SPECIFIC CELLULAR ANERGY IN HUMAN FILARIASIS. E.A.Ottesen*, P.F.Weller*, L.Heck*(intr. by C.H.Kirkpatrick**) LPD,NIAID,Bethesda, Maryland.

In order to understand the host response to the parasite Wuchereria bancrofti and its relationship to the variable manifestations of human filarial disease, cellular and humoral immune responses were investigated in 40 persons on a Pacific island where the disease is endemic.

Cell mediated immunity (CMI) was assessed in vitro as ^{3}H-thymidine incorporation by antigen-stimulated blood lymphocytes. Saline extracts of two filaria (Brugia malayi and Dirofilaria immitis) and streptococcal (SK-SD) and tuberculin (PPD) proteins were used as antigens. Immunoglobulins (Ig) were measured by radial diffusion (Ig-G,A,M,D), radioimmunoassay (IgE) or passive hemagglutination (filarial antibodies). Microfilaremia was quantified by a membrane filtration technique.

In filaria-exposed individuals without evidence of disease both filarial antigens induced brisk CMI responses with stimulation ratios (E/C) of 16.1±6.1 (mean±SEM) and 18.9±6.7. In contrast, lymphocytes from infected individuals generated significantly diminished responses (F/C 2.6±0.7 and 3.3±0.7; p<.005) to these same antigens. This diminished reactivity was antigen specific; maximal responses to either SK-SD or PPD were normal and not different in the two groups. No significant differences were found among the CMI responses of individuals with different clinical features of filariasis. Overall there were no significant differences in serum Ig or filarial antibody titers among the various infected and control groups, although specific antibody responses tended to be lower in infected children than in other groups.

The loss of antigen-specific reactivity by lymphocytes of infected individuals indicates that patients with filarial disease manifest a form of immune unresponsiveness affecting CMI similar to that recognized in certain other chronic infectious diseases.

SEROLOGICAL EVIDENCE FOR THE IN VIVO PRODUCTION OF LETHAL EXOTOXIN IN PATIENTS WITH PSEUDOMONAS INFECTIONS. M. Pollack, N. S. Taylor*, and L. T. Callahan*, Department of Microbiology, Naval Medical Research Institute, Bethesda, Maryland.

Pseudomonas aeruginosa (PA) exotoxin inhibits protein synthesis and is lethal for tissue culture cells and laboratory animals. Our unpublished data indicate the exotoxin is produced in vitro by most PA strains, yet there has been no evidence to date of its in vivo production in PA infections. In this study, exotoxin-neutralizing antibody was detected in patients' sera using a cytoxicity assay which employs mouse fibroblasts (L cells) and purified exotoxin. The titer is expressed as the $\log_2$ of the serum dilution eliciting 50% neutralization. Means ± standard errors include all results (negative = 1).

Serum neutralizing activity to exotoxin was found in all of 13 patients recovering from PA infections (5.79 ± 0.80) and in 4 of 7 patients with fatal PA infections (3.28 ± 2.68). In contrast, it was present in only 1 of 4 patients colonized and not clinically infected by PA (1.45 ± 0.90, p<.005 in comparison with PA infected patients), in 3 of 7 recovering from non-PA infections (1.44 ± 0.57, p<.001), and in 6 of 14 normal healthy controls (1.97 ± 1.25, p<.001). Serial titer rises were demonstrated in 2 survivors of PA infections, and neutralizing activity was associated with the IgG fraction of patients' sera as determined by Sephadex G200 and DEAE chromatography and resistance to reduction by 2-mercaptoethanol.

Thus, PA exotoxin, which is produced by most strains in vitro, is also produced in vivo in human pseudomonas infections in sufficient quantity to elicit antibodies. The role of the exotoxin in these infections needs to be further defined.

INTERFERON MOLECULES IN HUMAN BIOLOGICAL FLUIDS; RELATION OF THEIR STRUCTURAL CHARACTERISTICS TO THEIR CELLULAR SOURCE. G.E. Panagos,* J.A. O'Malley,* and W.A. Carter, Roswell Park Memorial Institute, Buffalo, New York.

Our study was designed to determine the structural characteristics of interferon molecules present in the biological fluids of Varicella-Zoster-infected cancer patients, before and after treatment with the interferon inducer, polyinosinic-polycytidylic acid ($rI_n \cdot rC_n$). We have previously detected differences in the degree of hydrophobicity of interferon molecules derived from different types of known normal cells by the use of several hydrophobic chromatographic systems (Jankowski, et al. J. Virol. 16, 1124, 1975; Davey et al., Biochemistry, Feb. 1976). Therefore, by chromatographing interferon derived from biological fluids of our patients, we can deduce its likely cellular source.

Serum and vesicle fluid interferon was chromatographed on tryptophan-agarose which, we have shown, binds human fibroblast interferon hydrophobically and which does not retain human leukocyte interferon. Serum drawn prior to $rI_n \cdot rC_n$ treatment contains extremely small quantities of three molecular forms of interferon which can be eluted from tryptophan-agarose: one with affinity characteristics of leukocyte interferon, another with those of fibroblast interferon, and a third, never before observed (possibly derived from lymphocytes). After treatment with 9 mg/kg of $rI_n \cdot rC_n$, the patients' serum contained greatly increased levels of all three molecular components of interferon. Vesicle fluid interferon, often moderately elevated before $rI_n \cdot rC_n$ treatment, also consists of the three molecular forms, all of whose levels increase after treatment. Thus, it appears that at least three cellular targets of the inducer elaborate interferon in man: leukocytes, fibroblasts, and a third target whose nature is currently under investigation.

TRACE METAL AND LIPID LEVELS DURING ROCKY MOUNTAIN SPOTTED FEVER IN THE GUINEA PIG. M.C. Powanda, E.C. Hauer,* R.E. Whitmire,* J.P. Fowler,* L.A. Harris* and R.H. Kenyon*. USA Med Res Inst of Infectious Diseases, Frederick, MD.

The purpose of this study was to determine if selected aspects of host metabolism could be used as indicators of the severity of vasculitis, the presumed cause of death in Rocky Mountain spotted fever. As in many other infections, plasma copper and ceruloplasmin were significantly elevated while plasma zinc and iron were significantly depressed during overt illness (elevated body temperature, rickettsemia). The magnitude of these alterations in trace metals appeared to reflect the severity of the vascular lesions of the cremaster muscle and ear. Thus, plasma trace metal patterns may be useful as a nonspecific index of the presence and persistence of vasculitis. In contrast to other infections, an initial rise in plasma copper occurred prior to overt illness and massive lesion development which was not associated with increased ceruloplasmin. The origin, possible ligand binding and significance of this increment in plasma copper remain to be determined.

Plasma triglycerides values were elevated on day 1 and free fatty acids by day 2; both reached a peak on day 7 and returned to control levels by day 10 when overt illness had disappeared but microscopic evidence of vasculitis remained. There was no change in cholesterol concentrations at any time. The increase in plasma triglycerides may be due to a decrease in endothelial cell lipoprotein lipase, an enzyme considered to regulate triglyceride uptake from blood. Plasma triglyceride and/or free fatty acid levels may thus be specific indicators of small vessel endothelial cell dysfunction during Rocky Mountain spotted fever.

RADIOIMMUNOASSAY FOR SCHISTOSOMIASIS USING PURIFIED EGG ANTIGEN. R.P. Pelley* and K.S. Warren**. Division of Geographic Medicine, Department of Medicine, Case Western Reserve University, Cleveland, Ohio.

Many sophisticated means of detecting antibodies (e.g. fluorescence, radioisotopes, biochemical reactions) have been developed but the sensitivity and specificity of serological tests for schistosomiasis are no better today than they were in 1919. The present study involves the use of a purified antigen isolated from Schistosoma mansoni eggs on the basis of its strong interaction with antiserum from chronically infected mice. Starting with crude soluble egg antigens the major serological antigen (MSA_1) was isolated by Concanavalin A affinity chromatography, labeled with ^{125}I, and purified to a single PAGE band on DEAE cellulose. Radioimmunoassay (RIA) was performed by the Farr technique utilizing 5-10 ng MSA_1 and 5 µl human serum. Sera from 135 St. Lucians relatively lightly infected with S. mansoni and 69 uninfected St. Vincentians were previously tested at CDC (AJTM&H 22:189, 1973) by a battery of tests. In comparison with the best CDC test [fluorescent antibody (FA)], RIA was more specific (100% - none of the St. Vincent sera were +), and more sensitive in ages 5-9 64/44 (%+RIA/%+FA), ages 10-14 82/72 and adults 98/76. When tested with sera from 40 heavily infected Kenyan children (ages 5-14) RIA suggested a relation to intensity of infection as they were 100% positive. Finally RIA on sera of patients matched for age and intensity of infection with S. mansoni, japonicum, and haematobium demonstrated that MSA_1 is quantitatively specific for S. mansoni. The MSA_1 RIA is the first assay to successfully use a purified antigen to overcome the problems of specificity and sensitivity in the serodiagnosis of a major helminth pathogen of man.

THE EFFECT OF SPLENECTOMY ON PHAGOCYTOSIS OF STREPTOCOCCUS PNEUMONIAE TYPE II IN INFANT SPRAGUE-DAWLEY RATS AND THE INFLUENCE OF PNEUMOCOCCAL VACCINE IN THIS SYSTEM. Arthur J. Provisor*, John M. Allen*, and Robert L. Baehner**, Indiana University School of Medicine, Department of Pediatrics, J.W. Riley Hospital for Children, Indianapolis, Indiana.

The increased susceptibility of both adult and infant splenectomized rats to pneumococcal infection has been documented. Two mechanisms for this have been suggested: 1) filtering effect of the spleen; 2) synthesis of opsonins in the spleen. To study the role of the spleen in opsonization, splenectomy was performed on half of a group of 18 infant rats, the other half having a sham procedure. Sera were obtained 4 weeks post-surgery. Utilizing ^{14}C type II pneumococci and peritoneal neutrophils obtained from control rats, we directly measured the rate of uptake of pneumococci in the presence of untreated sera, sera treated with EGTA and $MgCl_2$ to preserve the alternate pathway, and sera heated to 50° C for 30 minutes to preserve the direct complement pathway. No differences were seen between the 2 groups. The same procedure was carried out on a group of 16 one-week old rats (50% sham-50% splenectomy) except that 4 days post-surgery, these animals received subcutaneous immunization with 10^8 whole heat killed type II pneumococci. In the untreated sera and sera treated with EGTA and MgCl2, there was a marked increase in uptake of bacteria in the sham group when compared to the splenectomized group. No difference between the sham and splenectomized rats was seen with sera heated to 50° C. These studies suggest that in nonimmunized infant rats opsonization of type II pneumococci is not dependent on splenic tissue. The enhanced opsonization observed after immunization appears to require a functioning spleen.