- 103 *Plasma Adrenocorticosteroid Levels During Experimental Infections.* STANFORD B. FRIEDMAN, LOWELL A. GLASGOW and LEE J. GROTA, Univ. of Rochester Sch. of Med., Rochester, NY.

Adrenocortical function during infection has not been well defined in man, and patient care itself may interfere with meaningful steroid measurements. Animal models provided the opportunity to relate plasma adrenocorticosteroid (corticosterone - CS) levels to the natural course of fatal infections in mice inoculated IP with (1) a malarial parasite—*P. berghei*, (2) a bacteria—*D. pneumoniae*, or (3) 2 strains of a murine enterovirus—encephalomyocarditis (EMC) virus. In *P. berghei* infection animals survived through day 6, and CS levels increased 4- to 6-fold on day 5 and 6. Enlargement of the spleen and parasitemia precedes this rise in CS by 24 h. In the more acute infection with *D. pneumoniae* (deaths occurred within 72 h) increases in CS levels of a similar magnitude were found at both the 'peak' and 'trough' of the adrenocortical rhythm. CS levels and virus titers were determined in individual animals infected with one LD₅₀ of EMC virus, and correlated with the outcome of infection. Modest elevations of CS occurred only during the terminal phases of infection. Elevation of CS did not occur during the viremic phase, and were observed only after clearance of virus from the blood. A more virulent strain of EMC virus which was lethal in 48-72 h resulted in marked elevation of CS by 24-48 h.

- 104 *Leukocyte Maturation: G-6-PD Activity and NBT Dye Reduction.* JOSEPH A. BELLANTI, BRIGITTE E. CANTZ, MEI C. YANG, HORST VON THADDEN and ROBERT J. SCHLEGEL, Georgetown Univ. Sch. of Med. and Stanford Univ. Sch. of Med.

Evidence was presented at these meetings last year that human leukocyte G-6-PD activity decayed at significantly greater rates in newborns than in adults. Quantitative NBT dye reduction was correspondingly low provided collection, incubation and measurement times were carefully controlled. The present studies reflect longitudinal maturation of these functions as shown in the table.

Age	New-born	1-2 mos.	6-12 mos.	12-18 mos.
No. tested	20	11	16	11
NBT (mean Δ OD)	0.125	0.092	0.097	0.119
No. tested	9	6	5	6
G-6-PD (mean) (μ moles/g protein)	78	27	9.7	55
Age	1.5-4 years	4-10 years	10-14 years	Adults
No. tested	18	19	8	18
NBT (mean Δ OD)	0.144	0.139	0.175	0.206
No. tested	15	14	6	24
G-6-PD (mean) (μ moles/g protein)	56	98	148	141

These results show a fall in both G-6-PD activity and NBT dye reduction during early months of life followed by a continuous increase with maturation. In contrast, levels of 6-PGD activity remain constant. Lability of G-6-PD and diminished NBT dye reduction are also present in chronic granulomatous disease, an immune deficiency disorder associated with defective bactericidal activity of leukocytes [BELLANTI *et al.*, *Pediat. Res.* 1970]. These findings offer an explanation for the increase in resistance known to occur with age.

- 105 *Zoster Immune Globulin.* P. A. BRUNELL, A. ROSS, L. MILLER, M. COHEN and A. SCHMERLER, NYU Sch. of Med. and Meadowbrook Hosp.

It has been reported that susceptible children were protected against varicella if they received two ml of zoster immune globulin (ZIG) within 72 h following household exposure. In a double blind study to determine the minimum *modifying* dose of ZIG, 0.5 ml of either ZIG or immune serum globulin (ISG) was given to exposed household contacts. It was found that the four children who received ISG developed 161, 285, 286 and 407 vesicles while the children who received ZIG had 0, 2, 4 and 4 vesicles. All seven children who developed vesicles had rises in complement-fixing (CF) antibody. The ZIG which was used in these studies was prepared from plasma of pretested convalescent zoster donors who had CF antibody titers of 1:256 or greater. Pretesting of donors was eliminated subsequently in order to expedite the production of ZIG. Although plasma was obtained when highest antibody titers were expected, only 20 of 35 plasma collections had CF titers high enough ($\geq 1:128$) to be used for ZIG preparation. The dose of this and subsequent lots of ZIG will be estimated by comparing their antibody content with that of the reference lot for which the minimum protective dose was determined in these studies.

- 106 *Growth in Agarose of Human Cells Infected With Cytomegalovirus.* DAVID J. LANG and LUC MONTAGNIER, Duke Univ. Med. Center, Dept. of Ped., Durham, N.C. and Institut du Radium Biologie, Orsay, France.

Cultured cells transformed by certain DNA and RNA viruses develop the ability to multiply when suspended in a medium gelled with agarose. Normal fibroblasts obtained from human diploid lines do not grow in agarose medium but will do so after being transformed by Rous sarcoma virus. We report here that after infection by human cytomegalovirus (CMV) human diploid fibroblasts acquire the capacity to grow in agarose medium for several generations. Clones of infected cells grew for weeks although in every case they ultimately underwent lysis, apparently related to persistent replication of virus. Virus was inoculated at very low multiplicities (high dilution) in an effort to select non-infectious defective virions still capable of inducing cell stimulation. Viral suspensions were also irradiated (UV-CMV) to preferentially suppress infectivity. Dilute or irradiated virus yielded similar colonies of replicating cells although permanent transformation was not achieved. One clone derived from UV-CMV infected cells was passaged four times before undergoing lysis. During these passages the cells exhibited alterations in morphology and orientation. Our inability to obtain permanently transformed cells after inoculation with irradiated or diluted CMV may be due to complementation or the failure of the CMV DNA to achieve integration with the cellular genome. If permanent cellular transformation by human CMV is achieved in subsequent experiments, it will still remain to demonstrate whether this phenomenon bears any relationship to neoplasia in man. It is of interest that CMV is taxonomically related to EB and Herpes simplex type 2 viruses which have been associated with human cancer.

- 107 *Sex and Susceptibility.* SUMNER BERKOVICH, State Univ. of New York, Downstate Med. Center, Dept. of Ped., Brooklyn, NY.

Susceptibility to viral infection is not only influenced by age but by sex as well. In the human, these inter-related factors are difficult to evaluate. We have developed a test system in adult mice, however, which does allow ease of observation and control over 1 or the other of these 2 variables. A significant difference in death rate has been found between mature, male and female animals infected with a virulent strain of Coxsackie B1 virus. This difference in susceptibility could be related to hormonal effects. To test this hypothesis, mice gonadectomized at birth were infected as mature animals with the test virus. Results are summarized below.

	Unoperated		Sham-Castrated		Castrated	
	No.	Death rate (%)	No.	%	No.	%
Male	42	41	28	61	26	4
Female	37	11	32	6	33	9

Neonatal gonadectomy and the consequent hormonal changes did not exert an apparent effect on the response of the female to the infecting virus, whereas the adverse effect of maleness was negated by this procedure. The premise relating differences in susceptibility to hormone effects, therefore, is partially true. The increased susceptibility of the male is a sex-related characteristic.

- 108 *The Use of the Nitroblue Tetrazolium Reduction (NBT) Test in Diagnosis and Treatment of Bacterial Endocarditis.* B. H. PARK, M. A. SOUTH, F. F. BARRETT, J. R. MONTGOMERY, L. HEIM and R. A. GOOD, Ped. Res. Labs., Variety Club Heart Hosp., Univ. of Minnesota, Minneapolis and Dept. of Ped., Baylor Univ., Houston, Texas.

Despite the recent advances in the diagnostic and therapeutic armamentarium, the bacterial endocarditis remains one of the most challenging clinical problems. The conventional diagnostic methods for bacterial endocarditis often either fail or delay in the diagnosis and treatment of this disease. A new diagnostic test for the bacterial endocarditis is urgently needed in order to initiate the antibiotic therapy promptly. We have found the NBT test [PARK *et al.*, *Lancet* ii: 532, 1968] to be a useful diagnostic aid in these clinical situations. Patients with proven bacterial endocarditis and those with presenting symptoms suggestive of bacterial endocarditis were studied with the NBT test. The results were compared with those of controls. The absolute number and percentage of the NBT positive neutrophils were found to be regularly increased in seventeen patients with bacterial endocarditis (means: $3,150/\text{mm}^3$, 24.5%) as compared with normal control and patients with non-bacterial disease ($439 \pm 187/\text{mm}^3$, $6.86 \pm 2.87\%$). Furthermore, the clinical response to the antibiotics therapy was well correlated with the results of the NBT test. The simplicity and instant results are other advantages of NBT test as a diagnostic aid in bacterial endocarditis.

- 109 *Clinical and Laboratory Studies on Marmosets Experimentally Inoculated with Human Serum from Hepatitis Patients.* C. LIU, C. T. CHO, D. W. VOTH, D. J. SVOBODA and S. HEMPLER, Univ. of Kansas Sch. of Med., Kansas City.

It has been reported that hepatic changes and abnormal liver function tests occurred in marmosets injected with human serum from acute infectious hepa-

titis patients [DEINHARDT, F. *et al.*, *J. exp. Med.* 125: 673, 1967]. Using the Barker agent supplied to us by Drs. DEINHARDT and HOLMES, we have confirmed and extended their experimental findings. Inoculated marmosets showed SGOT and SGPT elevations and hepatic parenchymal cell changes after an average incubation period of 20 days. Duration of acute hepatitis lasted for about 6-8 weeks. Although infected marmosets usually did not appear clinically ill, 5 out of 47 animals died during the acute stage of illness.

Relative neutropenia with lymphocytosis was seen in differential counts of peripheral blood in marmosets with acute hepatitis. Corticosteroids given during acute hepatitis did not appear to alter the clinical course favorably or detrimentally. However, feeding of alcoholic beverages to marmosets with acute hepatitis or during early convalescence resulted in exacerbation of hepatic enzyme elevations and deaths.

Recently we have recovered a similar agent (Lyddon) by inoculating marmosets with the acute serum collected from one of our hepatitis patients. The incubation period for initial passage was 2 months. On subsequent passages, the incubation periods were shortened to 19-21 days. There was suggestive evidence from rechallenge studies that antigenic differences might exist between the Barker and Lyddon agents. (Supported by NIH Grant HD 02567.)

- 110 *Microbiological Studies in a Primitive Society: Significance of Escherichia coli from Yanomamö Indians.* WARREN C. EVELAND, Dept. of Epid., Sch. of Pub. Health; WILLIAM J. OLIVER, Dept. of Ped.; and JAMES V. NEEL, Dept. of Human Genetics, Univ. of Michigan, Ann Arbor, Mich.

The Yanomamö Indians of the northern Amazon tributaries constitute one of the largest unacculturated tribes of South America. The first sustained contact of Caucasians with a village of this tribe was in 1950; many villages still have had no or minimal contact with civilization. Stool samples obtained in 1967 during a field expedition to two recently contacted villages 250 miles apart were studied for serotypes of *E. coli*. Using standardized techniques described by the NCDC, six colonies were isolated from each of 72 samples and typed against 148 presently known serotypes of *E. coli*. Only 204/432 isolates were typable. Eight strains of enteropathogenic *E. coli* were isolated from 13 subjects; serotypes 055, 0111, and 0126 occurred in samples from both villages while five other enteropathogenic serotypes were confined to one of the two villages. Thus far ten new serotypically unique strains of *E. coli* have been isolated. For 8/10 of the new serotypes, positive reactions were confined to one village only; for 2/10, positive reactions were recognized in samples from both villages. *E. coli* of stool samples from an urban population of S. America (Cali, Columbia) gave negative reactions to the new serotypes. Although these observations substantiate the ubiquity of certain *E. coli* serotypes, a more pertinent observation is the finding of at least ten new serotypes, and the relative uniqueness of these between villages and apparent absence from a control population. These investigations are one aspect of an effort to understand primitive man in his ecosystem; they suggest that the 'internal milieu' must be as well studied as the 'external'.

- 111 *Antistaphylococcal Substance(s) from a Pharyngeal Organism.* KATHERINE SPRUNT, GRACE LEIDY, WINIFRED REDMAN, Columbia Univ., NYC.