α-fetoprotein concentrations in the rat normally declined abruptly after birth to approximately half of the prenatal level by 2 to 3 days of age, in accord with the loss of fetal membranes at delivery; the α-fetoprotein level then remained relatively constant until the rat was 6 to 8 days of age, after which synthesis of the protein was increasingly suppressed. Marked suppression of α-fetoprotein synthesis in the rat could be induced in the first week of life either by cortisone or by sham operations; epinephrine, corticosterone, testosterone, progesterone and estradiol had no observable effect on synthesis. Participation of the adrenal in the suppression noted to follow surgery was indicated by the observation that adrenalectomy did not inhibit α-fetoprotein synthesis. Subcutaneous injection of cortisone into the pregnant rat suppressed α-fetoprotein synthesis in the fetus *in utero* as did sham operations on the pregnant rat. (APS)

65 *Cord Blood Gamma M as a Screening Test for Congenital Viral Infections.* JOHN L. SEVER and HEINZ W. BERENDES*, NIH, Bethesda, Md.

Elevated levels of gamma M have been found in newborns with a number of congenital infections including syphillis, toxoplasmosis, rubella, and cytomegalic inclusion disease (CID). The frequency of this finding in normal and infected children was studied with specimens from the Collaborative Perinatal Research Study. Cord sera from 1000 children at 10 collaborating institutions were tested. 29 had elevated gamma M; 14 of these children had abnormalities including unexplained jaundice with hyperbilirubinemia; mental and motor retardation; hepatosplenomegaly; skeletal malformations; cataracts and strabismus with nystagmus; failure to thrive; and other significant findings. One of these children had congenital toxoplasmosis. Tests of children with congenital infections showed high cord blood gamma M for rubella (6 of 9), CID (2 of 3), toxoplasmosis (2) and generalized herpes (1). Maternal infections also were associated with high gamma M in the cord of children for rubella in the first trimester (9 of 37,6 of these were abnormal), and serological evidence for maternal toxoplasmosis (4 of 5,1 child abnormal). Other maternal infections during pregnancy did not result in significant elevation of cord gamma M including varicella (6), mumps (14) and rubeola (17). There was no elevation of gamma M in children with erythroblastosis (6), congenital leukemia (3) and mongolism (19 of 20). One mongoloid child had high gamma M and bronchial pneumonia and peritonitis. Only 1 of 36 children with congenital heart disease had high gamma M, and this child had congenital rubella. The simple gel diffusion determination of gamma M in cord blood and in the newborn should be useful as an initial screening test when considering congenital viral infections. (SPR)

66 *Immunologic Consequences of Congenital Rubella.* LOUIS Z. COOPER*, STEBBINS B. CHANDOR*, ALBERT B. OCKERSE*, DONALD FEINSTEIN* and SAUL KRUGMAN, New York Univ. Sch. of Med., New York, N.Y.

The immunologic consequences of maternal rubella have been correlated with the clinical and virologic data accumulated on 350 children followed since the 1964 epidemic. Mothers and their infants with rubella-associated defects have had persistence of rubella serum neutralizing and hemagglutination-inhibition antibodies. Antibody titers among these children have remained at levels $\geq$ to those in their mothers. In contrast, most children who are clinically normal, despite maternal rubella, have not produced rubella antibody. This relationship of fetal infection to congenital defects and persistent antibody production is supported by a study of 3 sets of twins; 5 of the children have anomalies and antibody, 1 child is normal and has no antibody. Alterations in serum immunoglobulin levels most commonly elevations of IgM and in one instance production of a small molecular weight (approximately 7S) IgM, and a decreased incidence of positive skin reactions to oidiomycin indicate that congenital rubella produces a spectrum of immunologic abnormalities similar to that which it produces in other organs. (SPR)

67 *Cells of Human Colostrum: In Vitro Studies.* CLIFTON W. SMITH* and ARMOUND S. GOLDMAN*, Univ. Tex. Med. Br., Galveston, Tex. (introduced Warren F. Dodge).

The types and behavior of human colostral cells both *in vivo* and *in vitro* were studied. Samples obtained from thirty individuals consistently revealed neutrophils, lymphocytes and macrophages. The relative frequency of these cells varied with time following delivery. The most abundant cells were macrophages.

Cultures in Leighton tubes without phytohemagglutinin (PHA) revealed many macrophages and vacuolated cells resembling colostral corpuscles. These two cell types could not be clearly separated morphologically. Both types adhered to glass surfaces; however, only those typical-appearing macrophages showed ameboid motion. Cell cultures with PHA uniformly displayed all stages of lymphoblastic transformation. Synthesis of deoxyribonucleic acid by these lymphoblasts was evidenced by radioautography of cells previously exposed to thymidine-H^3. It is concluded that living lymphocytes and macrophages are constituents of normal human colostrum. Studies of the immunologic functions of these cells will be described. (Supported by NIH Grant 5 RO1 HD 00735-03) (SPR)

68 *Specific Local Antibody Defect in Chronic Mucocutaneous Candidiasis.* RICHARD A. CHILGREN*, RICHARD HONG and PAUL G. QUIE, Univ. of Minn. Sch. of Med., Minneapolis, Minn.

Immunological defense mechanisms were studied in 3 patients with chronic mucocutaneous candidiasis of at least 9 years duration. None had systemic candidiasis or increased susceptibility to other infections.

Agglutination titers of standardized suspensions of heat-killed *Candida albicans* were measured in concentrated parotid duct saliva samples. Two patients' samples contained no agglutinating antibodies and one had a titer of only 1:4, in spite of gross oral infection. In contrast, parotid fluid from 6 patients who had recovered from *C. albicans* oral infection had titers ranging from 1:16 to 1:400. Despite the absence of agglutinating antibodies to *C. albicans*, isohemagglutinins and sheep cell agglutinins were present, and a normal immunoglobulin pattern was found (IgA present in normal amounts, IgG and IgM not detected).

The patients' sera, however, contained levels of agglutinating antibody ranging from 1:64 to 1:256 and had normal amounts of immunoglobulins. These findings are consistent with the known lack of correlation between local and circulating antibody levels and further suggest a specific deficiency in local antibody response to Candida.

Furthermore, the chronically infected patients failed to demonstrate delayed hypersensitivity to 2,4-DNFB, Candida, OT, SKSD, mumps and Trichophyton, whereas controls reacted to at least two of the antigens. Since IgA is the predominant salivary immunoglobulin, these patients may present another example of IgA deficiency in association with abnormalities of cellular immunity. Similar observations have been reported in ataxia-telangiectasia and thymectomized rodents. (SPR)

69 *Modification of Graft-Versus-Host Reaction with Anti-Lymphocyte Serum.* VINAI SUVATTE* and JOHN H. GITHENS. Univ. Colo. Med. Ctr., Denver, Colo.

The serious mortality and morbidity of the graft-versus-host reaction (GVHR) is a deterrent to the clinical use of bone marrow transplantation. In the present study, the GVHR has been significantly modified in mice by using anti-lymphocyte serum. A model of GVHR was produced in Balb/c newborn mice by injection of 6×10^6 viable adult C57Bl spleen cells intraperitoneally within the first 24 hours. Antilymphocyte serum was prepared by giving 3 intraperitoneal injections of 300×10^6 washed lymphocytes from adult C75Bl (mouse) lymph nodes and thymus into rabbits at weekly intervals. The antiserum was inactivated and absorbed with washed mouse red cells until free from hemagglutinins. The effectiveness of rabbit antimouse lymphocyte serum (RAMLS) was tested in vitro by cytotoxic and indirect fluorescent antibody tests, and in vivo by production of persistent lymphopenia. Thirty-one newborn Balb/c mice were treated with 0.1 ml. RAMLS intraperitoneally after the injection of 6×10^6 adult C57Bl spleen cells by 2 schdules: group I was started on day 3 and group II on day 1. The same dose was then administered every other day through the first 21 days. The incidence of runting and the mortality rate were compared with a spleen cell injected group of 26 newborn mice which received normal rab-incidence of runting and the mortality rate at 21 days bit serum by the same dose schedule. In group I the were reduced from 88 % in the controls to 35 % in the anti-lymphocyte serum treated group. In group II the incidence of runting and the mortality rate at 21 days were reduced from 90 % in the controls to 0 % in the treated group. The results in both groups were statistically significant and indicate that the GVHR can be completely blocked by the early administration of anti-lymphocyte serum in mice. (SPR)

70 *Macrophage Formation from Isolated Lymphocytes in Tissue Culture.* HAROLD W. LISCHNER*, Temple Univ. Sch. of Med. and St. Christopher's Hosp. for Children, Philadelphia, Pa. (introduced by Victor C. Vaughan, III).

Circumstantial evidence for the conversion of lymphocytes into macrophages has accumulated for half a century, but attempts to demonstrate this change in tissue cultures of isolated lymphocytes have met with failure. In these latter studies lymphocytes were exposed in columns to glass, silicon, cotton or nylon surfaces during their separation from granulocytes and monocytes. Other data suggest that either contact with a foreign surface is injurious to lymphocytes or an important minor population of lymphoid cells sticks to such surfaces. For example, column-separated lymphocytes are not agglutinated by isoantisera to leukocytes, and they transform poorly into blastoid cells upon specific stimulation in tissue culture.

Lymphocytes were therefore purified, without significant surface contact, by magnetic removal of iron-laden phagocytes after incubation of whole defibrinated blood with micro-filings of iron. Erythrocytes were sedimented with the aid of gelatin. The resulting lymphocytes were agglutinated well by isoleukoagglutinins, and they underwent blastogenesis as readily as unseparated lymphocytes. One to 3 % macrophages regularly appeared in suspension cultures of these lymphocytes even though the preparations initially contained less than 0.2 % nonlymphoid nucleated cells. In monolayer cultures varying proportions of the lymphocytes attached to the surface and began to enlarge soon after culture. Other lymphocytes never did adhere to the glass. After one week most of the *adherent* cells had the typical morphologic features of macrophages and were phagocytic. It is apparent that at least one class of lymphocytes is capable of conversion into macrophages in the absence of nucleated cells. (Supported by NIH Grants AM-9112, AM-6469 and T1 HD-66) (SPR)

71 *Experimental Fetal Growth Retardation.* WILLIAM A. BLANC, Columbia Univ., Babies Hosp., Col. of Physicians and Surgeons, New York, N.Y.

Reduction of uteroplacental blood flow in one uterine horn of pregnant rats was achieved by Wigglesworth's technique (ligation of uterine vessels at lower end of one horn on the 17th day of pregnancy). Cesarian section was performed on the 21st day. Fetal death or stunting occurred in fetuses located near the ligation. The most stunted fetus was compared with the corresponding fetus in the normal horn and a statistical analysis of 35 such pairs was carried for fetal weight and organ/fetal weight ratio of all organs. Stunted newborns had a mean weight of 2.77 g vs. 4.76 for controls. The liver, lungs, and kidneys, were the most stunted, and were affected more than the fetus as a whole, whereas the brain, placenta, and heart were least affected. The ratios for thymus, spleen, pancreas, and submaxillary gland were not statistically different from those of controls. These observations are, in part, comparable with those made in human newborns in maternal hypertension and 'placental insufficiency'. Histologically the organs differed little from those of control fetuses, except for lack of glycogen in livers of stunted fetuses. Even very stunted organs appeared to have matured normally. These experiments extend and confirm Wigglesworth's data on the weight of liver, brain, and placenta. They support the suggested relationship between uterine blood flow and fetal growth. They show an interesting discrepancy in the effect on growth and on maturation. (SPR)

72 *Phenylalanine Hydroxylase Activity in Hyperphenylalanemia.* MARGARET E. O'FLYNN*, PARVIN JUSTICE* and DAVID Y.Y. HSIA*, Children's Memorial Hospital and Northwestern, Chicago, Ill. (introduced by Robert B. Lawson).

The recent widespread screening for phenylketonuria among newborn infants has lead to the recognition that not all instances of hyperphenylalanemia is caused by phenylketonuria (PKU). Thi spaper describes enzyme studies in four patients with hyperphenylalanemia.

Case 1 is a patient with 'classical' PKU. Case 2 is an infant who was found to have a plasma phenylalanine (PPA) of 17 mg % at three weeks who showed 'mild' PKU. Case 3 is a 35y-ear-old retarded female with