805 EXPERIMENTAL ESCHERICHIA COLI MENINGITIS IN NEWBORN GUINEA PIGS. Robert E. Sinai, Melvin I. Marks, Chic H. Pai. McGill University-Montreal Children's Hosp., Departments of Pediatrics and Microbiology, Montreal, Quebec.

Newborn albino guinea pigs (GP's) were used as an animal model for Gram negative neonatal meningitis (NM). Twenty-four GP's 4-5 days old were inoculated intranasally with 10^{8-9} E. coli K1 organisms (MIC 0.1 ug/ml gentamicin) originally isolated from human NM. Bacteremia occurred in 50% of GP's within 48h, and meningitis (defined by positive CSF culture) was detected in 80% of bacteremic GP's within 5 days. Seven of 8 (88%) GP's with meningitis died within 7 days. All animals were sacrificed at 7 days post-inoculation and brains were examined histologically. There was 100% agreement between positive or negative CSF culture and presence or absence of inflammatory meningeal reaction. The majority (88%) of GP's with meningitis also had ventriculitis.

Two further experiments revealed that the results (rate of bacteremia, positive CSF, mortality) were reproducible. A group of animals was also treated with gentamicin (2.5 mg/kg IM q12h) begun after diagnostic cisternal puncture at 16h post inoculation. Mean serum and CSF gentamicin levels were 3.6 and 0.16 ug/ml, respectively. Mortality in meningitic GP's was reduced by about 60% with gentamicin therapy. These results suggest that the newborn GP may be a useful model to study pathogenesis and efficacy of therapeutic agents in E. coli NM, since a) meningitis can be induced in a predictable and reproducible manner and b) the non-lethal sampling of CSF permits assessment of therapeutic responses and measurement of antibiotic levels.

806 ENZYMATIC ASSAY OF PENICILLIN G. Arnold L. Smith, Ian Rosenberg and David H. Smith, Children's Hospital Medical Center, Dept. of Med., Boston, Mass.

Microbiologic methods of quantitating penicillin require indicator strains resistant to other antibiotics, are influenced by non-antibiotic serum factors, and require overnight incubation; features which limit the clinical usefulness. Since aminoglycosides can be quantitated with R-factor mediated enzymes, we examined the quantitation of penicillin with plasmid mediated β-lactamase. Osmotic shockates prepared from E. coli 1100/RK5 contain β-lactamase activity which is active against penicillin G, ampicillin, methicillin, oxacillin, cloxacillin and carbenicillin; converting them to their respective penicilloic acids. Penicilloic acid will reduce arsenomolybdate in the presence of trace amounts of Hg^{++}, yielding a blue complex which absorbs at 750 nm. We constructed a spectrophotometric assay for penicillin G based on quantitating β-lactamase dependent production of penicilloic acid. The assay reliably quantitates penicillin G in 0.2 ml serum in concentrations between 0.25 and 100 μ q/ml within one hour. Non-β-lactam antibiotics at maximum therapeutic concentrations do not interfere with the assay. Mixtures of β-lactam antibiotics can not be accurately assayed. Very high concentrations of aminoglycosides (approx. 100 μ q/ml) increase the blank value, but reliable (relative standard error of 5%) quantitation of penicillin G is possible. Preservative agents commonly added to blood specimens do not interfere with the assay. We conclude that plasmid mediated β-lactamase can serve as a reagent in the rapid, specific quantitation of penicillin G.

807 PHARYNGEAL IMPLANTATION OF ALPHA HEMOLYTIC STREPTOCOCCI (α-STREP) IN NEONATES IN AN ICU. Katherine Sprunt, Grace Leidy and Winifred Redman. Columbia University, Coll. of Phys. and Surg., Dept. of Pediatrics, Div. of Infect. Dis. New York City.

Pharyngeal implantation of a carefully selected strain of α-strep (#215) was attempted in 14 neonates with high titer abnormal colonization of the pharynx with potential pathogens (e.g., coliforms, pseudomonads, staphylococci) to convert the flora to "normal" (α-strep predominant). Serial semi-quantitative culture samples from 346 neonates in an ICU showed strong positive correlation between high titer abnormal colonization of the pharynx and infection. To date, no demonstrable systemic infection occurred in those infants with "normal" pharyngeal flora. All but one of the 14 infants received a single dose, approximately 10^6 colony forming units (CFU), of strain 215. The youngest was 8 and the oldest 64 days old when given the strain. The pharyngeal flora of 10 of the infants became "normal" within 2-3 days. In 5, implantation is considered responsible for the change because only the implant type was recovered initially. In the other 5 infants a low (4% or less) to moderate (5-15%) proportion of their strep initially resembled the implant. The strain was not recovered from 4 infants. Included in the 4 is the first infant given the strain who received too low an inoculum (10^2-10^4 CFU). Another was given cholamphenicol within 24 hrs of the implant. The data show 1) Implantation can be carried out in high titer abnormally colonized infants 2) "Normal" flora is attained in 2-3 days 3) The implant strain can be identified in mixed strep populations 4) No adverse effect of the implant strain has been noted.

808 EFFECTS OF GROUP B STREPTOCOCCI ON LUNG FLUID BALANCE IN UNANESTHETIZED SHEEP. Mildred T. Stahlman, Ragnar Olegard, and Kenneth L. Brigham. Newborn Lung Center and Pulmonary Circulation Center, Vanderbilt Univ., Nashville, TN.

Sepsis and pneumonia with group B streptococci (GBS) has become an increasingly important cause of morbidity and mortality among newborn infants. Because the associated clinical syndrome resembles endotoxemia with respiratory distress as a prominent feature, the effects of infusing GBS on lung fluid balance were studied in unanesthetized sheep. Sheep were prepared as described previously (J. Clin. Invest. 54:792, 1974) and during each experiment, lung vascular pressures, lung lymph flow, lymph and plasma protein concentrations and arterial blood gases and pH were measured. After a steady baseline period, 10^9-10^{11} GBS suspended in 100ml saline was infused over 30 minutes. GBS caused chills, fever, a 45% increase in pulmonary artery pressure, a 75% increase in lung lymph flow and a 10% decrease in lymph: plasma (L/P) protein concentration. GBS also caused Pa_{O2} and Pa_{CO2} to fall and pHa to increase. Heat killed and live organisms had similar effects. The increase in lung lymph flow with decreased L/P protein concentration is like the previously reported response to mechanically increased lung vascular pressures (Circ. Res. 37:271, 1975). It is concluded that intravenous GBS causes increased fluid filtration from lung microvessels primarily by increasing microvascular pressure. This effect could be an important part of the GBS septicemic syndrome in neonates, and may be mediated by an "endotoxin-like" substance.

809 TRANSFER FACTOR FOR THE PREVENTION OF VARICELLA-ZOSTER INFECTION IN CHILDHOOD LEUKEMIA. Russell W. Steele and Luis Canales. Dept. of Pad., Brooke Army Medical Center, San Antonio, Texas.

Herpes group viruses are pathogens which cause significant morbidity and mortality in the host who has compromised cellular immune function. Preliminary studies, employing non-human primates and herpes simplex virus type 1 challenge, demonstrated the ability of human dialyzable transfer factor (TF_d) to protect against fatal viral infection. The present studies were designed to evaluate possible efficacy of specific human TF_d in preventing or attenuating varicella-zoster (VZ) infection, usually chicken pox, in children with acute lymphoblastic leukemia (ALL).

TF_d was prepared following leukapheresis of adult donors who were convalescing from chicken pox. Recipients were children with ALL, 12 in remission and 3 in relapse; a single injection of TF_d was given equivalent to 10^8 lymphocytes per 7 kg body weight. Prior to and following TF_d injection, the following VZ specific parameters were measured: lymphocyte blastogenesis, cytotoxicity and leukocyte inhibitory factor (LIF) production, and indirect fluorescent and CF antibody titers.

No patients in relapse converted immune responses while 10/12 in remission developed positive reactivity in at least one assay of cell mediated immunity (CMI); 3/12 were positive in all 3 parameters of CMI and 8/12 in 2 assays. Cytotoxicity was the most consistently positive test following TF_d administration. No patients developed VZ antibody.

A large double-blind trial of TF_d vs placebo designed to evaluate clinical protection is now in progress.

810 PLASMA INFUSION CORRECTION OF OPSONIZATION FOR PNEUMOCOCCAL MENINGITIS. Russell W. Steele, Gualberto Marrero, and Luis Canales. Dept of ped. Brooke Army Med. Ctr., San Antonio, TX.

It is well appreciated that children with sickle cell disease (SCD) experience a strikingly increased susceptibility to pneumococcal meningitis and septicemia. One factor of major etiologic importance appears to be a defect in serum components which enhance phagocytosis (opsonins).

A new assay employing acridine orange as a vital stain for bacteria to examine phagocytosis and bactericidal capacity of neutrophils was used to evaluate a SCD patient with type 14 pneumococcal meningitis which remained culture positive for 8 days into penicillin therapy. The patient subsequently received infusions of fresh frozen plasma from donors with type 14 pneumococcal antibody. Results were as follows:

	Pre-infusion		Post Infusion	
Serum Supplement	Phagocytic index	Percent Kill	Phagocytic index	Percent Kill
None	0.83	61%	0.58	54%
Autologous serum	0.69	64%	1.80	90%
Control serum	1.95	91%	2.13	87%

These assays of neutrophil phagocytosis and bactericidal capacity, specific for the pneumococcus infecting the patient, revealed a deficiency of serum opsonization which could be corrected with infusions of fresh frozen plasma. These data therefore support the need for large controlled clinical trials of plasma infusions for treating pneumococcal meningitis and sepsis in patients with SCD. Studies of this nature are in progress.