normal serum IgA levels. 30 age-matched controls were negative for all the above tests.

The fundamental defect in A-T is probably immunologic. An altered immunologic response could lead to multiple system disease as a direct result of autoantibody destruction. It is also possible that deficient immunity may permit an infectious agent to cause widespread tissue damage with secondary production of autoantibodies. (Supported by NIH AI-07726 and National Tuberculosis Foundation.)

4 *Nasal Immunization of Asthmatic Children With Killed Influenza Vaccine.* RICHARD W. NEWCOMB and JERRY ELLER, Children's Asthma Research Inst. and Hosp. and U.S. Army Med. Res. and Nutrition Lab. (introduced by David Pearlman).

Since exocrine antibodies are produced and secreted in the nose in response to local antigens, and may be important for local immunity, we investigated the effectiveness and safety of nasal vaccination with a killed influenza virus (monovalent 'Hong Kong' strain) in 12 children with asthma and 2 normal adults. Significant hemagglutination-inhibition (HAI) and neutralizing antibody activities occurred in serum and nasal fluids of all subjects whether given 2 doses of antigen nasally, or one subcutaneously, then one nasally. Serum and nasal fluid titers were highest, however, in unvaccinated, naturally infected children. No adverse reactions to the killed virus were found; specifically, no aggravation of asthma, no increased sensitivity to bronchoconstricting chemicals, and no sensitization to egg antigen were discovered. Fractionation of nasal fluids by gel filtration revealed that most HAI and neutralizing antibody activity accompanied exocrine γA. Reduction and alkylation of these fractions sufficient to convert 11S exocrine γA to 75 γA decreased both antibody activities, but the activities of γG-rich fractions were not thus decreased.

These data suggest that nasal immunization with killed influenza virus is both safe and effective in producing nasal antibody in asthmatic children, although no conclusions can be drawn regarding whether the antibody levels attained are protective, or how long they will persist. (Supported by USPHS grant AI-08500 and NIAID contract PH 43-62-477.)

5 *A Sensitive Microtiter Test for Detecting Rheumatoid Factor in Children.* JOHN KATTWINKEL and REBECCA H. BUCKLEY, Duke Univ. Sch. of Med., Durham, N.C.

Serologic tests helpful in confirming rheumatoid arthritis in adults are most often negative in juvenile rheumatoid arthritis (JRA). Since rheumatoid factors (RF) have been shown to have iso- as well as heteroantigenic specificities, this study was designed to determine whether a test employing human gamma globulin with a number of isoantigenic determinants would permit more frequent detection of RF. Human O, Rh+RBC were sensitized with an incomplete anti-D antibody (Ri) known to be positive for a number of Gm isoantigens. Dilutions of sera (heated at 56 °C for 30 min) were made in V-bottomed microtiter plates, 0.1% (Ri) cells added, the plates centrifuged and tilted 35° from the verticle for 20–30 min before reading. Sera from children with no disease, acute rheumatic fever (ARF) and JRA were tested with sensitized sheep cells (SSC), the Hyland latex slide test, and (Ri) cells. A titer of 1:32 was considered positive for the SSC and (Ri) tests.

Results

	No. of children	Average age	(Ri) Cells+	SSC+	Latex+
Controls	37	8.3 yr	4 (11%)	3 (8%)	0
ARF	10	8.9 yr	0	0	0
JRA	11	7.8 yr	5 (46%)	3 (27%)	2 (18%)

The findings confirm those of others showing a low but definite incidence of RF in normal children. The (Ri) rest detected RF more frequently in JRA than the SSC and latex tests. The data suggest: (1) the (Ri) test may be helpful in the differential diagnosis between ARF and JRA, and (2) with the significantly ($p < 0.005$) higher percentage of (Ri) positive reactors in the JRA group that this test may be a more sensitive laboratory aid than those presently available for confirming JRA.

6 *An* in vitro *Test for Detection of Specific IgE-mediated Penicillin Reactions in Children.* ZACK H. HADDAD and JOEL KOROTZER, Los Angeles County-University of So. Calif. Med. Center, Dept. of Ped., Los Angeles, Calif. (introduced by John James).

The diagnosis of untoward immediate hypersensitivity reactions to penicillin(s), thus far had to be made presumptively on, at best, subjective clinical impression for lack of a simple, rapid, sensitive, reliable and reproducible *in vitro* immunological test. The objective of this work aims at fulfilling most of these criteria. Sera from 20 children clinically sensitive to penicillin and/or ampicillin, were incubated on a slide with a suspension of rat mast cells and various concentrations of penicillin, ampicillin, or penicilloyl-polylysine, under optimal conditions of pH and temperature. The cells were then examined microscopically for degranulation. A total number of 100 cells were counted over several randomly selected fields. The same model also was employed, using a monospecific goat antiserum to human myeloma IgE, instead of the specific drug. On incubation of equal volumes of cell suspension and serum from sensitive children, both the suspected drug and anti-IgE caused *specific* degranulation of the cells in a statistically significant fashion: average number of degranulated cells in presence of sensitive sera and drug or anti-IgE was *3–4 times* the number of altered cells in the absence of drug or anti-IgE, or in the presence of equivalent concentrations of anti-IgG, IgA, and IgM. Control sera from nonsensitive matched children caused no significant degranulation. Our data indicate that: 1. This *in vitro* test can be used for rapid detection of penicillin-specific IgE antibodies. 2. Human IgE can sensitize rat cells *in vitro*, affording a test which may obviate skin testing for penicillin allergy in children.

7 In vitro *Transformation of Cord Blood Lymphocytes by Antigens.* SANFORD LEIKIN, Children's Hosp. of D.C., and Geo. Washington Univ. Sch. of Med., JACQUELINE WHANG-PENG, NCI, Bethesda, Md., and JOOST J. OPPENHEIM, NIDR, Bethesda, Md.

Newborn human lymphocytes respond vigorously to immunologically specific stimulation with homologous cells in mixed leukocyte cultures and to non-specific stimulants. Certain maternal infections can be transmitted to the human fetus and induce antibody produc-

tion suggesting that the placental barrier is not impermeable to certain antigens. However, little is known about placental transfer of antigens in normal pregnancies. For this reason leukocyte cultures from random cord blood samples were incubated with various antigens, and transformation was assayed by tritiated thymidine (TdR^3H) uptake.

Analysis of variance of the response of triplicate cultures revealed that a ratio of 3:1 or greater of uptake of TdR^3H by stimulated to unstimulated cultures was statistically significant ($p < 0.01$). The number of newborns tested that manifested such a significant *in vitro* response was 5/21 with streptolysin 0, 5/15 with Type I pneumococci, 4/17 with Group A type 12 streptococcal cell wall extract, 2/14 with *E. coli*, and 3/9 with *S. enteritides* endotoxin. Examinations of metaphases in cord blood cultures from male infants revealed that the response of cord blood lymphocytes to these antigens could not have been due to the passage of maternal cells across the placenta. This 14–33% incidence of significant stimulation of cord blood lymphocytes by these common bacterial antigens is therefore either due to inborn cellular immunity or to transplacental transfer from the maternal circulation and prenatal sensitization of the fetus by these antigens. (Supported by: NIH Grant No. HD-04273.)

8 *Cell-mediated Immune Response* in vitro. SAMUEL P. GOTOFF and SOMSAK LOLEKHA. Dept. of Ped., The Abraham Lincoln Sch. of Med., Univ. of Illinois, Chicago.

Studies of the mechanism of cell-mediated immune responses have been hampered by the lack of *in vitro* systems. While the inhibition of macrophage migration model has advanced our understanding of delayed hypersensitivity reactions, the technique is complex and cumbersome. We have recently developed a simple test for measuring cell-mediated immune responses which depends on the aggregation of peritoneal exudate cells (PEC) in suspension cultures.

PEC from guinea pigs with delayed hypersensitivity aggregate when the cells are cultured with the appropriate antigen. Diphtheria toxoid, PPD, egg albumin and keyhole limpet hemocyanin have been used in this system. Aggregation appears at 6 h and reaches a maximum at 24–48 h which is comparable to the time course of cutaneous delayed hypersensitivity reactions. This *in vitro* model also correlates with another cell-mediated response, allograft rejection. PEC from strain 13 guinea pigs previously grafted with skin from strain 2 animals aggregate in the presence of strain 2 cells. Aggregation does not occur with mixtures of PEC from guinea pigs of the same strain or different strains without prior grafting.

Peripheral blood leukocytes, spleen cells or lymph node cells from sensitized animals cultured with antigen synthesize a factor which causes aggregation of PEC from nonsensitive guinea pigs. The aggregating cells are macrophages, and the titer of macrophage aggregation factor (MAF) is determined by serial dilution. Macrophage aggregation *in vitro* provides a simple semiquantitative test for cell-mediated immune reactions and permits further analysis of the mechanism involved.

9 *A Mechanism for Inhibitory Effects of Diverse Compounds on* in vitro *Antigenic Lymphocyte Stimulation and Histamine Release From Leukocytes.* CHARLES D. MAY, Dept. of Ped., New York Univ. Sch. of Med., New York, NY.

Ethanol (E), nicotinamide (N), cyclic 3′,5′adenosine monophosphate (AMP) and theophylline (T) are examples of dissimilar compounds found to inhibit the fundamental cellular responses of antigenic histamine release and lymphocyte stimulation. As glucose is a prime source of energy and intermediates essential to cellular responses, the effects of the compounds on glucose utilization by leukocytes were determined, and corresponding inhibition of glucose metabolism was revealed. The molar concentrations required for comparable degrees of inhibition were closely similar for the two cellular responses and glucose utilization. To ascertain loci of inhibitory actions, the effects on conversion of glucose labeled with ^{14}C at various sites to $^{14}CO_2$ were compared in suspensions of intact leukocytes and extracts of leukocytes devoid of cell membrane or nuclei. E and N were inhibitory mainly with intact cells and appear to affect glucose transport across the cell membrane. AMP and T also inhibited conversion by cell-free extracts and thus enzyme systems within the cell. Inhibition of glucose utilization by leukocytes is a common property of inhibitors of cellular responses, including E, N, AMP, T, and we found likewise for cortisol, colchicine, and chloroquine. The loci of action of the diverse compounds in the complex steps in utilization of glucose by leukocytes differ.

The *in vitro* systems employed to compare cellular responses with glucose utilization are useful in fundamental studies and also convenient for screening of compounds for activity in these processes.

10 *Isoimmune Neonatal Neutropenia Due to a New Neutrophile-specific Antibody.* EVA RADEL, DAN G. HANDELSMAN and PARVIZ LALEZARI, Depts. of Ped. and Hematol., Montefiore Hospital and Medical Center and Albert Einstein Coll. of Med., New York (introduced by Laurence Finberg).

Maternal isoimmunization to fetal leukocytes has been implicated in instances of neonatal neutropenia. However, leukoagglutinins often exist in pregnant women and are not associated with clinical symptomatology in the newborn. It has therefore been suggested that when neutropenia, sepsis, and maternal leukoagglutinins coexist, the latter are coincidental and the neutropenia is the result, and not the cause, of sepsis.

A newborn infant, in whom sepsis was suspected, had almost complete absence of circulating polymorphonuclear neutrophiles, persisting for 5 weeks. An antibody was detected in the serum of both mother and infant which agglutinated the neutrophiles of the infant and father. It did not react with eosinophiles, lymphocytes, platelets, or with the mother's neutrophiles. Two neutrophile-specific antigens have been described previously in association with neonatal neutropenia. Neutrophile typing of the family and unrelated donors demonstrates that this antibody is distinct and thus constitutes a third neutrophile-specific antigen. The more common leukoagglutinins which react with many types of cells may be benign, but antibodies specifically directed against neutrophiles may produce profound neutropenia and may thus predispose the neonate to severe infection.

11 *An X-linked Recessive 'Malignant' Reticuloendotheliosis.* JOHN M. FALLETTA, DONALD J. FERNBACH, DON B. SINGER, Baylor Coll. of Med., NOMIE