IMMUNOLOGY

673 USE OF THE THROMBOELASTOGRAPH (TEG) IN DIAGNOSING DISSEMINATED INTRAVASCULAR COAGULATION (DIC) IN THE NEWBORN. Margaret N. Watkins, Joseph A. Caprini, and Thomas H. Gardner. (Spon. by Lowell Glasgow) University of Utah Medical Center, Salt Lake City, Utah and Evanston Hospital, Northwestern University, Evanston, Illinois.

The TEG is an automated and very sensitive technique for analyzing whole blood clotting time, requiring only 50 lambda whole blood. Its use in the newborn period has not been previously examined. We utilized thromboelastographic tracings in conjunction with standard coagulation studies to make the diagnosis of DIC in 6 of 13 critically ill newborns with severe birth asphyxia or respiratory failure from hyaline membrane disease. 7 healthy newborns served as a control population for normal values. Samples were drawn at 12 and 24 hrs of age in the control group and every 12 hrs for at least 72 hrs and up to 120 hrs in the study group. The control group showed a generally hypercoagulable state on samples drawn at 12 hrs of age as manifest by low R values and high MA and angle values. By 24 hrs of age the TEG conformed more to the normal adult curve. These patterns persisted after priming with Celite. The trend of the TEG in the study group was for the R values to increase, the MA and angle to decrease with time and the blood to become more hypocoagulable. The trend was accentuated in patients with DIC, progressing to a flat line in some patients. TEG patterns correlated well with elevations of fibrin split products and decreasing or low fibrinogen levels and platelet counts. This test is a technically simple, inexpensive and very rapid technique for diagnosing hyper- and hypo-coagulable states of blood clotting in the newborn.

674 OXIDANT MECHANISM FOR SULFONE INDUCED AGRANULOCYTOSIS Robert M. Weetman, Mary P. Brown, Laurence A. Boxer, Robert L. Baehner, Indiana University School of Medicine, J.W. Riley Hospital for Children, Department of Pediatrics, Indianapolis.

The antimalarial sulfone, dapsone, produces hemolysis and agranulocytosis. A metabolite of dapsone, 4-amino-4'-hydroxylaminodiphenyl sulfone (DDS-NOH) generates superoxide anion and hydrogen peroxide in vitro. To study a potential oxidant mechanism for agranulocytosis, we examined the effect of DDS-NOH on altering stem cell proliferation in vitro. Human bone marrow cells (2 X 10^5 cells/culture plate) were exposed to DDS-NOH for one hour at 37°C, washed, and then cultured in semi-soft agar for 14 days. The majority of colonies were granulocytic and their number (mean ± SD) were:

	Control	1mM DDS-NOH	0.1mM DDS-NOH	0.01mM DDS-NOH
BM#1	119 ± 10	2 ± 3	74 ± 14	-----
BM#2	129 ± 8	0 ± 0	68 ± 6	124 ± 18
BM#3	63 ± 10	0.3 ± 0.5	34 ± 11	52 ± 11

Trypan blue exclusion by nucleated marrow cells was impaired at 1mM DDS-NOH. With 0.1mM DDS-NOH, addition of superoxide dismutase (SOD) or lactoperoxidase, sodium iodide, and SOD further decreased colony numbers, while catalase partially restored colony formation. These studies demonstrate that dapsone induced agranulocytosis may be mediated by oxidant damage to bone marrow stem cells and suggests that SOD may enhance sulfone induced oxidant injury.

675 INDUCTION OF CERVICAL CARCINOMA IN THE MOUSE BY PROLONGED GENITAL EXPOSURE TO FORMALIN INACTIVATED HERPES SIMPLEX VIRUSES TYPES 1 AND 2 (HSV-1 AND HSV-2). W. Budd Wentz, James W. Reagan, Alfred D. Heggie. (Spon. by John H. Kennell) Case Western Reserve Univ. Sch. Med., Depts. of Reproductive Biology, Pathology, and Pediatrics, Cleveland, Ohio.

Seroepidemiological evidence has implicated genital infection with HSV-2 as a possible cause of cervical carcinoma in women and we found in a pilot study that prolonged genital application of formalin inactivated HSV-2 induced this lesion in mice. The present study was done to extend these observations and to investigate the oncogenic potential of HSV-1 in this mouse model. HSV-1 and HSV-2 stocks were prepared from infected HEp-2 cell cultures and were inactivated with formalin. Formalinized control fluids were made in the same way from uninfected cultures. Cotton pledgets saturated with inactivated virus or control fluid were placed in the vaginas of CFW Swiss mice 5 times a week. Cervical smears were examined biweekly and the mice were autopsied after 80 weeks of treatment. The results summarized in the table show that prolonged genital contact with inactivated HSV-1 and HSV-2 induces cervical dysplasia and carcinoma in the mouse and support the concept that these viruses are oncogenic in the genital tract.

	Cervical Lesions Induced by HSV-1 and HSV-2				
Treatment Group	None	Cervical Dysplasia	Microinvasive Carcinoma	Invasive Carcinoma	Total No. of Mice
HSV-1	7(16)*	25(55)	6(13)	7(16)	45
HSV-2	4(11)	25(65)	5(13)	4(11)	38
Control	20(100)	0	0	0	20

*No. of mice(% of mice exposed). Supported by NIH Grant CA 16706.

676 ACTIVITY OF THE ALTERNATIVE PATHWAY OF COMPLEMENT (AP) IN THE NEWBORN. David H. Adamkin, Ann E. Stitzel, Joan R. Urmson, Mary Lou Farnett and Roger E. Spitzer. University of Louisville, Norton-Children's Hospital, Department of Pediatrics, Louisville, KY and State University of New York, Upstate Medical Center, Department of Pediatrics, Syracuse, NY

Cord sera (CS) from 94 normal newborns were studied. Concentrations of properdin convertase, never before measured in the neonate, were at adult levels. In accord with other studies, serum levels of C3, properdin, factor B and C3-C9 activity were markedly depressed. Despite these low levels, however, CS was able to support complete activation of its own AP when incubated with zymosan or cobra venom factor. Thus, formation and stabilization of sufficient factor B-containing enzyme can occur to mediate complete consumption of that amount of C3-C9 contained in CS. This latter process, however, does not result in effective lysis of a target cell. Nearly 75% of CS had rabbit erythrocyte CH_{50} titers more than 2 S.D. below the mean for adult sera. This deficiency is only partially corrected by the addition of excess purified C3-C9 to CS. Addition of factor B and P is more effective but reconstitution with all of the above is necessary to achieve maximal lysis. These data suggest that although the AP of the neonate is completely intact, its activity is suboptimal. Further, although the entire pathway is limited, the major deficit appears to be in the ability to generate an adequate number of stable and active enzymatic sites on a target cell membrane.

677 LUMINOL ENHANCED CHEMILUMINESCENCE OF NEONATAL GRANULOCYTES AND MONOCYTES. D. Anderson, E. Marquez, J. Huntsberger, Dept. of Human Development, Michigan State University (Spon. by W.B. Weil), East Lansing, MI.

Clinical investigations of the functional activity of neonatal phagocytes have been inconclusive, partly due to difficulties obtaining sufficient numbers of cells from clinical samples. Luminol enhanced chemiluminescence (CL) has been useful in assessing intracellular and opsonphagocytic function with small numbers of purified cell populations (ICAAC-Oct 1977; Abst #209 & 210). We adapted an assay to allow accurate CL measurements using $5x10^5$ phagocytes (2.5 ml blood samples) in a 1 ml reaction mixture of PBS (pH 7.2), 10µl BSA (1 mg/ml) or superoxide dismutase (5 mg/ml), luminol [10^{-8}] and preopsonized zymosan (Z:P-50:1). Phagocytosis associated CL correlated with ingestion of zymosan particles. Phagocytosis-associated peak CL values for neonatal PMNs & monocytes was less ($p<.01$) than corresponding adult values. CL by phagocytizing monocytes of both neonates and adults was ~50% of PMN CL values. (Healthy neonates(12) PMN=$1.1x10^5$ CPM, Mono-.55 x 10^5 CPM. Adults(20) PMN=$2.5x10^5$ CPM, Mono=$1.1x10^5$ CPM.) The contribution of superoxide (O_2^-) to CL responses, as assessed by superoxide dismutase CL inhibition, in concurrent experiments was similar in neonatal and adult phagocytes (~50% reduction of PMN and Mono CL). These observations demonstrate that in healthy neonates phagocytosis-related CL and O_2^- generation is less than in adults. Since spontaneous PMN CL values are also less than corresponding adult values, it would appear that the reported enhanced oxidative metabolism of neonatal PMNs is dependent in part on factors other than those determining CL activity.

678 VOLUMETRICALLY DISTINCT SUBSETS OF MONONUCLEAR PHAGOCYTES IN CHILDREN AND ADULTS. Edward B. Arenson, Martin B. Epstein, Philip Herzog, and Robert C. Seeger. UCLA School of Medicine, Departments of Pediatrics and Radiological Sciences. Los Angeles.

There is increasing evidence that cells of the mononuclear phagocyte system (MPS) are heterogeneous; we therefore asked whether subsets of the MPS could be identified volumetrically in human blood. Adherent cells were purified from human mononuclear leukocytes by centrifugation and brief adherence to plastic; these cells were composed of >90% monocytes by simultaneous tests for naphthyl butyrase activity and phagocytosis of yeast. Coulter volume distribution analysis of purified cells was performed using a multi-parameter cell sorter. Two major volume peaks, M_1 and M_2, were present in approximately equal proportion in all normal individuals tested, and a larger third peak, M_3, was often present as a minor population. Mean volumes of M_1 and M_2 were approximately $200\mu^3$ and $500\mu^3$ respectively with the range of M_1 overlapping that of lymphocytes. Volume distributions were essentially constant when analyzed consecutively for the same individuals. Mean volumes and volume distributions were similar in adults and children. Preliminary functional comparison of M_1 and M_2 indicated that M_1 has a relatively reduced phagocytic rate. These studies support our hypothesis that there are identifiable subsets of MPS cells in human blood. These subsets now can be analyzed and also can be separated for ontogenic and functional studies.