainland.
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This descriptive comparative design study was done with a convenience sample of 44 Puerto Rican women of which 26 were island Puerto Ricans and 18 were mainlanders, recruited from Shelters and court records. Various self-report instruments and face-to-face interviews assessing abuse and psychological effects of abuse were used. (Conflict Tactics Scale, Recent Life Changes Questionnaire, Post-Traumatic Stress Disorder Questionnaire, Beck Depression Inventory, Sheehan Patient Rated Anxiety scale, Sheehan Disability scale, Sheehan Stress and Social Support scale). T-tests and chi-square tests were used to compare various demographic characteristics between island and mainland Puerto Rican women. T-tests were also used to compare differences in the severity of physical and nonphysical abuse, PTSD, depression, anxiety, levels of acculturation, life changes, functional disability, and perceived social support and stress between groups. Chi-square tests examined differences in frequency of depression and anxiety of the women. Statistical significance was determined at ($= .05$).

Results: Demographic characteristics age from 17 to 58 years (mean=32). Mainland Puerto Rican women, reported significant higher monthly income (mean=1038) than those of the island (mean=251) ($t=6.82, p<.001$). 59% of the sample were nonphysically abused. t-tests revealed a significant difference in the severity of nonphysical abuse between the islanders and the mainlanders ($t=-3.054, p<.05$), but not in the severity of physical abuse. Puerto Rican women were more likely to continue the relationship (96%); while mainland Puerto Ricans were more likely to separate or divorce the person (50%) [$\chi^2(2)=22.3, p<.001$]. Puerto Rican women in Puerto Rico reported more frequent (39.2) and higher levels of posttraumatic stress symptoms (4.4) than those in the United States (28.2 and 2.8, respectively), but the difference was significant only in the severity of the symptoms ($t=-2.31, p<.05$). In conclusion, women in the island were more likely to stay in the relationship and had more severe nonphysical abuse and had less financial resources. More research is needed in order to determine how culture, financial and religion affect the response to abuse. There is also need for nursing interventions to decrease abuse and its effects. (Supported by 0NINR).



Structure of Cephalic Sense Organs of *Dirofilaria immitis*.

A-46

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Although previous studies have described the anterior nervous system of nematodes like *Caenorhabditis elegans*, *Strongyloides stercoralis*, and *Ascaris lumbricoides*, there is no detailed description of the anterior nervous system of tissue-dwelling parasitic nematodes. This study was conducted to describe the ultrastructure of the cephalic sense organs and anterior nervous system of *Dirofilaria immitis*, the heartworm of domestic dogs. Scanning electron microscopy and vital staining have revealed the presence of eight cephalic papillae, arranged around the buccal orifice as an inner and outer ring of four papillae each, and two amphids. Serial sections taken approximately fifty microns from the anterior tip of *D. immitis* adult and transmission electron microscopy indicate the presence of modified cells around the esophagus which assumed radial and circumpapillary patterns. Radial nerve projections are present surrounded by the cytoplasm of these cells suggesting a network of supporting cells around the papillary nerves. Also, there

are structural differences between the cephalic papillae and the amphids. One of these differences is that a modified cilium is embedded in reticular material that fills the upper part of the papilla while nine cilia are observed within the amphid. These results suggest the presence of an organized and complex sensory system which appears to integrate and coordinate the mechanical and chemical stimuli from the environment outside the worm. Supported, in part by the RCMI Award RR-03051 from the division of Research of Resources, NIH, SIGMA XI Student Grant, and the Associate Deanship of Biomedical Sciences and Graduate Studies.



A-47 Prevalence of *Schistosoma mansoni* antibodies in Puerto Ricans with Inflammatory Bowel Disease.

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Background: Inflammatory Bowel Diseases (IBD), Crohn's (CD) and ulcerative colitis (UC) are more prevalent in industrialized countries. Dysregulation of the immune system has been related to the pathogenesis, and a predominance of Th-1 response has been reported in CD. Parasitic infections in childhood may modulate future immune responses. Lack of exposure to these infections may prevent the development of a proper response to other antigens in the future. Schistosomiasis is associated to a Th-2 response. A decreased prevalence of infection with this parasite in Puerto Rico and an increased prevalence of IBD support this hypothesis.

Aims: To determine the prevalence of *Schistosoma mansoni* antibodies in Puerto Ricans with IBD and controls.

Methods: Serum from IBD patients and controls was screened for *S. mansoni* antibodies to *S. mansoni* adult microsomal antigens (MAMA) using the Falcon ELISA test. All positive samples were confirmed by Western Blot. The protocol was approved by the IRB of the MSC. Results: 101 IBD patients (CD:42,UC:59) and 109 controls were tested. There were no differences in age, gender and geographic location between cases and controls. 7/210 (3%) were confirmed positive, 4 controls (3.6%) and 3 IBD (3%) ($p=1.00$). All IBD cases had UC (3/59 or 5%, $p=0.26$).

Conclusions: No difference in the prevalence of S

mansoni antibodies was observed between controls, IBD, UC or CD. Our results do not support the hypothesis that parasitic infections in childhood protect against IBD. Because of the low prevalence of *S. mansoni* infection, a much larger sample would be needed to show significance. Supported in part by an RCRII award 1P20RR11126 from the National Center for Research Resources, NIH.

to that in the younger patients. In addition, elderly patients had a tendency towards more liver related deaths. This data could support treating selected elderly patients with Interferon. Funded, in part, by RCMI RCRII award IP20 RR11126 from the NIH.

A-48 Clinical Outcome of Chronic Hepatitis C (CHC) in the Elderly.

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Background: Data on the natural history of CHC in elderly patients is limited. The NIH consensus panel recommended Interferon therapy in these patients only under investigational protocols.

Objectives: To study the epidemiologic data, risk factors, biochemical and histologic parameters, response to treatment and outcome in hepatitis C patients older than 60 years old.

Methods: The records of 31 patients older than 60 years (elderly) and 42 randomly selected patients <60 years from the University Hospital Hepatitis Research Clinics were reviewed. All patients were HCV positive and most of them had a liver biopsy. Data regarding sex, age, risk factors, symptoms, histologic findings, response to interferon and outcome were collected in all patients. Analysis of the data was performed using Student's t test for normally distributed variables and Wilcoxon's Rank-Sum test for normally distributed variables. The Fisher's Exact Test was used to compare categorical variables.

Results: There was no difference in gender, biochemical parameters and interval from the risk factor until the time of the biopsy between both groups. Patients older than 60 years were less likely to be IVDU ($p < 0.006$). Elderly patients were more likely to have fibrosis or cirrhosis ($p < 0.002$), although the difference in the interval from the risk factor and the liver biopsy in both groups was not statistically significant ($p = 0.81$). Response to Interferon was equal in both groups (14% in elderly patients vs. 16% in patients <60 years). Elderly patients were more likely to be dead as compared with the younger patients (6 cases vs. 1 control ($p < 0.01$)) and there was a tendency towards liver related deaths in the elderly group (4 vs. 1, $p = 0.14$), even though their follow up time was shorter (2 years in elderly patients vs. 4 years in patients <60 years).

Conclusions: Although elderly patients were more likely to have cirrhosis, response to Interferon was similar

A-49 Folic Acid Supplementation Does Not Prevent Ribavirin Induced Anemia:

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Background: Therapy with interferon (INF) and ribavirin has proven effective for hepatitis C. 14-21% of patients on ribavirin have a drop in hemoglobin (Hgb) of 4 gms ascribed to hemolysis. Folic acid (FA) has been used in chronic hemolysis.

Aims: Evaluate the effectiveness of FA supplementation in preventing ribavirin-induced anemia.

Methods: Patients enrolled in treatment protocols with INF and ribavirin were randomized to receive FA 1mg qd or no supplementation (control(C)). Ribavirin dose was 1,000mg for weight <70kg and 1,200mg for >70kg. Hgb, reticulocyte count, LDH and bilirubin were measured at baseline, 2,4,6,8,12,16,20 and 24 weeks. Ribavirin was reduced to 600mg for Hgb <10 and discontinued for Hgb <8mg. Haptoglobin was measured at baseline and 24 weeks in 24 patients (FA:9, C:5). Data was analyzed using ANOVA for normally distributed variables and Wilcoxon's Rank-Sum Test for non distributed variables.

Results: 21 patients received (FA) and 22 did not. Groups were similar in age, gender and ribavirin dose. Mean base Hgb was 15.1 ± 1.0 for treated, 14.6 ± 1.5 for controls ($p > 0.5$). Mean drop in Hgb was 3.6 ± 1.3 for treated and 2.6 ± 1.2 for controls ($p = .004$). There was no difference between groups (treatment vs. controls respectively in relation to increase in reticulocytes (4.4% vs 4.4%), increase in LDH ($71 + 89$ vs $63 + 113$), increase in bilirubin ($0.5 + 0.3$ vs $0.5 + 0.8$) and decrease in haptoglobin (43.0 vs 21.0), $p > 0.05$. Five patients required ribavirin dose reduction, 4 females and 1 male, all in the treatment group. Four patients had a decrease in reticulocytes and 12 patients (FA) had an increase in haptoglobin during ribavirin treatment.

Conclusions: Treatment with FA 1 mg daily does not prevent ribavirin-induced anemia. A larger number of patients is needed to confirm this finding.

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Topical Antimicrobial Therapy in the Prevention of Early Childhood Caries.

A-50

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Early Childhood Caries (ECC) is microbiologically characterized by heavy infection of mutans streptococci (ms) on dental surfaces. Accordingly, it is reasonable to speculate that suppression of dental ms levels would decrease risk for ECC. On this basis, a randomized double blind placebo controlled pilot study was performed. The study population consisted of 31 subjects (age:12 to 19 mos; sex: 18F/13M) who were clients of a WIC clinic in Puerto Rico. Inclusion criteria included: (1) unremarkable medical history; (2) presence of 4 maxillary primary incisors (PMI) with no visible defects; (3) clinically caries free; (4) use of a nursing bottle at naptime and/or bedtime which contained a cariogenic substrate; (5) two consecutive ms positive cultures (utilizing MSB agar) from pooled PMI plaque. The subjects were randomized into 2 groups. The 15 subjects in the experimental group and the 16 subjects in the control group were evaluated every 2 months during the study period. At each evaluation, the subjects had 10% povidone iodine (experimental group) or placebo (control group) applied to their dentition. The placebo was commercial instant tea (without lemon or sweetener) and deionized water. Treatment failure was defined as the appearance of a white spot lesion(s) on any of the PMI during the study period. The mean duration of observation to treatment failure was 155 days; the mean duration of observation for treatment success was 217 days ($p=0.26$). Five of the 16 control subjects and 0 of the 15 experimental subjects experienced treatment failure (Fisher's Exact Test: $p=0.04$). The Kaplan-Mier estimate for incidence of treatment failure in the placebo group was 48% over 357 days ($p=0.02$). These observations suggest that topical antimicrobial reduces risk for the development of ECC in high risk children.



Oral Health Knowledge and DMFS in 12 Year Olds.

A-51

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The aim of this study was to assess dental caries, health knowledge and utilization of dental services of twelve

year old population in Puerto Rico, according to type of school. A probabilistic interpenetrating sample of schools stratified by eleven health regions, type of schools and grade profile, economic index and urban and rural zones was designed. In each region we planned to select with probability proportional to enrollment five public and one private elementary and intermediate schools. In each school 25 twelve year old children were randomly selected. Each student filled out a questionnaire and was clinically evaluated according to NIDR criteria for DMFS Index. Evaluations were performed by calibrated examiners. A total of 1,434 subjects from a population of approximately 70,000 were evaluated. The overall DMFS for Puerto Rico was 6.30. Statistically significant differences were observed in DMFS scores between public (6.69) and private (4.44) schools ($p < .05$). Also a greater proportion of students in private schools (27.3 vs. 17.6%) were caries free ($p < .05$). Children in private schools visited the dentist more frequently, reported less fear and fewer barriers of access than those in public schools. These differences were statistically significant ($p < .01$). In most aspects surveyed children in private schools had a greater knowledge of oral health and prevention of dental disease than those in public schools ($p < .05$). It is interesting to note that approximately 60% of children had little or no knowledge concerning the prevention of periodontal disease, as well as, the effect of tobacco (40%) and alcohol (60%) on oral health. Children in both groups indicated parents as their primary source of information concerning oral health. Findings suggest a need for educational programs geared towards the general community to promote oral health, emphasizing the prevention of periodontal diseases and oral cancer and their relationship to tobacco and alcohol use.



Variability in the Diagnosis of Incipient Interproximal Caries Using Bite-wing Film.

A-52

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Interproximal caries is usually diagnosed by means of a clinical and radiographic exam. The method mostly used for diagnosing incipient Interproximal caries is the bite-wing film radiograph. The purpose of this study was to assess the variability between faculty members of the U.P.R. School of Dentistry in diagnosing incipient Interproximal lesions by interpreting bite-wing radiographs. A faculty member with advanced training in Oral Radiology was selected as the gold standard. Fourteen examiners were selected from four clinical areas:

Oral Diagnosis, Restorative Sciences, Pediatric, and TEAM Dentistry. A sample of forty radiographs was selected from the record pool of the Dental School Clinic. Eight surfaces were examined per radiograph for a total of 320 surfaces. To determine inter-rater reliability each of the examiners evaluated the forty radiographs, and ten were interpreted twice by each examiner for intra-rater reliability. A scale of 0-3 was used to assess the carious lesion depth in the dental enamel (Pitts, 1984). Kappa statistics were computed using SPSS for Windows. The level of reproducibility obtained was evaluated according to Rossner (1995). The Kappa statistics for intra-rater reliability indicated excellent reproducibility (.89 and .79) for two examiners and good reproducibility for the gold standard (.70) and the other twelve examiners (.70 to .40). Kappa statistics for inter-rater reliability indicated good reproducibility for eleven examiners (.64 to .43) and marginal for three (.39 to .31). Discrepancies in the level of Kappa were observed between the different clinical areas. Calibrations are recommended to standardize diagnostic criteria among faculty members as means to improve the quality of the teaching-learning process.

A-53 Prevalence of Dental Fluorosis in a Non Fluoridated Community.

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Fluoridated water was administered in Puerto Rico to approximately 70 percent of the population in an inconsistent manner during the decade of 1970 and later discontinued during the mid '80s (Giusti, 1993). The aim of this study was to assess the prevalence of Dental Fluorosis of twelve year old population in Puerto Rico, according to geographical areas and zones, and type of school. A probabilistic interpenetrating sample of schools stratified by eleven health regions, type of schools and grade profile, economic index and urban and rural zones was designed. In each region we planned to select with probability proportional to enrollment five public and one private elementary and intermediate schools. In each school 25 twelve year old children were randomly selected. The Dean Index was employed to assess Dental Fluorosis following the National Institute of Dental Research criteria and methodology. Evaluations were performed by calibrated examiners. A total of 1,434 subjects from a population of 70,000 were evaluated. The overall Community Fluorosis Index (CFI) obtained was 0.53, 95% confidence interval (0.49;0.57). This value represents a borderline public health significance (NIDR,

1991). The values obtained for urban (0.55) and rural (0.50) were not statistically significant. The differences between public (0.55) and private (0.43) were not found statistically significant (95% CI). The CFI varied between geographic regions from a high of 1.25 to a low of 0.31. The prevalence of dental fluorosis for level 2 and higher, in public schools was 10% and 8% in private, not statistically significant (95% CI) and 11% in urban and 9% in rural schools, not statistically significant (95% CI). The prevalence for levels 3 and 4 was 3% both in public and private schools, and 3% for urban and 2% for rural. Dental Fluorosis is borderline in terms of public health significance. The CFI shows a great variation in the different regions and statistically significant differences were observed. Further studies requiring samples for each region and identification of sources of fluoride should be conducted.

A-54 Marginal Seal of Class V Amalgam Restorations.

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This study was undertaken to evaluate the degree of microleakage of Class V Amalgam restorations using three dentin bonding systems. Class V cavity preparations were cut on buccal and lingual surfaces of human molars with the occlusal margins in enamel and gingival margins in cementum. Teeth were randomly assigned to four experimental groups each lined with either; (A) Copal varnish (control); (B) Prime & Bond 2.1 light cured (LC); (C) ScotchBond MP self activated (SA) and (D) Prime & Bond 2.1 self activated (SA) adhesive. Specimens were coded and stored in water at 37°C for two weeks. Samples were thermocycled 2500 cycles, stained with Basic Fuchsin dye (0.5%), sectioned and evaluated for leakage under the microscope at 40X by calibrated evaluators. Redit analysis of the leakage data gave the following mean and standard deviations values for groups A-D: (A) 0.6415±0.093; (B) 0.3543±0.265; (C) 0.4125±0.263 (D) 0.5915±0.175. Barlett's Test showed that variances among groups were not homogenous at 0.10 level of significance. Newman Keul's Test for multiple comparisons among groups showed significant differences between groups A & B, A & C, D & B and D & C at 0.05 level of significance. All Groups experienced microleakage. Prime & Bond 2.1 (LC) and Scotchbond Multipurpose (SA) reduced initial microleakage significantly better than Prime & Bond (SA) and traditional Copal Varnish. Clinical research is necessary to evaluate if post-operative sensitivity is related to the presence of microleakage.

A-55 Oral Health Status and Treatment Needs in a Population of Hispanic Children and Adolescents with HIV Disease.

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The purpose of this pilot study is to assess the oral health status and treatment needs of approximately 200 HIV+ children and adolescents, residing in Puerto Rico. In addition, the study will determine the feasibility of conducting a larger epidemiologic study of the oral health status and treatment needs of approximately 800 children and adolescents of different age groups in the island of Puerto Rico. The specific aims of this study are: (1) to provide descriptive clinical data that will characterize the oral health status of a sample HIV+ children and adolescents in Puerto Rico and (2) to compare to the results of this study with those of a similar study that was conducted in Puerto Rico in the general 12 years old population. Oral health examinations are being performed by trained, calibrated examiners following NIDR diagnostic criteria and procedures. The data is being recorded into specially designed examination forms by trained recorders. The following oral health clinical parameters are being assessed on each of the subjects: dental caries, restored and missing teeth, oral hygiene, gingival-periodontal conditions, treatment needs, malocclusion and oral lesions. The parent-guardian consent and youngster's demographic profile, as well as the parents oral health knowledge and attitudes, are being obtained from a questionnaire that is administered prior to the patient's examination. Statistical analyses will be made using the SAS computer program. A detailed descriptive profile based on the clinical measures of the oral health status and current treatment needs of the subjects will be provided. Results will be stratified by age and gender. Bivariate as well as multivariate analyses will be performed in order to determine sociodemographic factors and oral health knowledge and attitudes associated with the clinical parameters. At the present time, 13 children and adolescents have been evaluated. Preliminary data will be presented and discussed.

A-56 Efecto del Midazolam Oral en el Comportamiento de Niños Pre-Escolares al Recibir Tratamiento Odontológico.

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El estudio piloto consistió de una muestra de 10

pacientes entre las edades comprendidas entre los 2 a 6 años, los cuales según la Academia Americana de Anestesiología fueron clasificados ASA I, que tenían historial de problemas de comportamiento al recibir tratamiento odontológico, siendo clasificados según la tabla de comportamiento de Frankl en las categorías 1(definitivamente negativo) y categoría 2(negativo). Cada paciente recibió un tratamiento odontológico integral, realizado por cuadrantes, a medida de lo posible.

Se les administró 0.5mg/Kg de peso de midazolam oral, diez minutos antes del procedimiento odontológico, evaluando el comportamiento del paciente durante el mismo, observando si existía variación en dicho comportamiento. Al mismo tiempo se evaluaron los signos vitales y la saturación de oxígeno.

Se observó que en el 90% (9 pacientes)de los casos el midazolam por vía oral es efectivo para mejorar el comportamiento de los pacientes que reciben tratamiento odontológico, moderando el comportamiento del paciente de las categoría 1 y categoría 2 a las categorías 3 (positivo) y 4 (definitivamente positivo). También se observó que en el 100% de los pacientes no existieron variaciones significativas en los signos vitales ni en la saturación de oxígeno.

Concluyendo que el midazolam por vía oral es efectivo y seguro administrado en pacientes pre-escolares que requieren recibir tratamiento odontológico para mejorar el comportamiento. En la mayoría de los pacientes no se requirió el uso del midazolam en las visitas subsiguientes.

A-57 Fluoride Varnish as an Alternative Preventive Treatment for Early Childhood Caries. Pilot Study.

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Even though we have experienced a marked improvement in the general oral health status of our population, caries prevention has not been effective in some age groups. Early childhood caries continues to be a significant health problem in Puerto Rico, especially in children from the rural areas and the lower socio-economic levels. The purpose of this study is to establish the need for massive dosage or exposure to fluoride varnish as an effective preventive and therapeutical measure in infants with high risk of having ECC. Seventy children between 12 and 48 months of age (39 males and 31 females) were recruited exhibiting high-risk behavior for ECC, i.e. continued bottle feeding during the night. The subjects

were treated three times in a 7 day period with an application of either a Fluoride Varnish (Duraflor) or a Placebo Varnish, prepared by the same company. Every child received at least one fluoride application. The experimental group received three fluoride varnish applications and the Placebo group, one fluoride varnish and two placebo varnish applications. The same procedures were repeated in a six-month interval and all the subjects evaluated a year after the first visit and examined for the appearance of any carious or white spot lesions. Results demonstrated overall efficacy of varnish applications but no difference was established between the two regimens. (This investigation was supported in part by a RCR II award, 1P20RR11123 and by CIDIC funds.)



Information Technologies at the Beginning of the XXIst Century: Implications for the Academic Health Centers.

A-58

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A scenario of the available information technologies in the early 21st century is presented. Using the techniques of foresight, issues management, and visual strategic envisioning, a scenario is constructed that describes the environment of the Academic Health Center in the era of smart devices and ubiquitous computing. Fundamental assumptions in the scenario are that Moore's law will remain accurate until *circa* 2010, that current silicon technologies will make a smooth transition into the quantum transistor realm, and that an appropriate economic model will be found for high-performance telecommunications via the Internet. This scenario involves wireless remote communications of smart microprocessor driven devices accessing a high-performance high-speed distributed metacomputing and telecommunications environment operating at O(GHz) speeds and communicating at O(Gbps) rates. Access to Terabyte or Petabyte sized databases for information, patient care, and research will be common. The need for secure, flexible, and accessible computing environments for education and clinical care are shown to be similar and inseparable. The need to re-structure the traditional healthcare teaching environment clearly arises from the analyses carried out under the assumptions present in this scenario. Policy recommendations resulting from this assessment are made to ensure a successful transition into the age of ubiquitous computing.

Desarrollo de Infraestructura de Comunicación y Computación en el C.P.R.S.

A-59

R. García García, E. Steininger y S. Aponte.
Proyecto Título III, C.P.R.S.

En noviembre de 1996 el Colegio de Profesiones Relacionadas con la Salud comenzó una transformación tanto a nivel administrativo como académico. Esto gracias al Proyecto Título III que le proveyó al Colegio la oportunidad histórica de colocarse a la vanguardia de la tecnología de computación y comunicación. Al día de hoy, el C.P.R.S., es la unidad del Recinto de Ciencias Médicas que se encuentra dictando pautas en el desarrollo de infraestructura y de mejoramiento de procesos a todos los niveles con una proyección de futuro y sin retorno. El Proyecto Título III provisionó en dos áreas fundamentales, a saber: la implantación de una infraestructura de comunicación y computación que ha provisto al Colegio de 350 espacios desde los cuales una computadora se puede conectar a la Red CPRS, que da acceso a programados específicos en nuestros servidores, al Internet, a los sistemas FRS, HRS y SIS, mas recientemente a las bases de datos de la biblioteca y a todos los recursos que nos provee la UPRNet. Esta infraestructura permite interconectar a todos nuestros programas académicos, aún cuando se encuentren en otros edificios como enfermería, su anexo, y el edificio principal. A su vez hemos equipado dos salones con 16 computadoras de alto rendimiento y con las facilidades de proyección, para el uso de los estudiantes, la facultad y el personal. Siendo lugar donde se ofrecen cursos y talleres de computadoras y multimedia. Al día de hoy contamos con más de 150 computadoras conectadas entre si, a diferencia del 1996 cuando las pocas que habían estaban aisladas, anticuadas, con pocas posibilidades de conexión y sin posibilidades de mantenimiento.



Osteopenia in Puerto Rican Patients with Crohn's Disease.

A-60

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Introduction: Crohn's disease (CD) is a chronic inflammatory disease of the GI tract, with peak occurrence between the ages of 15-35, age when maximum bone mass is achieved and any reduction would be more significant. Osteopenia (decreased bone density) has been reported frequently in CD. The exact mechanism is unknown but many factors may contribute. Purpose: To determine the prevalence of osteopenia in Puerto Rican patients with

Crohn's disease and correlate with risk factors. **Methods:** Bone mineral density was determined by DEXA in the lumbar spine and femur of 66 patients with CD. Albumin, calcium, phosphorus, and alkaline phosphatase were determined, and Vit. D, Vit. D3, and osteocalcin levels were also determined in a sample of the population. Severe osteopenia was defined as: Z score \leq -2.00 SD, osteopenia: -1.99 SD \leq Z score \leq -1.00 SD, normal: Z score \geq -1.00. CD and metabolic questionnaires were obtained and correlations were done between clinical parameters and bone density. The study was approved by the IRB of the Medical Sciences Campus.

Results: Severe osteopenia was found at hip in 41.8% (28) patients, in 43.3%(29) at lumbar spine and 31.3%(21) at femoral neck. Osteopenia was found in 28.4%(19) patients at hip, 23.9%(16) lumbar and 29.9%(20) at femoral neck. No correlation was found between degree of osteopenia and duration of disease, bone mass index, gender, surgeries, disease localization, age of disease onset, treatment, albumin, calcium, phosphorus, alkaline phos., osteocalcin, Vit D or Vit D3.

Conclusions: An increased prevalence of severe osteopenia and osteopenia was found in Puerto Ricans with Crohn's disease. No risk factors or predictors were identified. Routine testing for osteopenia in CD may be of help in the early diagnosis and therapy of this condition.

nursing care. The Ethnonursing qualitative methods used for this study included participant observation and semistructured interviews to elicit the experiences and perceptions of the informants. There were 40 informants for this study, 30 were Mexican American adults who receive care at a community based clinic and family members or friends who accompanied the informant to the clinic and ten nurses and other health care professions for environmental context. Data analyses involved Leninger's four phases of Ethnonursing Analysis of Qualitative Data. Phase four of data analysis resulted in an abstract model reflected expected relationships between the client and nurse. Components include care values of Mexican Americans familiarity or personalismos between the nurse and client resulting in confidence this combination leads to congruent care practices, well being and promotion of healthy lifestyles. Implications identified for nursing include continued understanding of the concept of confidence in caring for Mexican American clients in the promotion of health and well being. In understanding the cultural care needs of the people, nurses will be better able to promote healthy lifestyles



A-61 The Experiences of Mexican Americans Receiving Professional Nursing Care: An Ethnonursing Study.

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This paper discusses a research study exploring a Nursing phenomenon meant to uncover experiences of Mexican Americans who have received professional



**POSTER
PRESENTATIONS**

Women as Primary Caregivers to Frail Puerto Rican Older Adults.

P-1

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This qualitative study examines the circumstances in which 30 Puerto Rican middle-aged women who are primary caregivers carry out tasks of informal support to frail elderly relatives. Research methods included: structured and open questions and observation. Average number of caregiving years was eight. Results from the General Well-Being Schedule indicated that 57% of the carers were in moderate stress, 10% in severe stress and 33% reported positive well-being. Caregiving tasks performed almost every day were: light household cleaning, giving the old person a bath, and meal preparation. Tasks performed on a routine basis but not more than twice a week were: washing and ironing clothes, shopping for basic items such as groceries and medicines, taking the elderly for a ride or to buy needed articles, taking the elderly to medical appointments, and financial management. Half of the women expressed that they were moderately satisfied with the help received from their kin network and would like more assistance; 47% stated that they were rarely helped by relatives. Principal sources of conflict faced were: problems with family members or the care recipient, problems related to personal or health matters of the carer, job-related problems, and problems related to role as main carer. Findings provide insight into the dynamics of caretaking of frail elderly and the role of primary carer. Carers must make choices due to multiplicity of roles that are the result of strenuous decisions.

Implications of the Aging of Puerto Rico for the Preparation of Professionals in Gerontology.

P-2

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The rapid aging of the population of Puerto Rico in less than 30 years posed demands to public health as an academic discipline and practice. The Program of Gerontology of the School of public Health is the first-established academic graduate program in Puerto Rico to train professionals in gerontology. At first, it offered a graduate certificate degree and prepared professors at the different faculties of the Medical Sciences campus. Recently, and MPH in Gerontology has been added as well as elec-

tive and specialized courses in health promotion, death and dying, ethical and legal aspects of old age, and issues in gerontological research.

These courses are open to health professionals and also to persons from other backgrounds such as law, social work, and communications. The expansion of the curriculum has followed the recommendations of students and professionals in community agencies serving older adults as well as needs assessments of older adults carried out by professors and students. Both programs pursue to provide students with theoretical knowledge, skills to deal with older adults, and also sensitivize them to the needs and situation of the aged. This poster focuses on the various stages of the expansion of the curricular offering.

Respiratory and Na-Pump Activities of Cultured Aortic Bovine Endothelial and Rat Smooth Muscle Cells. Effect of Simvastatin and Angiotensin II.

P-3

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Cell components of the vascular wall are known to play an important role in the ethiology of cardiovascular diseases such as hypertension and atherosclerosis. However, some energetic parameters of these cells have not been well characterized. Bovine Aortic Endothelial cells (BAEC) and Rat Aortic Smooth Muscle cells (SMC) were grown to confluence, trypsinized and suspended in a BSS HEPES pH 7.4 buffer at 37 °C. Oxygen consumption (OC) was measured by means of a Clark-type O₂ electrode in 3 ml of buffer containing no substrate. Na-Pump activity (SP) was estimated in the presence of 1 mM Ouabain from the ouabain-sensitive O₂ consumption (OS), assuming a P/O ratio of 3 and a K⁺/ATP ratio of 2. OC (nmoles O₂/10⁶ cells x min) of BAEC was 1.29 ± 0.06 (30) and that of SMC 3.06 ± 0.16 (22). Ouabain inhibited respiration in both type of cells by about 25%. OS of BAEC was 0.37 ± 0.03 (30) and that of SMC 0.76 ± 0.09 (22). By contrast freshly isolated rat hepatocytes have been reported to show an OC of about 15 and OS close to zero. Nystatin (0.05 mg/ml), a Na⁺ ionophore, increased OS in both BAEC and SMC by 129% and 64% respectively, indicating a functionally responsive SP. Dinitrophenol (0.1 mM), a mitochondrial uncoupler, increased OC in both BAEC and SMC by 150% and 100% respectively, whereas 1 mM CN⁻ reduced OC to zero in both types of cells. Angiotensin II (0.6 iM) did not affect OC or OS in these cells, whereas 20 iM Simvastatin, an inhibitor of the de novo cholesterol synthesis, inhibited OC in SMC by 20%

without affecting OS, whereas this drug had no effect on BAEC. SP (nmoles K^+ / 10^6 cells x min) in BAEC was 4.44 and that of SMC 9.12. By contrast SP in hepatocytes has been measured to be 4.46. When expressed as moles K^+ / μ^2 cell surface area x hr, SP was 410 for BAEC, whereas that of SMC was 740 and that of hepatocytes 25. These results indicate that BAEC and SMC show a respiratory activity which is mainly mitochondrial, with well coupled mitochondria. The inhibitory effect of Simvastatin on OC of SMC might reflect an inhibitory action on cholesterol synthesis and it may represent an additional benefit of this drug in the treatment of vascular disease.



GABA-like Immunoreactivity in the Central Nervous System of *Aplysia californica*: A Double-labeling Study.

P-4

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Gamma-aminobutyric acid (GABA) is a 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. In molluscan model systems, 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_i) neurons in *Aplysia californica* using wholemount immunohistochemistry. GABA_i cell bodies were found in each of the major central ganglia, with the exception of the abdominal ganglion. A number of asymmetries were observed in the pattern of GABA_i neurons in bilateral ganglia. One unpaired GABA_i cell was located in the region of the buccal commissure. Double-labeling experiments, in which the cerebral-buccal connective (CBC) was backfilled with the tracer biocytin, revealed that this unpaired cell projects into the CBC. Additional buccal-cerebral interneurons (BCIs) were detected, including a pair of cells in each contralateral hemiganglion. Double-labeling experiments also revealed the presence of at least two GABAergic cerebral-buccal interneurons (CBIs) in the "crotch" region of the cerebral ganglion. All commissures in bilaterally paired ganglia were particularly rich in immunoreactive fibers. 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. No GABA_i 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 are consistent with the notion 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, MBRS, and RCMI.



The Problem of Specimen Flattening During Negative-Staining for Electron Microscopy.

P-5

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A major problem in the computer image analysis of macro-molecular complexes studied by negative-staining and electron microscopy (EM) is that the structures are often highly flattened on to the supporting substrate (a carbon or plastic film), thus preventing calculation of three-dimensional reconstructions of the protein electron density. To overcome this problem, we have recently compared the results of negative-staining of isolated fish muscle thick filaments stretched across a perforated (EM) grid both with and without a supporting carbon film. Negative-staining in which the filaments lie in a stain film stretched across a hole without a supporting carbon film yielded images in which the filaments were uniformly smaller in diameter and less flattened. The filaments in stain film had a mean diameter of 28 ± 1.3 nm (mean \pm standard deviation, $n=132$), while those filaments stained on carbon films had a mean diameter of 33 ± 2.8 nm ($n=104$). This was also confirmed by analysis of Fourier transforms of the filaments in which the position of the major reflection on the first layer line shifted consistent with a change in the radius at which the center of mass of the myosin heads lies from 11.2 ± 0.74 nm ($n=12$ transforms) in the stain films to 13.2 ± 0.82 nm ($n=10$ transforms) on thin carbon. Similar measurements are being made for isolated frog filaments. Supported by a 'Research Centers in Minority Institutions' award RR-03051, from the National Center for Research Resources (NIH), a 'Grant-in-Aid' Award (9607749S) from the American Heart Association of Puerto Rico, and 'Minority Basic Research Support' Grant (MBRS SO6 GM08224 from NIH).



A Breast Cancer Health Promotion Program for Older Puerto Rican Women.

P-6

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The paper focuses on a model about health promotion program for the early detection of breast cancer in elderly Puerto Rican women. The model was designed based on the findings of a national survey conducted to assess the knowledge, beliefs, and early detection practices of breast cancer of women in this age group and their perceptions of barriers associated to a noncompliance. The model intends to minimize barriers for early detection and increase the elderly women's compliance with recommended guidelines. It involves the combination of educational and environmental supports for actions and conditions conducive to health. It consists of the following components: (1) a culture and cohort - sensitive health education program for elderly women on breast cancer and assertive strategies for the client-physician relationship, (2) a training for primary-care health providers on current guidelines for women 65+ and barriers affecting compliance among older women in Puerto Rico, and (3) coordination of necessary support services to facilitate access to clinical breast examinations and mammograms. The model was implemented on a metropolitan area in Puerto Rico. The evaluation measured the progress on the plan implementation and assessed the immediate products, as well as the long term impact results. Results indicate a not significant increase in breast self examination and the need of external assistance to improve women's compliance with mamograms and clinical exams.



Estado de salud y capacidad funcional de la población de 65 años o más residente de la región universitaria de salud de Puerto Rico.

P-7

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La descripción de las características sociodemográficas y de salud de la población de edad avanzada es el primer paso para identificar las necesidades de servicios de salud y sociales y las variables predictoras de fragilidad y utilización de dichos servicios. El propósito principal de esta investigación es determinar el estado de salud y la capacidad funcional de la población de 65 o más residente en los municipios de Carolina, Trujillo Alto, Canóvanas

y Loíza. En la primera fase del estudio se entrevistó a 500 sujetos, seleccionados en forma aleatoria y sistemática por valor de la vivienda y número de envejecientes según el último censo. El cuestionario utilizado contenía los siguientes aspectos: sociodemográficos, utilización de servicios de salud, actividades del diario vivir, condiciones de salud, hábitos y problemas comunes del envejecimiento. Con la información recopilada se estimaron prevalencias a través de intervalos de confianza. En la segunda fase, se evaluó clínicamente una submuestra de 100 sujetos para determinar las variables asociadas con la necesidad de servicios de salud. La población estuvo compuesta en su mayoría por féminas, con mayor prevalencia de enfermedades crónicas que los varones. La dependencia funcional aumentó con la edad y se duplicó en los sujetos mayores de 75 años. De éstos un 80%, demostraron necesidad de evaluación y cuidado geriátrico integrado para identificar factores potencialmente modificables y evitar la discapacidad futura.



Applications of Soft X-ray and Other Microscopy Techniques to Elucidate the Structure of Parasitic Metazoa.

P-8

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Preliminary studies presented at the 1998 Research and Educational Forum were continued to elucidate more completely the structure of *T. spiralis* newborn larvae by the use of several microscopy techniques. Scanning electron microscopy revealed longitudinal, superficial cuticular ridges similar to those present in adult worms. Transmission electron microscopy disclosed a fully-formed body wall consisting of cuticle and muscle cells; bacillary bands in the hypodermal trunks are not fully developed; cephalic space is extensively innervated by axons, which apparently originate from cells near the nerve ring, and terminate as modified cilia in cephalic papillae and amphids. Similar cilia were detected in the posterior portion of some larvae, suggesting formation of caudal sensory organs. Triradiate esophagus, extending from the buccal capsule to the intestine, is enveloped by a primordial stichosome; many of the stichocytes contain secretory granules, which are also detectable by confocal microscopy. Intestine appears to be non-patent. Soft X-ray microscopy enabled

detection of ganglion-like structures in the mid-body of the larvae, and a large genital primordium. These results indicate that the internal organization of the newborn larva is comparable to that of mature muscle-stage larva, and that X-ray microscopy is a very useful technique to examine not only small metazoa, but also has great potential for application in the study of other types of biological specimens, 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-AC03-76SF00098; RCMI RR-03051, NIH; Office of Health and Environmental Research, U.S. DOE; and by the Cooperative Agreement DOE/EPSCoR DE-FC02-91ER75674).

(right side 875 vs. left side 91, periphery 60 pg/ml; receptively). A repeated MRI —5 months after the first one— confirmed the presence of the adenoma. The patient underwent transphenoidal surgery with successful removal of a pituitary microadenoma. She was discharged on glucocorticoid replacement therapy which was discontinued over the ensuing 9 months. She experienced catch up growth and remission of signs of CS after the surgery. She has remained off steroids for over 4 years now. Her most recent 24 hour urinary free cortisol levels, at 18 years of age, were normal at 110 mcg/24 hr, nl 21-143. We conclude, that growth failure could be the earliest sign of CS among children, which underscores the importance of close monitoring of growth rate throughout childhood.

P-9 Growth Failure: Hallmark of Cushing's Syndrome in Childhood

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Hypercortisolism impairs growth by either suppressing growth hormone secretion or by impairing action at target tissue site. This might present with growth failure in childhood. Growth failure as a distinct early feature of Cushing's Syndrome (CS) in a female with type 1 diabetes mellitus is presented. An 18 y.o. female was admitted to the University Pediatric Hospital at 9½ years of age with diabetes mellitus *de novo*. She presented with history of classical symptoms of diabetes mellitus plus hyperglycemia (204 mg/dl) without ketosis. Her physical examination at that time was remarkable for height between the 50th - 75th percentile and weight between the 75th - 90th percentile. There were no signs suggestive of CS. Over the ensuing 19 months she grew poorly (1.9 cm) —expected 8 cm— and crossed percentile moving down below the 50th percentile. In addition, she had experienced 6 Kg of weight gain during these 19 months and developed subtle signs that were felt to be suggestive of hypercortisolism. A screening urine collection showed increase urinary free cortisol levels (325 mcg/24 hr). A low/high dose dexamethasone suppression test revealed suppression of urinary free cortisol levels to high dose dexamethasone by 51% and 17 OH-corticosteroids by 48%. A magnetic resonance imaging (MRI) study of the pituitary gland demonstrated a normal pituitary gland. A computerized tomography scan of the abdomen cavity showed normal adrenal glands. However, biochemical profile was felt to be most compatible with CS secondary to a pituitary adenoma. She was referred for further evaluation to the National Institutes of Health at 12 9/12 years where an increase adrenocorticotropic hormone (ACTH) secretion from the right side of the pituitary gland was demonstrated by the inferior petrosal venous sinus sampling test

P-10 tDNA-PCR Amplification and AFLP for Generating Species-Specific Polymorphic Bands in *Plasmodium falciparum*, *Plasmodium berghei* and *Plasmodium yoelii*

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The tRNA genes contain highly conserved sequence motifs among the eubacteria. Since many tRNA genes are organized in tandem repeats, primers can be designed based on the conserved motifs at the 5' and 3' ends to amplify regions among adjacent tRNA genes. These intergenic regions are not expected to be conserved in sequence or length and hence provide a convenient target for generating polymorphic PCR-amplified bands between species. Using tDNA primers that are the complement of the 5' and 3' consensus sequences of eubacterial tRNA genes, polymorphic bands were amplified in *P. falciparum*, *P. berghei*, and *P. yoelii*. DNA prepared from Swiss albino mouse liver was used as a control template for the rodent malarial parasite species. A distinct pattern of polymorphic bands were observed between the different species. Preliminary results were also obtained by amplified fragment length polymorphism (AFLP) on genomic DNA of the three *Plasmodium* species. These results indicate that eubacterial tDNA primers and AFLP are valuable tools for generating diagnostic species-specific markers for *Plasmodium* species.

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Intrauterine Thrombotic Event in a Newborn.

P-11

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ences Campus.

Thromboembolic events in neonates are associated to congenital and acquired disorders. Congenital etiologies include deficiencies in the plasma inhibitor proteins, especially protein C, protein S, and antithrombin III. Acquired disorders include those related to umbilical catheters, cyanotic heart disease, and disseminated intravascular coagulation. We present the case of a newborn girl with an intrauterine thrombotic event. Upon delivery, she presented cyanosis, edema, and poor perfusion of the left forearm and hand with areas of necrosis. Protein C and S functional activity were reduced (39% and 16%, respectively). These suggested a type II protein C and S deficiency. Plasminogen (level and activity) and antithrombin III were normal. With continuous infusion of heparin and treatment with urokinase, the patient recovered with loss of one finger and limited movement of the wrist. Two skin grafts were required and the patient is receiving physical and occupational therapy. Deficiency of plasma inhibitor proteins must be highly suspected in newborns presenting thromboembolic events. Early diagnosis is important in view of the immediate treatment required for adequate recovery and the implications for long term therapy.

The Kinetics of Resistance of Anti-Fas Induced Apoptosis of Jurkat Cells Correlates with Mitochondrial Integrity.

P-12

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Some viruses have developed apoptosis-blocking mechanisms. We examined the kinetics of apoptosis and mitochondrial integrity of Jurkat cells chronically infected with HIV (J1.1 cells) and compared it with that of uninfected cells. Samples were treated with anti-fas (CD95) antibodies and cultured for 72h and harvested from 0 to 72h post-treatment and examined for apoptosis, mitochondrial transmembrane potential and integrity, and cellular morphology. Results show J1.1 resistance to apoptosis induction during the 72h of the kinetics study (0-8%). Percent values of mitochondrial potential (0-17%) and NaO staining (0-23%) correlated with protection from apoptosis. Jurkat cells maintained percent values of apoptosis dur-

ing the kinetics duration (0-16%) although baseline values were higher for these cells. However, they had increased impairment of mitochondrial function (4-83%) and membrane integrity (2-89%) as the time of treatment increased. Cell morphology correlated with apoptosis and mitochondrial alterations for both cell lines. Our results support the protective role of HIV from apoptosis induction on infected cells. Supported by NIH grants RR03051 and S06-GM08224.

Congenital CMV Presenting with Microcephaly

P-13

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Congenital cytomegalovirus (CMV) infection affects approximately 2 percent of all live births in the United States, with an estimated total of 40,000 live births infected each year. In Puerto Rico there is no recent study related to the prevalence of CMV transmission. If an intrauterine sonogram does not reveal characteristic findings a diagnosis of CMV may be missed due to the subtle clinical symptoms present in the mother, such as flu-like symptoms. The majority of neonatal cases are asymptomatic, and only 5 to 10 percent present symptoms at birth. Affected infants may present microcephaly, hepatosplenomegaly, neonatal jaundice, petechial rash, chorioretinitis, intracranial calcifications, intrauterine growth retardation, and sensorineural hearing loss. The diagnosis of CMV is confirmed by a positive culture or PCR in saliva or urine. Increase in IgM anti-CMV tiers within the first three weeks of birth is diagnostic for congenital CMV. We report a BBTAGA born to a 23 y/o mother G3P3A0 by C-section due to breech presentation. with gestational age of 37 6/7 weeks. The mother had bronchitis during the pregnancy, and an intrauterine sonogram revealed microcephaly. Physical examination revealed microcephaly, petechial rash and splenomegaly. Antibiotic therapy initiated due to possible clinical sepsis, and thrombocytopenia was present at birth. Increased IgM antibody titers for CMV and CMV urine culture were positive at birth. Head CT scan showed periventricular calcifications. Congenital CMV is rarely diagnosed in Puerto Rico. One must be aware that if the prevalence in Puerto Rico is similar to that of the United States, 1300 neonates of the 65,000 yearly live births may be affected with the condition. At our service the diagnosis is suspected in some neonates but proven in very few.

P-14 Production of Monoclonal Antibodies against *Blomia tropicalis*.

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The objective of this research was to produce monoclonal antibodies against whole body extracts from the storage mite *Blomia tropicalis* (Bt). The monoclonal antibodies were produced using standard techniques. One hundred mg of body mite extract was resuspended in 100 ml of Phosphate Saline Buffer (PBS), emulsified 1:1 with Hunter's Titer Max adjuvant and then, 200 ml of the antigen preparation was injected intraperitoneally (i.p.) in Balb/c mice. Twenty-eight days after, the animals received a booster i.p. of 50 mg of antigen in 100 ml of PBS. Three days after the booster, splenic cells from the immunized mice were fused with murine myeloma cells (Sp20/Ag-14) and the resulting hybrids were cultured in HAT selective medium. Culture supernatants from hybrids were used to determine the presence of antibodies against Bt. using ELISA. One antibody producing hybrid were obtained (5.6%) and it was cloned by limiting dilution producing nineteen clones. Seven of these clones (36.8%) were positive by ELISA and three were subcloned and selected as antibody-producers against Bt. Fourteen subclones has been identified as monoclonal antibody-producers. The isotype of these mAb has been determined as IgM by ELISA. Six of these monoclonal antibodies were concentrated by ascitic fluid technique. Western Blot technique is currently in progress to identify and define the antigens recognized by the monoclonal antibodies and determine cross-reactivity between Bt. and other mites. The monoclonal antibodies will be used to localize the recognized antigens in the body of mites.

P-15 Molecular Characterization of Group 1 Allergen Blot 1 from House Dust Mite *Blomia tropicalis*.

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A current focus in allergy research concerns several classes of proteins from house dust mite species, implicated as elicitors of the allergic response in asthma. One of these classes of proteins is the group I cysteine proteases. Using molecular biology techniques, allergens coding sequences from this protein have been cloned from *Dermatophagoides pteronyssinus* (DP), *D. farinae* (DF) and *Europhyphus maynei* (EM) allowing the sequence

analysis and potential manipulation of these proteins. The mite species *Blomia tropicalis* (BT) is an important source of allergens inducing allergic asthma in tropical regions. Many studies have been performed to characterize and identify the allergens in extracts from DF and DP. Unfortunately, little work has been done regarding the identification of the spectrum and relative clinical importance of BT allergens. To overcome this limitation, we have applied polymerase chain reaction (PCR) to amplify sequences from a cDNA library of BT (λ gt 11), prior to cloning and sequencing. Using the cysteine protease cDNA sequences of Der p 1 and Der f 1 and the genomic sequence of Eur m 1, we designed two primers from highly conserved amino acid regions: one corresponding to amino acids 23-29 of the amino terminal and the other corresponding to amino acids 288-294 of the C-terminus of the protein. PCR amplification from BT cDNA (35 cycles of 95°C/45s, 52°C/2m and 72°C/2min) gave a single product which was further amplified and cloned using PCR-Script plasmid vector. Deducted amino acid sequence analysis of this single product showed a 60% homology with cysteine proteases of other mites as DP, DF and EM. The level of primary sequence similarity obtained suggest that the protein from BT have the same gross secondary structure and enzymatic properties than cysteine proteases from DP, DF and EM.

P-16 Mapping of 5ht-like Immunoreactivity in the Central Nervous System of a Tropical Freshwater Prawn.

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The giant prawn *Macrobrachium rosenbergii* shares many characteristics of other Decapod crustacea that make them good model systems in neurobiology, with the added advantage of being better suited than other crustacea for developmental studies (Friedlander & Levinthal, J. Neurosci., 2, 1982). We wish to use this system to study the development of circuitry controlling specific behaviors. As a first step we are using wholemount immunohistochemical techniques to characterize the distribution of various transmitters and modulators in the central nervous system of the prawn. Here we report our observations on the distribution of serotonin-like immunoreactive (SLI) cells. The brain contains paired clusters of medium and small sized SLI cells located at the midline and small sized SLI cells scattered more peripherally. Each circumesophageal ganglion contains a single SLI cell, which sends its axon anteriorly towards the brain, and

extensive terminal arborizations of fibers traveling through the connectives. In the subesophageal ganglion, small paired SLI cells were found along the paths of SLI midline and lateral fiber bundles (MFB and LFB, respectively). A pair of thick SLI fibers that form part of the MFB exit the ventral nerve cord through one of the roots between the subesophageal ganglion and the first thoracic ganglion (T1). Cell bodies of axons traveling in the MFB include two pairs of large SLI neurons, one found at the midline in T5, the other in the first abdominal ganglion (A1). Each thoracic ganglion (1-5) appears to have at least one pair (in some cases two) of small SLI neurons with cell bodies placed laterally and axons projecting towards the midline. In addition to these small lateral neurons, T5 also has two pairs of large neurons, one in the midline, the other halfway between the midline and the lateral edge of the ganglion. The axons of the latter pair of neurons project horizontally for a short span and then anteriorly between the MFB and LFB. The six abdominal ganglia all seem to have a similar pattern of distribution of SLI neurons (with the exception of A1): at least two pairs of small cells, with fibers going across the midline and traveling anteriorly, with a neuropil region located laterally in each hemiganglion. A1 appears different only in that one pair of these SLI neurons is large in size. The system of SLI neurons in this organism seems well designed to produce widespread control or regulation of motor programs and/or behavior. Support: RCMi G12RR03051, NSF IBN9707091, Puerto Rico EPSCoR.

P-17 Stimulation of Na Pump Activity in Mouse Brain Astrocytes by Kappa, but not Delta Opioid Agonists.

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Receptors for delta, kappa and mu opioids have been identified on the astrocyte membranes both in culture, and in brain, where they are found near neuronal synapses that also have opioid receptors. Nonetheless, little is known of the functional role of opioids in astrocytes. Opioid agonists are known to inhibit adenylyl cyclase, thereby blocking synthesis of cyclic AMP and the subsequent activation of the cyclic AMP-dependent cascade, including the cAMP-dependent protein kinase. Moreover, activity of the Na,K-ATPase (also known as Na Pump) has been shown to be reduced by protein kinase A-mediated phosphorylation, although stimulation by inhibitors of adenylyl cyclase has not been shown. In the present study opioid agonists were applied to confluent cultures of neo-

natal mouse cortical astrocytes permeabilized with alamethacin. Activity of the Na Pump was determined as the ouabain-sensitive hydrolysis of an alternate substrate, p-nitrophenyl phosphate. The specific kappa1 agonist U-69593 (20 iM) was found to significantly stimulate activity, whereas the delta agonist DPDPE (20 iM) had no effect. Ouabain-insensitive phosphatase activity was not stimulated by the kappa agonist. Activation occurred in less than 5 minute. Western blot studies confirmed that the astroglial cultures contained only the $\alpha 1$ and $\alpha 2$ isoforms of the Na,K-ATPase, and lacked the $\alpha 3$ isoform that is absent from glia but expressed to high levels in neurons. The cells were predominately positive for GFAP, an astrocyte-specific antigen. The cultures also contained occasional small, bright cells that have not been identified. These results provide evidence that kappa opioids rapidly activate the Na Pump in cultured cortical astrocytes. This neurotransmitter-modulated response may contribute importantly to astrocytic removal of potassium from extracellular space following action potential activity.

P-18 Mu Opioid Agonist DAMGO Stimulates and PGE-1 Inhibits Na,K-Pump in SH-SY5Y Human Neuroblastoma Cells.

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The effect of prostaglandin E_1 and DAMGO ($[D-Ala^2, N-Me-Phe^4, Gly^5-ol]-Enkephalin$), a mu-opioid agonist, on Na,K-ATPase activity was examined in intact, retinoic acid-differentiated SH-SY5Y human neuroblastoma cells. Only the $\alpha 3$ isoform of Na,K-ATPase was found to be present in either control or differentiated cells. The cells were permeabilized with alamethacin (20 $\mu g/ml$), then treated with 1 iM PGE_1 \pm the mu-opioid agonist DAMGO (0.01-1 iM) for 10 min. Preincubation with PGE_1 inhibited maximal Na,K-ATPase activity to 40% of control, with peak inhibition occurring after 10 min pre-incubation. When 1 iM DAMGO was included in the pre-incubation mixture, activity was not different from control, whereas 10 nM DAMGO had no effect, and 100 nM restored activity only partially. The stimulatory effect of 1 iM DAMGO was blocked by naloxone (10 iM), and was reproduced by 150 iM 8-bromo-cyclic AMP. Production of cyclic AMP was 7.4 ± 0.38 pmol/mg protein during maximal inhibition with PGE_1 , and $3.4 \pm .23$ with both PGE_1 and DAMGO. Adding 5 mM IBMX raised the amount of cyclic AMP by 20-fold. These results demonstrate that opioid agonists modulate the activity of Na,K-ATPase measured under V_{max} conditions, especially in

the presence of heterologous inhibition. This effect may contribute to the neuroinhibitory effect of opioid neurotransmitters. [Supported in part by NIH MARC GM18554 and RR-03051].



P-19 **GAD65- and GAD67-like Immunoreactivity in the Circadian System of the Thirteen-Lined Ground Squirrel.**

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The suprachiasmatic nucleus (SCN) of the hypothalamus houses the principal pacemaker or biological clock of the circadian system in mammals. It receives information on environmental photoperiods directly from the retina via the retinohypothalamic tract (RHT), and from the intergeniculate leaflet (IGL) of the thalamus via the geniculohypothalamic tract (GHT). Several neuroactive substances (neurotransmitters/neuromodulators) are expressed by circadian system structures, and it has been reported that g-amino butyric acid (GABA) is the principal neurotransmitter [Moore and Speh: *Neurosci. Lett.* 150(1993)112-116]. We were interested in studying GABA's expression in the circadian system of the thirteen-lined ground squirrel (*Spermophilus tridecemlineatus*). Immunohistochemical procedures were used to study the expression of GAD65 and GAD67, two isoforms of glutamic acid decarboxylase (GAD), GABA's synthesizing enzyme. Squirrels housed under 12h L/ 12h D were anesthetized and perfused intracardially at 3 PM (n=7). Their brains were frozen and sectioned; sections of interest were stained for the isoforms. GAD65- and GAD67-like immunoreactive cells and axon terminals were observed in the SCN and the IGL, although the staining pattern differed in both structures. Experiments with both isoforms demonstrated a larger number of labeled cells, as well as more intense staining, in the IGL. GAD65- and GAD67-like neurons in the SCN were few and lightly stained. Experiments are underway to examine the expression of the two isoforms in animals housed under other photoperiods. (Supported by NIH grants MH-48190 and RR-03051.)



P-20 **Effect of Ambient Photoperiod on Neuropeptide Y-like Immunoreactivity in the Circadian System of the Ground Squirrel.**

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The suprachiasmatic nucleus (SCN) of the hypothalamus houses the major circadian clock in mammals. The SCN receives projections from the retina, the intergeniculate leaflet (IGL), and the dorsal raphe nuclei. Several neuroactive substances have been detected in the SCN, among them neuropeptide Y (NPY). The source of NPY immunoreactive fibers in the SCN is reported to be neurons of the IGL. In the present experiments variation of NPY expression with photoperiod was studied in a diurnal hibernator, the thirteen-lined ground squirrel (*Spermophilus tridecemlineatus*). Squirrels were housed under one of three photoperiod regimes: 12h L/ 12h D (n=3), 24h L (n=3), 24h D (n=3). After 1-2 weeks exposure to the regime, the animals were anesthetized and perfused intracardially at 3 PM. The brains were frozen and sectioned; sections of interest were stained immunohistochemically for NPY. All animals exhibited NPY-like immunoreactive terminals in the SCN and immunoreactive cell bodies in the IGL. Labeled cells in the IGL had a soma diameter ranging from 6 to 20 mm, with a mean of 14mm. The number of immunoreactive cells was greater in animals exposed to 24h D. Optical density measurements were performed in the SCN to compare expression of NPY for the different photoperiods. The SCN of animals housed under 12h L/12h D and 24h D displayed similar values of expression of NPY, while the SCN of animals kept under 24h L showed a slight increase in NPY expression when compared to the other two groups. Statistical analysis of the data did not demonstrate any significant variation. Some of our results on NPY expression in the SCN after different photoperiod regimes do not agree with those reported in nocturnal, non-hibernating rodents like rat and hamster. (Supported by NIH grants MH-48190 and RR-03051.)



P-21 **Growth Associated Protein Gap-43 and Neurofilament Expression in Frog Ganglion Cells After Optic Nerve Injury.**

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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 or crushed between eyeball and chiasm and Nissl stains of flat-mounted retinas were prepared at 2 week intervals after the operation. RGCs from control and experimental retinas were impaled with intracellular microelectrodes

filled with Lucifer Yellow. Retinas 6 weeks after axotomy contained dying cells, most of which had pyknotic nuclei. Apoptosis was also detected in the frog retina after axotomy. Injection of Lucifer Yellow into RGC somata in frog retinas after axotomy revealed a variety of sizes and dendritic morphologies. Some of the injected cells had dendritic processes that showed signs of sprouting, with growth cone-like structures at the tips. In the control retinas neurofilament and GAP-43 immunoreactivity was found only in the optic nerve fiber layer and in the optic nerve. One month after axotomy a subset of RGCs cell bodies and dendrites were labeled with antibodies against these two markers, in both cut and crush preparations. An additional population of smaller cells was also stained in the retina when the optic nerve was crushed. Three months after crush a profusion of regenerating fibers had penetrated the distal stump. When the nerve was cut, reconnection between the stumps was not observed at three months. At this time, neurofilament and GAP-43 stain remained high in the retinas where the optic nerve was cut, while it had diminished in the crush preparations. Surviving RGCs suffer dendritic remodeling that includes the appearance of growth cone-like structures. Supported by NIH MBRS S06 08224 and G12RR-03051.

P-22

Excitatory Amino Acid Transporters: Possible Targets for Anticonvulsant Therapy?

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High concentrations of glutamate (Glu), the major excitatory neurotransmitter in the brain, have been observed in many pathological conditions such as stroke, epilepsy, Huntington, and Amyotrophic Lateral Sclerosis. Excitatory amino acid transporters (EAATs) constitute the main mechanism for removing Glu from the synapse. Thus, up-regulation of EAAT activity could have profound therapeutic implications. Genetic and chemically - induced seizures alter EAAT activity. In rat hippocampal slices, veratridine (prevents Na⁺ current inactivation) produces epileptiform activity and causes a decrease in EAAT activity. *In vivo* inhibition of EAAT activity in rats treated with the convulsant kainic acid, increases susceptibility to further convulsive stimuli. G-proteins can regulate EAAT activity. Metabotropic glutamate receptors (mGluRs) are a possible source of EAAT regulation. In-

deed, L-AP4 (mGluR-III agonist) reverses the inhibitory effect of veratridine on EAAT activity. Similar effects are observed with the mGluR-I/II antagonist, MCPG, and in synaptosomes from mice with seizures. These results point to the involvement of EAATs in epileptic activity and to its regulation as a possible therapeutic target.

K⁺-induced Rapid Changes of Rectification in Kir of Glial (Müller) Cells.

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The rectification ratio, I_{KIN}/I_{KO} , of K⁺-inward rectifying channels (Kir) is different in endfoot and soma, as well as in different areas of retina during steady state conditions in Müller (radial glial) cells (Skatchkov et al., 1999 in press). We report here that I_{KIN}/I_{KO} ratio is changed rapidly and reversibly during physiologically relevant changes of [K⁺]_o in subsecond time. Quick changes in [K⁺]_o in sequences: 3-1-3-10-3 mM, repeated again in subsecond time resulted in an almost two-fold decrease of I_{KIN}/I_{KO} (1.88 ± 0.22, n=7) in isolated cells from retinal center (85-140 μm length). In contrast, peripheral cells (30-60 μm) demonstrated almost no changes in their I_{KIN}/I_{KO} ratio. We conclude, first, that rapid relief of Kir rectification during physiologically functional (time and K⁺ concentration) might be a feedback mechanism of potassium spatial buffering, making it more rapid and focused. Second, the mechanism of rectification changes may be related to changes of K⁺ and/or SMP/SPD concentrations as well as an interaction between K⁺ and polyamines at glial Kir channel sites. Supported, NIH-RCMI G12RR03035, NINDS, NSF-EPSCoR and DFG (849/3-2 and BMB+F (IDZL, 01KS 9504 project C5).

Spatial Localization of Polyamines in Glia and Neuronal Kir Channels in Hippocampus.

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Disruption of glial function blocks both inhibitory (Skatchkov et al., 1998, 7th Puerto Rico Neurosci. Conf.) and excitatory (Fonnum et al., 1997, *Glia*, 21:106) synaptic transmission in neurons. Several mechanisms involving the neuronal-glial relationship have been proposed to

explain this phenomenon. For example, paired-pulse facilitation in CA1 area of hippocampus is observed after treatment with glial blockers, and also with the addition of spermine/spermidine (SPM/SPD). Therefore, the question arose: where is SPM/SPD localized in the hippocampus and how does this compare to the distribution of SPM-dependent receptors and channels? To address this question, hippocampal slices were prepared for immunocytochemical visualization of SPM using anti-SPM (Laube and Veh, *Glia*, 1997, 19:171) and anti-Kir2.4 polyclonal antibodies to determine their spatial distribution. Here we demonstrate that glial cells in area CA1 (astrocytes) are predominantly and selectively stained with SPM antibody and that SPM/SPD is removed by gliotoxin or accumulated after SPM perfusion. Previously, we have shown functional co-expression of SPM and K^+ inwardly rectifying channels (Kir) in glial cells (Biedermann et al., 1998, *Glia*, 23: 209; Skatchkov et al., 1998, *Soc. Neurosci.*, 24:1329). Furthermore, here we describe spatial co-localization of polyamines in glia near neurons expressing SPM-dependent Kir-2.4 type channels. This suggests that a source of polyamines (SPM/SPD) controlling neuronal Kir rectification is glia. Supported, NIH-RCMI G12RR03035 (projects A and B), NINDS, NSF-EPSCoR, DFG (849/3-2 and BMB+F (IDZL, 01KS 9504 project C5).



P-25 Possible Abnormal Retinal Aging in Human Residents of Puerto Rico.

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Rhesus monkeys raised in Puerto Rico undergo a process of retinal aging not observed in rhesus monkeys raised in more northern climates. In the peripheral retina of Puerto Rican rhesus monkeys large cells (most likely retinal ganglion cells) are lost during aging while smaller cells (most likely glia) proliferate, resulting in an overall increased cell density. Since the rhesus monkey visual system is for many purposes a good model of the human visual system, it is reasonable to inquire whether the retinas of human residents of Puerto Rico also exhibit this abnormal process of aging. For this purpose retinas were obtained from three donated cadavers (73 and 83 year old females, 86 year old male). These individuals were born and died in Puerto Rico. Another set of retinas for a 73 year old male was obtained from the Lions Central Florida Eye Bank. Retinas were removed from the eyes and mounted as whole mounts on microscope slides with the ganglion cell layer uppermost. The retinas were stained on the slide with cresyl violet which labels all RNA in endoplasmic

reticulum and nucleoli. The former is mainly present in the cell body and its staining thus reveals the shape of the soma. Using a camera lucida, fields 80 x 80 μ m were analyzed at the intersections of a 2 x 2 mm grid superimposed on the retina. The outline of each soma within the 80 x 80 μ m field was traced. Afterward, the area of each soma outline was determined and the equivalent circle diameter was computed. Our results show that the retina obtained from Florida and one of the Puerto Rico residents (83 year female) retained large cells and did not show increased cell density in the periphery. A second Puerto Rico resident (73 year female) showed a decreased number of large cells with possible increased peripheral density while a third (86 year male) showed a complete loss of large cells and increased density in the peripheral portion of the ganglion cell layer. These results indicate the need to examine more residents of Puerto Rico in more detail in order to determine the extent of this abnormal process. Supported, in part, by NIH grants MH-48190 and RR-03051.



P-26 Bilateral Intraocular Injection of GABA Reduces Phototaxis in *Rana pipiens* via a GABA_A Receptor.

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The positive phototaxis of the frog *Rana pipiens* depends on ON-type retinal ganglion cells (see Kicliter, Kay and Chino, *Brain Res.* 210:103-113, 1981). In *Ambystoma tigrinum*, another amphibian, application of 500 μ M GABA to the retina blocks the ON component of ON/OFF retinal ganglion cell responses to light flashes, while the OFF component is left intact (Zhang and Slaughter, *J. Neurophysiol.* 74:1583-1592, 1995). The present experiments explored the possibility that GABA application to the retina might affect phototaxis in *Rana pipiens*. GABA was injected bilaterally into the vitreous body in amounts sufficient to produce concentrations of 5-500 μ M over the retina. Injections at 500 μ M reduced positive phototaxis by 6-16 percent, as measured by a light/dark choice in a Y-maze, from pre-injection levels of 80-90 percent choice of the lighted arm. Reduction in phototactic preference was also observed in frogs injected at 50 μ M, but not at 5 μ M. Nor was the effect observed in sham injected frogs. The reduction in phototaxis could be blocked with simultaneous injection of either picrotoxin or bicuculline, but not saclofen, at 100 μ M. Since picrotoxin and bicuculline block the GABA_A receptor while saclofen blocks the GABA_B receptor, the present findings indicate that the

effect of GABA on the retinal network underlying phototaxis is mediated through a GABA_A receptor. Supported, in part by NIH grants MH-48190 and RR-03051.

Retinal Aging in Rhesus Monkeys from Puerto Rico.

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Rhesus monkeys in Puerto Rico are exposed to increased levels of ultraviolet radiation as a consequence of geography. Since ultraviolet light has been shown to have several deleterious effects on the eye, we examined the ganglion cell layer for evidence of change. Monkey eyes were enucleated soon after death and fixed by immersion in 4% paraformaldehyde. After postfixation, retinas were removed from the eye, mounted on microscope slides and stained for Nissl substance as whole mounts. Five monkey retinas selected for detailed analysis had ages of 5, 11, 12, 18 and 21 years at death. Retinas were studied by sampling at the intersections of a 2 mm X 2 mm grid which was overlaid on the retina so that one line passed through both fovea and optic disc. The sampling area extended from approximately 2 mm nasal to the optic disc to 4 mm temporal to the fovea. Vertical distances studied extended approximately 8 mm above and below the line connecting the fovea and optic disc. The retina was not sampled in the macular region; the closest sample was made at approximately 2 mm from the fovea. At grid intersections within this sampling area the somata of all cells in the ganglion cell layer were drawn in an 80 mm X 80 mm field, using a camera lucida with an oil immersion objective. Evidence was found of increasing cell density in the older retinas. Cell size histograms prepared from the combined samples in each retina indicate that smaller cells in the 5-7 mm diameter range account for much of the increase. This research was supported, in part, by NIH grants MH-48190 and RR-03051.

Tubular Localization Of Ca²⁺ Channels In Crustacean Skeletal Muscle Fibers.

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The disruption of the transverse tubular system in vertebrate skeletal muscle has been used to correlate the localization of Ca²⁺ channels with some role during E-C coupling. Because the structure of the T-system and the E-C

coupling mechanism of crustacean muscle are different from vertebrate muscle, we attempted to detubulate crustacean muscle. We examined the electrophysiological, mechanical and ultrastructural effects of the formamide (FMD) procedure (del Castillo and Escalona de Motta. J. Cell Biol 78:787, 1978) in tonic muscle fibers of the crustacean *Atya lanipes*. Contractility was rapidly inhibited in the presence of FMD (2M). When transferred back to normal saline, mechanical activity was partially recovered during the first 5-8 min, but it was irreversibly lost after 30-45 min. Caffeine (4mM) induced sustained contractures after FMD treatment, indicating that the contractile machinery was intact. Strontium spikes and currents (V_{max}), and membrane capacitance (C_m) were reduced after FMD treatment; electronmicrographs showed a swelling of the T-tubules. Lanthanum, a marker of the T-tubule system, penetrated the tubules in control muscles but failed to enter in muscles treated with FMD. These observations provide direct evidence that FMD produces detubulation and indicate that the Ca²⁺ channels required for E-C coupling are mainly located in the T-tubules. (Supported by NIH NS-07464, RR03050 and GM-08224).

Experimental Calibration Standards With Enhanced Green Fluorescent Protein Used in Quantitative Flow Cytometry Analysis as a Function of Ph Environment.

P-29

Y. Gerena, L. Díaz, G. Santiago, I. Hernández, A. Schwartz and E. Fernández-Repollet.

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Enhanced Green Fluorescent Protein (EGFP) expressed in living cells can be quantitated by flow cytometry using experimental calibration standards labeled with EGFP. When performing quantitative fluorescence measurements by flow cytometry it is important to know whether the calibration standards are responsive to environmental changes (i.e. pH) as well as their stability with time. These issues were investigated in a set of experimental EGFP calibration standards (FCSC, San Juan, PR) which were equilibrated in a series of Coulter Balanced Electrolyte Solution buffers which pH was adjusted across a range that covered from 5.0 to 9.0. The EGFP standards were also equilibrated in a physiological solution (pH 7.2) for a prolonged period of time (6 months) to test their stability. The fluorescence intensity (FI) of the EGFP calibration standards was analyzed on a FACSort flow cytometer (BDIS, San Jose, CA) at the same instrument settings (FL1 PMT 625). Our data revealed that the FI of the EGFP standards remained unchanged across a pH range

of 6.0-9.0. A small reduction (<10%) in FI was observed at pH 5.0. The FI of the EGFP standards maintain at pH 7.2 remained stable throughout the duration of our experiment. Studies are in progress to determine whether a similar response to pH changes is observed with free EGFP protein as well as in cells expressing EGFP. Supported in part by RCMI Grant No. 612-RR-03051 and MBRS Grant No. 5-S06-GM08224.

P-30 **Characterization of Potassium Channels Mediating Outward Currents in Frog Muscle Membrane by Novel Toxins From Marine Sponges.** K.V. Alejandro^{2*}, O. Barreto-Chang, R. Rosa, A.D. Rodríguez, And G. Escalona De Motta. University of Puerto Rico, Río Piedras Campus, ¹Dept. of Chemistry, and Medical Sciences Campus, ²School of Medicine and Institute of Neurobiology, San Juan.

Well identified potassium channel subtypes may be specifically blocked by compounds and toxins acting as potassium channel inhibitors. This work aims to characterize the K⁺ channels of fast skeletal muscle fibers recording the macroscopic currents elicited by depolarizing voltage stimuli applied to patches of frog sartorius muscle membrane. Currents recorded in the presence of 10, 15 and 20 mM TEA showed a dose dependent blockade of outward currents without any alteration in the inward currents. This observation confirms that these currents are conducted via TEA-sensitive, potassium channels. These channels were identified as IK channels as the specific inhibitor Tityus toxin in the crude venom of *T. serrulatus* inhibited an average of 93.8% of the current at 10 pg/mL, 61.3% at 0.10 pg/mL and 24.7% at 0.001 pg/mL. Observations were made in the presence of four bromopyrrole alkaloids isolated from specimens of Caribbean sponges of the genus *Agelas*, clathrodin (CLA) and oroidin (ORO), respectively, the non-brominated and di-brominated monomers, and scep trin (SCP) and dibromoscep trin (DBS), respectively, the mono- and di-brominated dimers. Application of both CLA and DBS reduced the amplitude of inward sodium currents, producing complete blockade in 6 (CLA) to 30 (DBS) min. Outward currents were also inhibited, CLA producing a 50% inhibition in 5 min while DBS blocked 60% of the current after 30 min. SCP and ORO exerted only a moderate blocking effect on both current components, inhibiting nearly 30% of the currents after exposure periods of more than 40 min. Published observations from our laboratory indicate that these compounds interact with the sodium channel macromolecule. The present pharmacological observations suggest that the chemical structure of these alk-

aloids allows them to interact with similar affinities with both the sodium and the potassium channels present in this muscle membrane. Supported by NIH Grants GM08102 and MH48190.

P-31 **Frog (*RANA PAPIENS*) Gastric *Muscularis Externa* As A Model Assay For Pharmacological And Morphological Studies: Characterization Of Cholinergic And Adrenergic Receptors.**

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The contractility of gastrointestinal smooth muscle is influenced by both cholinergic and adrenergic outflows conducted through the peripheral nervous system and the localized neuronal plexi. We used isolated gastric *muscularis* muscle strips from the frog *Rana pipiens*, to examine the relative roles of these outflows on the spontaneous contractile activity exhibited in these muscles. Four functional stomach regions were identified, the upper cardiac or upper region being the most active. Strips from this region (1 and 2) exhibited both tonic and phasic responses to ACh (10nM to 10mM) and rhythmic spontaneous contractions. This contractile activity was inhibited by atropine, a non-specific antagonist, suggesting the presence of cholinergic muscarinic receptors. Using the specific blockers pirenzepine (M₁), AF-DX 116 (M₂) and 4-DAMP (M₃), we demonstrated that muscarinic receptors of the M₃ subtype mediate both spontaneous and ACh-induced contractions in this muscle. Application of norepinephrine (NE) inhibited both the amplitude and the frequency of these spontaneous contractions. This inhibitory effect was blocked in the presence of propranolol, a b-adrenergic antagonist but not in the presence of tolazoline, an a-adrenergic antagonist muscles blocked with the M₃ muscarinic blocker 4-DAMP, no direct NE effect was observed. Use of the specific a- and β-receptor agonists revealed a potent inhibitory action for the β-agonist isoproterenol. These results indicate that this preparation is a sensitive and reproducible assay for compounds acting on muscarinic M₃ subtype, cholinergic receptors. It is proposed that adrenergic input exerts a negative modulatory effect on this ACh-mediated spontaneous contractility of amphibian gastric muscle. Supported by NIH Grants GM08102 and MH48190.

P-32 **Effect of Water-and Ether-Soluble Fractions of Koso-Toxin On Frog (*RANA PIFIENS*) Stomach Contractility.**

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Kousso is a potent anthelmintic remedy used in NE Africa and primarily prepared from flowers of *Banksia* trees. Though it is highly effective destroying tapeworms, it has poor cathartic action (Grieve, 1998). Koso-toxin (KTx), its principal active constituent, is said to be neurotoxic and has been seen to cause tetanus in frog skeletal muscle (Dajas, unpublished observations). We examined the effect of water- and ether- soluble fractions, obtained from a crude kousso extract, on the spontaneous phasic contractions, of *muscularis externa* strips dissected from the cardiac region of frog (*Rana pipiens*) stomach. Previously, we showed that this activity is enhanced by ACh and depends on extracellular calcium (Ca_o) concentration. Records of these spontaneous contractions were obtained on a polygraph, under partially isometric conditions, with the aid of a mechano-electrical transducer. Application of a 10mg/mL dilution of the water-soluble KTx (w-KTx), in cumulative doses from 7mg/mL to 200mg/mL, stimulated the force of contraction and reduced their frequency both in normal and in high (2X) Ca_o Ringer's. Low (0.25X) Ca_o levels reduced contractile activity both in the presence and in the absence of w-KTx. Contrary to these observations, addition of ether-soluble KTx in concentrations as low as 0.1mg/mL decreased the amplitude of contractions eventually causing a complete block. These observations suggest the existence of two different toxic components in these fractions, a paralytic one possibly related to koso-toxin and a calcium-dependent stimulatory one possibly responsible for the neurotoxic effects of kousso. Supported by NIH Grants GM08102 and MH48190.



P-33 **Effect Of Liver Extracts From Caribbean Reef Fishes On The Contractility Of Frog (*RANA PIFIENS*) Gastric Smooth Muscle.**

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Maitotoxin (MTX) is a water-soluble toxin produced by the marine dinoflagellate *Gambierdiscus toxicus* and

found in the body of various herbivorous reef fishes in contrast with other dinoflagellate polyether toxins which are sodium channel activators, MTX acts as a calcium channel agonist. As the contractility of intestinal and gastric smooth muscles is critically dependent on the influx of calcium from the extracellular medium, this tissue is an ideal experimental model to evaluate the mechanism of action of MTX and MTX-like compounds from these marine organisms. In our laboratory we have characterized the gastric *muscularis* muscle dissected from the stomach of frog *Rana pipiens* as a useful preparation to assay toxins and novel compounds acting on the receptors and channels of smooth muscle membrane. Previous studies with this model have shown the possible presence of MTX in liver extracts obtained from barracuda (genus *Sphyraena*) In this work we have studied the effects of liver extracts obtained from Caribbean reef fishes from the genera *Haemulon* (grunts), *Holocentrus* (squirrelfishes) and *Acanthurus* (surgeonfishes or doctorfishes), were collected during a period of eight months (August 1994 to March 1995). Fishes caught in a given month were pooled for the preparation of liver extracts following standard procedures to obtain palytoxin (PTX), ciguatoxin (CTX) and MTX. The latter samples (F66-F73) were dissolved in 20% formamide to a final stock concentration of 10mg/mL. Increasing concentrations (7-100mg/mL) were applied to muscle strips and their effects were observed on the spontaneous activity of the muscle. Isometric recordings of the force and frequency of these contractions were done under control conditions and in the presence of the liver extract samples to observe their effects on the spontaneous activity of *muscularis externa* muscle strips. Results obtained in this study showed at least two types of responses: an increase in the force and/or frequency of contractions, and a moderate decrease in these responses. The effects observed were both concentration- and time-dependent. At low concentrations (7-15mg/mL) effects were seen with longer exposure time (10-30 min.). The most active samples were those obtained from fishes collected in November and December. These findings correspond to those observed in previous studies with barracuda samples. Further studies will probe the factors responsible for the apparent seasonality of the activity of these fish extracts. Supported by the NIH Grants GM08102 and MH 48190.



Diagnosis of Malaria by PCR.

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Malaria is the world's most important parasitic infection, with an estimate of 300-500 million cases and between 1.5 to 2.7 million deaths annually. This disease was a serious health problem in Puerto Rico during the first half of the century, but the Island became the first tropical country in America to eradicate the disease and was certified malaria-free by WHO, in 1962 (Miranda and Casta, 1997). However, more than 200 imported cases of malaria have been reported to the Health Department of Puerto Rico since eradication (Informes epidemiológicos mensuales 1975-1992). Traditionally, the diagnosis of *Plasmodium sp.* was performed by microscopic examination of blood smears, a simple technique that depends on the direct detection of parasites in Giemsa-stained slides. This is a report of a 45-years-old female patient admitted to a San Juan Area Hospital with an 8 day history of daily chills and fever, myalgia, nausea and vomiting. Upon admission, peripheral blood smears displayed multiple intraerythrocytic ring-shaped trophozoites (approximately 50% parasitemia), highly suggestive of *Plasmodium falciparum*. In addition, the Polymerase Chain Reaction (PCR), was used as a complementary method for the detection of malaria parasites and confirmation of post-treatment parasite clearance.

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Identification of an *mdr* gene in *Plasmodium yoelii*.

P-35 I. Ferrer-Rodríguez, G. Gervais, J. Pérez and A. E. Serrano. University of Puerto Rico, School of Medicine, Department of Microbiology and Medical Zoology.

Amplification and mutations in the *pfmdr1* gene are partially responsible for the development and spread of resistance to antimalarial drugs in *Plasmodium*. Although *pfmdr1* has been associated with chloroquine and mefloquine resistance in human malaria, neither the mode of action of quinoline containing compounds, nor the mechanism by which the parasite has managed to develop resistance are well understood. Our "in vivo" murine malaria system includes strains of the rodent plasmodia para-

site *P. yoelii*, naturally resistant to chloroquine, as well as four related lines with different profiles of drug resistance, which may help to elucidate and understand the drug resistance mechanisms in *Plasmodium*. A DNA fragment (530pb) from the *mdr* gene of *P. yoelii* was amplified by PCR using primers specifically designed to amplified a fragment of similar size of the *mdr* gene of *P. berghei*. The PCR product was cloned into the pCR2.1 TOPO TA® cloning vector. Sequence analyses showed that this fragment contains an open reading frame that shares 93% identity at the amino acid level with the *pbmdr1* gene and 57% homology with the *pfmdr1* gene. Additional experiments will be performed using this fragment as probe, to determine the chromosomal location of the gene and to measure the gene copy number in the collection of drug sensitive and drug resistance strains of *P. yoelii*. Furthermore, the 530bp fragment was cloned into the expression vector pDS56E. Results from preliminary expression experiments show that pDS56E-*pymdr* produces an induced band of approximately 21 kda. This recombinant peptide will be purified and inoculated into rabbits in order to produce specific antibodies for the localization and characterization of the PyMDR protein within the parasite.

This project was partially supported by Associate Deanship for the Biomedical Sciences and Graduate Studies, NHI/MBRS grant GM08224 and by RCMC award PR03051 of the NCRN-NHI and the institutional CIDIC award.

Sustained Release Spheres Prepared by Cross Linking Technique.

P-36 S. Pica and E.S. Ghaly. School of Pharmacy, Medical Sciences Campus, University of Puerto Rico.

The use of sustained release formulations provides prolonged therapeutic blood levels of a drug and a consistent clinical response. It is convenient due to cost effectiveness, reduction of side effects and patient compliance. In this study, sustained release theophylline spheres were prepared by cross linking technique using Carrageenan and cross linking agents. The effects of different cross agents (potassium chloride, KCl; calcium chloride, CaCl₂; aluminum sulfate, Al₂(SO₄)₃) on drug release were investigated. As the polymer level is increased in the formula, the size of the spheres decreased and the bulk density increased. The invitro dissolution data showed that the drug release was increased as the percent of the polymer was increased from 2% to 4%. The percent drug released from spheres containing 2% polymer and KCl as cross linking agent was 92.3% while the percent

drug release from spheres containing 4% polymer was 100.97% at 2 hours of testing dissolution. Additionally spheres containing 2% polymer and prepared with $Al_2(SO_4)_3$ as cross linking agent released 67.4% drug at 2 hours while spheres containing 4% polymer release 91%. The cross linking of the polymer with cations produces a tridimensional network that controls the drug release.



P-37 Enhancement of Glipizide Solubility and Characterization Using Sensitive Instruments.

M. Sánchez and E.S. Ghaly. School of Pharmacy, Medical Sciences Campus, U.P.R.

The effect of different molar ratios (1:1, 1:3, 3:1) of Poloxamer:Glipizide and Beta-cyclodextrin (B-CD):Glipizide molar ratios on the dissolution rate of water insoluble drug (Glipizide) were studied. Two different preparation methods (physical mixture and coprecipitate method) were investigated. Infra-Red spectroscopy (IR), X-Ray diffraction (XRD), differential scanning calorimetry (DSC) and Scanning electron microscopy (SEM) were used as instruments to investigate the physicochemical properties of the different molar ratio preparations. The results indicated that it was feasible to prepare a solid dispersion (coprecipitate method) of glipizide with each polymer. The solubility of glipizide was increased in poloxamer and (B-CD) preparations with exception of (B-CD):glipizide (1:1) and (B-CD):glipizide (1:3) coprecipitate mixture. Also the solubility of the drug was increased with the increase of polymer concentration. An increase of poloxamer:Glipizide molar ratio from (1:3) to (3:1) produced an increase in drug dissolution from 73% to 100% at 3 hours of testing dissolution. The XRD, ESM and IR for poloxamer: glipizide and (B-CD):glipizide preparations suggested a possible drug-polymer interaction. The (DSC) results indicated a possible solidification of the drug from a crystalline to an amorphous system that should be less stable and consequently more soluble.



P-38 Theophylline Sustained Release Matrix Using Hydroxyethylcellulose Polymer.

E.B. Caro, P. Anzalota and E. S. Ghaly: School of Pharmacy, Medical Sciences Campus, U.P.R.

The direct compression method has been extensively used for sustained release tablet manufacturing. In this study theophylline tablets were prepared with a hydroxyethylcellulose polymer to form a matrix for a sustained release system. Five batches were manufactured and were tested for drug release. In the first three

batches, different percent of polymer (20%, 30%, 40%) were incorporated to theophylline formulation in order to select the best level of polymer which released the drug in a controlled rate. Additional two batches were prepared with different drug level (25% and 40%) and 40% polymer in order to explain the mechanism of drug release. Theophylline tablets were evaluated for their physical properties and invitro drug release. The data obtained indicated that in general, the 40% polymer was the best level for obtaining sustained delivery of drug from the theophylline tablets. Tablets containing 10% polymer released 66% of drug while tablets containing 40% polymer released 51.2% drug at six hours of testing dissolution. Also the use of different percent of drug showed that the mechanism of drug release follows the diffusion model for inert porous matrix. Hydroxyethylcellulose polymer has potential for controlling the release of theophylline from tablets.



P-39 UPR-School of Dentistry's Adolescent and Adult Patient Population Profile.

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The purpose of this study is to obtain a profile of the patient population treated in the UPR-School of Dentistry between the years 1992 and 1995. The profile includes epidemiological characteristics, medical and oral conditions and oral treatment needs. The information was gathered from the records of 600 patients that included the oral examination charting form, the dental and medical history, treatment plan and patient informed consent form. A calibration exercise was performed to standardize the data gathering process utilizing a customized data form. Data analysis was performed with an Epi-Info 6 computer program. General characteristics of the population: 69.2% Females ages 47 to 56, medical condition ASA I 59.0% with no oral lesions. The 24% of patients reported allergies. Patients were mostly interested in preventive care and operative treatment. Tooth conditions; the most absent #16, most carious #31, least carious #32, most restored #15. The dental occlusion classification that prevailed was Class I Angle. The radiograph most used was an intraoral full mouth series 67.7%, followed by the panoramic 21%. In conclusion, the information contained in the patient record may be utilized as one of parameters to evaluate the patient care system of a dental school.



P-40 **Calidad de Vida en el Residencial Jardines de Cataño y Factores que Afectan a la Misma.**

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En el trabajo de investigación realizado a la comunidad del Residencial Jardines de Cataño tiene como objetivo principal observar la calidad de vida que llevan los residentes del residencial y observar los factores que afectan a la misma.

Se tomó una muestra de 20 familias a razón de 2 familias por estudiante, donde la mayoría de la población estaba constituida por personas mayores de 60 años. Se utilizó un cuestionario para obtener datos tales como: nivel económico estilos de vida y el ambiente en que residían con el propósito de detectar algunos problemas que se encontraban afectando a dicha comunidad. Entre los hallazgos o problemas identificados se encontró lo siguiente: que la comunidad manifestaba altas incidencias de depresión, condiciones respiratorias, mantenimiento inadecuado de la salud, manejo inadecuado de desperdicios sólidos, presencia de vectores, animales realengos, aguas estancadas, insectos y mal olor. Como parte de nuestras intervenciones en la comunidad, realizamos planes educativos individualizados para cada familia, dependiendo de sus necesidades. A nivel macro trabajamos en una feria de salud, realizamos referidos a la trabajadora social de la comunidad entre muchos otros. Este trabajo nos brindó la oportunidad de desarrollarnos profesionalmente, ya que nos brindó nuevas vivencias y poder despertar el interés en los residentes dirigido a la prevención y mantenimiento de la salud, ya que los problemas de los residentes se proyectan o son parte del sistema social de un país.



P-41 **Asociación entre los niveles disminuidos de glucosa y colesterol sanguíneos y las condiciones siquiátricas en Puerto Rico.**

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Abstracto. Objetivo. Determinar si los niveles de glucosa y colesterol en suero están asociados con la enfermedad mental.

Trasfondo. Los síntomas físicos de la hipoglicemia y de la hipocolesterolemia se asemejan en muchos casos a las expresiones fisiológicas de algunas enfermedades mentales. Además, muchas de las personas que padecen de alguna condición siquiátrica pueden presentar niveles disminuidos de glucosa y colesterol sanguíneo debido a

su ingesta inadecuada de alimentos o alguna the adrenergic system on exercise tolerance and on the progression of heart failure, transgenic mice have been subjected to a treadmill exercise tolerance test. Mice with hypertrophic cardiomyopathy (HCM) and mice with significantly increased basal contractility due to overexpression of the β_2 adrenergic receptor (β_2) were used for this study. The hypothesis being tested is that HCM mice will have limited exercise capacity while β_2 mice will have an enhanced ability to perform treadmill exercise. HCM, β_2 , and wild type control mice (n=6-8) were tested using a custom-built mouse treadmill with a shock stimulus at the back of the belt. An infrared detector system was used to record the number of times the mice steps off the back of the belt (hits per minute). After a two-week acclimation period, the mice ran for 11, one hour sessions at a speed of 20 meters/minute on a 10% incline, and the number of hits per minute for each mouse were averaged. Results show that the ability of the HCM mice to do exercise is limited, as seen by the high number of hits per minute compared to control mice (19.03 + 2.22 vs. 12.63 + 2.03, $p < 0.05$). In contrast, mice overexpressing the β_2 adrenergic receptor performed significantly better than controls (3.97 + 0.91 vs. 12.63 + 2.03, $p < 0.001$). The decreased exercise tolerance in the HCM mice is consistent with other signs of heart failure observed in older male HCM mice, including chamber dilation and decreased contractility. For future studies, we will cross-breed the HCM and β_2 mice to determine if the cardiac dysfunction and exercise intolerance can be diminished. Despite theoretical concerns that the excess adrenergic receptors might impair the ability of the β_2 transgenic hearts to coordinate physiologic stress signals or that the increased contractility might push the transgenic hearts into ischemia, the enhanced exercise ability we observed in the β_2 mice suggests that it is possible to manipulate cardiac adrenergic signaling in such a way as to improve exercise ability over basal levels.



Exercise Tolerance in Transgenic Mice with Altered Cardiac Contractility.

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Heart failure is characterized by very poor contractility of the heart muscle. One of the hallmarks of heart failure is exercise intolerance. The role of the adrenergic system in the progression of heart failure has long been a

topic of debate. To investigate the role of activating the adrenergic system on exercise tolerance and on the progression of heart failure, transgenic mice have been subjected to a treadmill exercise tolerance test. Mice with hypertrophic cardiomyopathy (HCM) and mice with significantly increased basal contractility due to overexpression of the β_2 adrenergic receptor (β_2) were used for this study. The hypothesis being tested is that HCM mice will have limited exercise capacity while β_2 mice will have an enhanced ability to perform treadmill exercise. HCM, β_2 , and wild type control mice (n=6-8) were tested using a custom-built mouse treadmill with a shock stimulus at the back of the belt. An infrared detector system was used to record the number of times the mice steps off the back of the belt (hits per minute). After a two-week acclimation period, the mice ran for 11, one hour sessions at a speed of 20 meters/minute on a 10% incline, and the number of hits per minute for each mouse were averaged. Results show that the ability of the HCM mice to do exercise is limited, as seen by the high number of hits per minute compared to control mice (19.03 + 2.22 vs. 12.63 + 2.03, p<0.05). In contrast, mice overexpressing the β_2 adrenergic receptor performed significantly better than controls (3.97 + 0.91 vs. 12.63 + 2.03, p<0.001). The decreased exercise tolerance in the HCM mice is consistent with other signs of heart failure observed in older male HCM mice, including chamber dilation and decreased contractility. For future studies, we will cross-breed the HCM and β_2 mice to determine if the cardiac dysfunction and exercise intolerance can be diminished. Despite theoretical concerns that the excess adrenergic receptors might impair the ability of the β_2 transgenic hearts to coordinate physiologic stress signals or that the increased contractility might push the transgenic hearts into ischemia, the enhanced exercise ability we observed in the β_2 mice suggests that it is possible to manipulate cardiac adrenergic signaling in such a way as to improve exercise ability over basal levels.

P-43 **Percepciones que Tiene el Personal de Enfermería de sus Experiencias, Sentimientos y Necesidades Educativas al Brindar Servicios a Personas con Vih/Sida - Proyecto Piloto.**
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La enfermedad del VIH/SIDA constituye un problema de salud pública en Puerto Rico. Entramos a un nuevo milenio donde aun esta enfermedad continua impactando

hombres, mujeres, adolescentes y niños del mundo. En Puerto Rico hay 22, 851 adultos y niños afectados con SIDA (CLETS, 1999). Se estima sin embargo que puede haber sobre 60, 000 personas infectadas que no conocen su estatus. La población más afectada lo son hombres y mujeres en su etapa más productiva que comprende los 25-44 años. Lo que se pensaba era una enfermedad de un grupo estereotipado y rechazado en nuestra sociedad, se ha convertido en un problema de todos.

Desde el inicio de la epidemia, la enfermería ha jugado un papel importante en el cuidado directo a personas con VIH/SIDA ya que usualmente es este profesional el proveedor primario de servicios de salud. El cuidado que se requiere brindar a las personas infectadas y afectadas por el VIH/SIDA debe ser uno integral y humanitario. Estudios previos han examinado los conocimientos y actitudes de las enfermeras y enfermeros que brindan cuidado a personas con esta enfermedad (Bennet, 1995, Newton, 1995, Wissen & Woodman, 1994). En Puerto Rico sin embargo no se ha estudiado detenidamente las variables percepciones, experiencias, sentimientos y necesidades educativas y destrezas de los profesionales de la enfermería. Ante los nuevos retos que la epidemia puede traer para el nuevo milenio y la necesidad de preservar la calidad y expectativas de vida de las personas afectadas utilizando los nuevos antiretrovirales y otras terapias, enfermería debe estar mejor equipada para ofrecer cuidado de calidad esta población.

Este estudio piloto examinará las variables mencionadas y será conducido utilizando una metodología cualitativa conocida como fenomenología con enfermeras y enfermeros trabajando en diferentes escenarios de salud. Los resultados podrían arrojar información relevante para diseñar e implantar nuevos modelos educativos y estrategias de intervención dirigidas a mejorar la práctica y por ende el cuidado a personas con VIH/SIDA en Puerto Rico.

Alternative Medicine and HIV.

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HIV infection is a devastating disease, making those afflicted with it desparate for any glimmer of hope even in unorthodox medicine.

We have been approached by NIH to investigate the

claims of a local individual residing in the Virgin Islands who is offering a herbal tea preparation made up of a variety of plants, for individuals suffering from HIV. Claims were made that this tea has boosted the immune response of these patients and increased the CD4 count. A series of experiments were designed to test the effect of this tea *in vitro* on human lymphocytes separated from the blood of healthy volunteers. The lymphocytes were incubated at 37°C in PHA for 48 hours. At the end of this period, 4×10^4 cells were seeded in wells in microtiter plates. The herbal tea was lyophilized, and 100mg of the freeze-dried material was reconstituted in 100ml of PBS. A ten-fold serial dilution of this preparation was added to the plates and incubated for 48 hours. The plates had controls of the herbal preparation in PBS, as well as lymphocytes without the tea preparation. At the end of this period, MTT was added to the wells and the plates were further incubated for 4 hours, then read on a V-max plate reader. There was no evidence to show that the herbal tea had any stimulatory effect on the lymphocytes. Serial dilutions of the herbal tea were also tested on lymphocytes infected with HIV, but the results also showed no evidence of an increase in the proliferation of lymphocytes. Supported in part by a "Research Centers in Minority Institutions" award, RR-03051, from the National Center of Research Resources NIH. One of us (JPR) gratefully acknowledges the Puerto Rico Alliance for Minority Participation (AMP) financial support to carry out this research project.

Role of Phosphorylation Domain on the Basal Activity of the Recombinant Dol-P-Man Synthase from *S. Cerevisiae*.

P-45

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Earlier work with isoproterenol treated cells, and with cAMP-dependent protein kinase (PKA) deficient chinese hamster ovary (CHO) cell mutants, have suggested that Dol-P-Man synthase (DPMS) activity could be regulated by a PKA phosphorylation signal. DPMS gene (DPM1) in *S. cerevisiae* codes for a protein of 267 amino acids of M_r 31 kDa whose predicted sequence contains one PKA site (i.e., serine 141). *In vitro* phosphorylation of the recombinant DPMS enhanced the synthase activity by 3-6 fold. The rate, as well as the magnitude of catalysis were higher with the phosphorylated enzyme. There was no change in the K_m for GDP-mannose, but the V_{max} was increased by 6-fold. The k_{cat} as well as the k_{cat}/K_m were also increased with the phosphorylated enzyme. Immu-

noprecipitation of DPMS followed by SDS-PAGE, autoradiography and immunoblotting with anti-phosphoserine antibody established DPMS as a M_r 31kDa phosphoprotein. We have now constructed two mutants in which serine 141 and serine 142 have been replaced with alanine by PCR site-directed mutagenesis (S141A and S142A). These genes were cloned into pET3(a) vector and expressed in *E. coli* BL21 (DE3)pLys S. A high level of expression of the mutant and the wild type enzymes was observed. The rate of catalysis at 22°C was lower in both mutant enzymes relative to the wild type. This difference was dramatically increased when the assay was carried out at 37°C, where S142A showed ~50% activity and S141A only 5%. These results suggest that serine 141 and serine 142 are located in the catalytic domain of the enzyme and serine 141 plays an essential role for maintaining the DPMS activity. Supported by the CIDIC funds, and Elena A. Carrasquillo is supported by the NIH-NIGMS pre-doctoral fellowship F31GM17177.

Molecular Characterization of 'endosymbionts' of *Dirofilaria Immitis*.

P-46

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Previous studies have identified intracellular bacteria in several species of filariae. These organisms appear to be organotropic, being detected to date only in the hypodermis, rachis, and oocytes, and seem to be vertically transmitted from one generation of filariae to another. The association between these organisms and their filarial host is unknown, but may be similar to that of insects and their endosymbionts. Since these organisms may represent a new taxonomic group, this study was conducted to determine their taxonomic position by the use of molecular biology techniques. *Dirofilaria immitis* adults obtained from naturally infected dogs were dissected to obtain organs which contain the 'endosymbionts' (uterus and ovaries); testes, organs without the 'endosymbionts', served as controls. DNA was extracted from the filariae and the 'endosymbionts'. The 16S rRNA gene of these 'endosymbionts' was amplified by PCR with primers that amplified most eubacterial 16S rDNA's, and the data was used to determine phylogenetic relationship between the organisms and the *Rickettsiales*. The PCR products from ovaries and uteri, which produced a band of approximately 1.5 kb in agarose gel electrophoresis, were purified and sent for sequencing to Robert Wood Johnson Medical School DNA Synthesis and Sequencing Laboratory,

Piscataway, New Jersey. A 1495 base sequence of the 16S rRNA gene, obtained by alignment of the partial sequences, was submitted in the Gene Bank, and can be accessioned by number AF088187. The 3.5 version of the PHYLIP software package was used to infer the phylogenetic relationship of these 'endosymbionts' by constructing a phylogenetic tree of the 16S rRNA gene and those of other Proteobacteria. The results indicate that the organisms of *D. immitis* may be endosymbionts belonging to the alpha subdivision of the Proteobacteria, are most closely related to the *Rickettsiaceae*, and apparently to belong to the *Wolbachia* group. Supported, in part by the RCMC award RR-03051 from the division of Research of Resources, NIH.

Structure of Cephalic Sense Organs of *Dirofilaria immitis*.

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Although previous studies have described the anterior nervous system of nematodes like *Caenorhabditis elegans*, *Strongyloides stercoralis*, and *Ascaris lumbricoides*, there is no detailed description of the anterior nervous system of tissue-dwelling parasitic nematodes. This study was conducted to describe the ultrastructure of the cephalic sense organs and anterior nervous system of *Dirofilaria immitis*, the heartworm of domestic dogs. Scanning electron microscopy and vital staining have revealed the presence of eight cephalic papillae, arranged around the buccal orifice as an inner and outer ring of four papillae each, and two amphids. Serial sections taken approximately fifty microns from the anterior tip of *D. immitis* adult and transmission electron microscopy indicate the presence of modified cells around the esophagus which assumed radial and circumpapillary patterns. Radial nerve projections are present surrounded by the cytoplasm of these cells suggesting a network of supporting cells around the papillary nerves. Also, there are structural differences between the cephalic papillae and the amphids. One of these differences is that a modified cilium is embedded in reticular material that fills the upper part of the papilla while nine cilia are observed within the amphid. These results suggest the presence of an organized and complex sensory system which appears to integrate and coordinate the mechanical and chemical stimuli from the environment outside the worm. Supported, in part by the RCMC Award RR-03051 from the division of Research of Resources, NIH, SIGMA XI Student Grant, and the Associate Deanship of Biomedical Sciences and Graduate Studies.

Head and Trunk Stabilization in Monkeys during Treadmill Locomotion.

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The brain requires frames of reference (e.g., stabilized body segment, external reference point) to determine body orientation in space. The necessary frame often changes, however, due to locomotor mechanics. During quadrupedal overground locomotion by Indian monkeys, the head rotates on a stabilized trunk during walking, but the trunk rotates on a stabilized head during galloping (Dunbar & Badam, Neurosci. Biobehav. Rev., 22:541-546, 1998). Do the same movement patterns occur during in-place locomotion? Head and trunk pitch rotations were measured and yaw rotations estimated from high-speed (100 and 200 frames/s) 16 mm movie films of 3 adult African vervet monkeys (*Cercopithecus aethiops*) locomoting on a treadmill. While similar under both conditions during walking, head and trunk rotational patterns occurred during treadmill gallops that were never observed during overground gallops. Specifically, the head occasionally pitched (>20N) and yawed (~45N - >180N) through several degrees, even though the trunk was also experiencing large pitch rotations (20N - >30N). During treadmill locomotion, the immediate visual surroundings remain fixed relative to the monkey. The ability to move the head and trunk simultaneously without disturbing balance and orientation implies that visual input alone can provide the reference frame. Films provided by Dr. J. Vilensky. Funded by RCMC Award RR-03051, and the Assoc. Deanship of Biomed. Sci., UPR Medical School.

Efecto del Midazolam Oral en el Comportamiento de Niños Pre-Escolares al Recibir Tratamiento Odontológico.

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El estudio piloto consistió de una muestra de 10 pacientes entre las edades comprendidas entre los 2 a 6 años, los cuales según la Academia Americana de Anestesiología fueron clasificados ASA I, que tenían historial de problemas de comportamiento al recibir tratamiento odontológico, siendo clasificados según la tabla de comportamiento de Frankl en las categorías 1(definitivamente negativo) y categoría 2(negativo). Cada paciente recibió un tratamiento odontológico integral, realizado por cuadrantes, a medida de lo posible. Se les administró 0.5mg/Kg de peso de midazolam oral,

diez minutos antes del procedimiento odontológico, evaluando el comportamiento del paciente durante el mismo, observando si existía variación en dicho comportamiento. Al mismo tiempo se evaluaron los signos vitales y la saturación de oxígeno.

Se observó que en el 90% (9 pacientes) de los casos el midazolam por vía oral es efectivo para mejorar el comportamiento de los pacientes que reciben tratamiento odontológico, moderando el comportamiento del paciente de las categoría I y categoría 2 a las categorías 3 (positivo)

y 4 (definitivamente positivo). También se observó que en el 100% de los pacientes no existieron variaciones significativas en los signos vitales ni en la saturación de oxígeno.

Concluyendo que el midazolam por vía oral es efectivo y seguro administrado en pacientes pre-escolares que requieren recibir tratamiento odontológico para mejorar el comportamiento. En la mayoría de los pacientes no se requirió el uso del midazolam en las visitas subsiguientes

AUTHORS INDEX

| <u>AUTHOR</u> | <u>ABSTRACT/POSTER NO.</u> | <u>AUTHOR</u> | <u>ABSTRACT/POSTER NO.</u> |
|--------------------------|-------------------------------------------------------------------------------|-----------------------|-------------------------------|
| Acosta H, | A-47 | Corretjer G, | P-17 |
| Adamsons K, | A-19, A-20, A-30, A-32, A-33, A-35, A-36, A-37, A-38, A-39, A-40, A-41, | Crespo K, | A-51, A-52, A-53, P-39 |
| Agosto J, | A-54 | Crouch-Ruiz E., | A-34, A-45 |
| Alejandro KV, | P-30 | Cruz M, | A-47, P-40 |
| Almaguel F, | P-5 | Cruz Y, | P-40 |
| Alvarado de Ballester C, | A-8 | Cruz E, | P-12 |
| Amigó LA, | A-44, P-46 | Cruz P, | A-40 |
| Antoun MD, | P-44 | Dajas F, | P-32 |
| Anzalota P, | P-38 | De la Vega A, | A-29, A-32, A-35, A-38, A-240 |
| Aponte R, | P-3 | De Jesús A, | A-30, A-37 |
| Aponte S, | A-59 | De Jesús R, | A-48 |
| Arroyo J, | A-49 | De Andino R, | P-7 |
| Arroyo-Calderon Y, | P-6 | Del Llano AM, | A-25, P-12 |
| Banerjee DK, | A-27, P-45 | Delgado R, | P-12 |
| Barreto-Chang O, | P-30 | Díaz N, | A-2, A-17 |
| Barry A, | A-18 | Díaz Ríos M, | P-4, P-17 |
| Bernstein J, | P-3 | Díaz AM, | P-14, P-15 |
| Blagburn J, | P-21 | Díaz L, | P-29 |
| Blanco RE, | P-21, P-31 | Dunbar DC, | P-48 |
| Blasini I, | A-13 | Eaton MJ, | P-23, P-24 |
| Boltermann RJ, | A-11 | Echevarría R, | P-43 |
| Bonet N, | A-16 | Elías A, | A-51, A-53 |
| Bonnemann C, | P-25 | Elías AE, | A-53 |
| Brown J, | P-8 | Escalona De Motta G, | P-30, P-31, P-32, P-33 |
| Burgos J, | A-36 | Escobar P, | A-36 |
| Camacho L, | P-19 | Febo I, | A-55 |
| Caraballo EV, | A-25 | Ferchmin P, | P-22 |
| Carmona R, | A-7 | Fernández-Repollet E, | P-29 |
| Caro E.B, | P-38 | Ferrer-Rodríguez I, | P-34, P-35 |
| Carrasquillo EA, | P-45 | Flores I, | P-15 |
| Carrión N, | P-40 | Font A, | A-1 |
| Castillo J, | A-56, P-49 | Freeman K, | P-42 |
| Castro FJ, | A-48, A-49 | Frontera RL, | A-12 |
| Chinea B, | A-48, A-60 | García I, | P-13, P-40 |
| Cintrón K, | A-18 | García García R, | A-11, A-59 |
| Claudio OI, | P-22 | García L, | P-11 |
| Collazo-Santos ME, | P-14 | Gerena Y, | P-29 |
| Colon I, | A-19 | Gervais G, | P-35 |
| Colón-Rivera CI, | P-42 | Ghaly ES, | P-36, P-37, P-38 |
| Concepción CB, | P-13 | GilbertoOrtiz J, | P-26 |
| Conde J, | P-7 | Giusti JC, | A-51, A-52 |
| Córdova G, | P-34 | Gómez Y, | A18 |
| Corey Specht S, | P-17, P-18 | Gómez G, | A13 |
| Correa Luna A, | A-57 | González C, | A-51, A-53 |
| | | González MJ, | A-28 |
| | | González R, | A-15, A-16, A-58 |

| <u>AUTHOR</u> | <u>ABSTRACT/POSTER NO.</u> | <u>AUTHOR</u> | <u>ABSTRACT/POSTER NO.</u> |
|----------------------|-----------------------------|----------------------|----------------------------|
| González H, | A-48, A-49 | Moore R., | P-42 |
| González A, | A-13 | Mora E.M., | A-21, A-24, A-26 |
| González-Pijem L, | P-9 | Mora E., | A-23 |
| Haasp J, | A-11 | Mora C.I., | P-15 |
| Haddock L, | A-60 | Morales Y., | P-39 |
| Haddock F, | A-54 | Morales A.B., | A-3, P-43 |
| Hawayek J, | A-39 | Morales J., | P-39 |
| Hernández I, | P-29 | Morales L., | P-40 |
| Hernández CM, | P-16 | Moronne M., | P-8 |
| Herrera L., | A-23 | Moshé S., | P-22 |
| Hillyer G., | A-47 | Muns M.A., | A-29 |
| Jiménez Y., | A-10 | Muñoz C., | P-2 |
| Julian Y., | A-18 | Muñoz H., | A-49 |
| Kensler R.W., | P-5 | Nair A., | P-8 |
| Kensler A., | P-5 | Nazario C.M., | A-23, A-28 |
| Kicliter E., | P-25, P-26, P-27 | Nieves-Méndez D.E., | A-46, P-47 |
| Kozek W.J., | A-44, A-46, P-8, P-46, P-47 | Nieves-Rivera F., | P-9 |
| Kozek W., | P-12 | Núñez A., | A-4 |
| Krusek J., | P-23 | Oharriz J., | A-60 |
| Larabell C., | P-8 | Oliver M., | A-22 |
| Larota L., | A-52 | Oliver-Vázquez M., | P-6 |
| Lavergne J.A., | A-25, P-12, P-44 | Oliver-Vázquez O., | P-7 |
| Lebrón S., | P-39 | Orkand R.K., | P-17, P-23, P-24, P-28 |
| Leinwand L., | P-42 | Ortiz G., | P-28 |
| Libreros C., | P-40 | Ortiz J.G., | P-22 |
| Linares N., | A-2, A-5, A-9 | Ortiz N., | A-15 |
| López R., | P-2 | Osorio F., | A-14 |
| López L., | A-50 | Otero A., | P-39 |
| López Del Valle L., | A-57 | P.M., | P-28 |
| López-Enriquez S.R., | A-21, A-26 | Padró N., | P-40 |
| Lozada R., | P-40 | Peraza N., | A-56, P-49 |
| Lozano R., | A-39 | Pérez C., | A-60 |
| Lugo N., | P-19, P-20, P-25, P-27 | Pérez J., | P-35 |
| Lugo-López R., | P-33 | Pérez-Delboy A., | A-33 |
| Machuca M., | P-39 | Pérez-Ramos J., | P-44 |
| Maldonado N., | P-40 | Pérez-Rivera M.J., | P-41 |
| Martín M. | A-16 | Pica S., | P-36 |
| Martínez J.A., | A-27 | Preston A., | A-18 |
| Martínez A., | A-12 | Quiñones-Morales L., | P-33 |
| Medina N., | A-53 | Ramírez D., | P-43 |
| Meléndez E., | P-40 | Ramírez M., | A-60 |
| Méndez A., | P-45 | Ramos L., | A-11, A-18 |
| Meneses I., | A-18 | Ramos G., | A-17 |
| Meyer-Ilse W., | P-8 | Ramos Ramos Z., | P-44 |
| Miller M.W., | P-4 | Rehner S., | P-10 |
| Mojica P., | A-23, A-24 | Reichenbach A., | P-23, P-24 |
| Montealegre F., | P-15 | Rentas Y., | A-7 |
| Monterrubio J., | P-28 | Reyes G., | A-13, A-14, P-11, P-13 |

| <u>AUTHOR</u> | <u>ABSTRACT/POSTER NO.</u> | <u>AUTHOR</u> | <u>ABSTRACT/POSTER NO.</u> |
|------------------------|----------------------------|------------------------|----------------------------|
| Ríos M.E., | A-49 | Severin E., | A-25 |
| Ríos I., | A-52, P-39 | Singer D., | A-19 |
| Rivera Y., | A-56, P-49 | Skatchkov S., | P-23, P-24 |
| Rivera L., | A-16 | Sosa M.A., | P-16 |
| Rivera E., | P-40 | Soto S., | P-28 |
| Rivera H., | A-53 | Soto I., | P-21 |
| Rivera J., | A-14, A-41 | Soto B., | A-10 |
| Rivera R., | A-51 | Sperber E., | P-22 |
| Rivera J.R., | A-34 | Steininger E., | A-59 |
| Rivera-Jiménez J., | P-33 | Suárez J.C., | A-23 |
| Rivera-Tubéns H., | A-17 | Suárez E., | A-22 |
| Rivero M., | P-43 | Suárez-Pérez E., | P-6, P-7 |
| Rodríguez A.D., | P-30 | Suess E., | P-4 |
| Rodríguez L., | P-39 | Szeto A.C., | P-10 |
| Rodríguez A., | A-24 | Tapia V., | A-9 |
| Rodríguez C., | A-18 | Toledo N., | A-55 |
| Rodríguez Ortiz E.M., | A-43 | Tolosa J., | P-26 |
| Rodríguez D., | P-45 | Tormos H., | P-39 |
| Rodríguez E., | A-54, P-17 | Toro C., | A-51, A-52, A-53 |
| Rodríguez-Centeno A., | P-32 | Torres S., | A-45 |
| Roldan R., | P-45 | Torres E.A., | A-49 |
| Romaguera J., | A-31 | Torres R., | A-30, A-33 |
| Romerop J.C., | A-11 | Torres M., | A-48 |
| Rosa A., | A-60 | Torres E., | A-20 |
| Rosa M.E., | A-6 | Torres E.A., | A-47, A-48, A-60 |
| Rosa R., | P-30 | Tosteson T., | P-33 |
| Rosario R., | P-7 | Valcalcel M., | P-13 |
| Rosario G., | P-12 | Vázquez G., | P-34 |
| Ross C., | A-42 | Vázquez-Dubeau D., | A-20, A-38 |
| Rubio-Dávila M., | P-31, P-32, P-33 | Vega R., | P-7 |
| San Miguel R., | A-14 | Veh R.W., | P-23, P-24 |
| Sánchez Ayendez M., | P-1, P-2, P-6 | Velazco G., | A-41 |
| Sánchez M., | A-22, A-26, P-37 | Vélez H., | A-14, A-22 |
| Santiago P., | A-30, A-33, A-41 | Vélez-Almodóvar H., | P-6 |
| Santiago R., | A-31 | Velisek L., | P-22 |
| Santiago C., | A-5, A-9, P-10 | Vidal L., | P-20 |
| Santiago G., | P-29 | Villamil J., | P-39 |
| Santiago-Borrero P.J., | P-11 | Weinstein P., | A-57 |
| Santos L., | P-39 | Weinstock J., | A-47 |
| Schwartz A., | P-29 | Woolcock Rodríguez K., | P-18 |
| Schwarz S., | A-38 | Zoucha R., | A-61 |
| Seise N., | A-18 | Zuazaga C., | P-28 |
| Serrano A.E., | P-10, P-34, P-35 | | |