MOLECULAR BASIS OF C4 POLYMORPHISM. *P. Teisberg*, B. Mevåg, B. Olaisen,* Institute of Forensic Medicine, University of Oslo and Med. dept., Rikshospitalet, University of Oslo, Oslo.

The genetic polymorphism of human C4 is a very complex system. In a Caucasian population approximately 10 different haplotypes occur with appreciable frequency, and a large number of different rare variants may be found. This large variation is due to the occurrence of molecular variants and to the existence of different combinations of variants in haplotypes. The majority of chromosomes express two C4 loci, but chromosomes expressing one C4 locus are fairly common, and we have recently shown that a few chromosomes express three loci.

In the present study various common C4 gene products were isolated from serum by immunoprecipitation. After reduction the C4, α, β and γ polypeptide chains were studied by two-dimensional electrophoresis. Isoelectric focusing was performed in the first dimension and SDS polyacrylamide gradient gel electrophoresis in the second. The charge differences behind the electrophoretic polymorphism were shown to reside in the α chain. Charge variation closely mirroring the chain differences were also found in a 49,000 dalton fragment of the α chain, possibly C4d. The basic β chain could not be studied in detail, but no differences were observed with regard to molecular weight or charge of the γ chains of the different C4 gene products.

THE THIOLESTER SITE OF HUMAN C4: IMPLICATIONS OF SEQUENCE HOMOLOGY WITH HUMAN α_2M AND HUMAN AND GUINEA PIG C3. *R.A. Harrison, M.L. Thomas*,* and *B.F. Tack,* Children's Hospital and Harvard Medical School, Boston, Massachusetts.

α Proteolytic digestion of C4, radiolabeled at either the "active-site" thiol or at its reactive acyl group, was studied. At low elastase concentrations rapid digestion of the α-chain occured and a 45000 molecular weight C4d-like fragment containing the radiolabel was generated. At high elastase concentrations, the digestion of (^{14}C) methylamine (MA)-inactivated C4 bound to activated thiol-Sepharose yielded a 25000 molecular weight fragment still containing the radiolabel. Both fragments were isolated by gel filtration chromatography. C4d (ela25), radiolabeled with (^{14}C)MA and (^{3}H) iodoacetate, had incorporated 0.83 mole of MA/mole iodoacetate. Comparison of the N-terminal sequence of each fragment with that recently reported (Cambell et al. *Bioscience Reports 1,* 423, 1981) showed these fragments to be produced from cleavages close to the N-terminus of C4d. C4d (ela45) is therefore similar to the physiologically-derived C4d fragment whereas C4d (ela25) has lost a large C-terminal segment. Extended sequence analysis of C4d (ela25) showed (^{3}H) counts to be released at position 21 and (^{14}C) counts at position 24. These data confirmed the originally calculated (^{14}C): (^{3}H) incorporation ratio and further indicated that the incorporation of each radiolabel was limited to single sites in C4. Comparison of the sequence obtained with those determined for the thiolester sites of C3 and α_2M showed that in addition to conservation of the Gly-Cys-Gly-Glu-Glu sequence considerable homology, largely C-terminal to thise site, exists. However, whereas in C3 and α_2M this region is hydrophobic, four basic residues occur in C4. Additionally, while a high degree of homology between human and guinea pig C3 exists N-terminal to this site, there is little homology between C3, C4, and α_2M. We therefore propose that the highly conserved region is essential for maintenance of the thiolester site and that the variable regions may, in part, be responsible for specificity in the binding reaction.

ACUTE LUNG INJURY FOLLOWING INTRAVASCULAR COMPLEMENT ACTIVATION; ASSOCIATION WITH TOXIC OXYGEN METABOLITES FROM NEUTROPHILS. *Gerd O. Till*, Kent J. Johnson, Robin Kunkel and Peter A. Ward* Department of Pathology, University of Michigan Medical School, Ann Arbor, Mich.

Systemic complement activation by cobra venom factor results in the transient appearance in serum of chemotactic activity and a concomitant profound neutropenia. Within 30 min acute lung injury develops, as measured by increases in lung permeability. Morphologically, there is intrapulmonary, intracapillary neutrophil sequestration, damage and/or destruction of endothelial cells, evidence of vascular basement membrane damage, and intraalveolar hemorrhage. None of these changes is realted to the presence of contaminating amounts of phospholipase A_2 in the cobra venom preparation. The acute lung injury following intravascular infusion of cobra venom factor is neutrophil-dependent as demonstrated by the protective effects of neutrophil depletion. The injury also appears to be related to the production by stimulated neutrophils of O_2^- and H_2O_2, as reflected by the protective effects of superoxide dismutase and catalase. These data suggest that the pulmonary vasculature is readily damaged by the intravascular generation of C5a which, in turn, activates neutrophils, causing the generation of toxic oxygen metabolites. This mechanism may be important in lung injury that is seen in the acute respiratory failure syndrome and a variety of other lung injury states.

FREEZE-FRACTURE ANALYSIS OF THE MEMBRANE LESION OF COMPLEMENT. *J. Tranum-Jensen,* Anatomy Dept. C, University of Copenhagen, Denmark, and *S. Bhakdi,* Institute of Medical Microbiology, Giessen, W.-Germany.

Analysis of single and complementary freeze-fracture replicas prepared by techniques of unidirectional and rotational shadowing served to clarify the structure and membrane insertion of the human C5b-9(m) complex, generated by lysis of antibody coated sheep erythrocytes with whole human serum in excess. Membrane samples were frozen without cryoprotection as 40 um fluid films in a propane jet at -170 °C, or they were frozen in nitrogen slush after cryprotection wtih glycerol.