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THE USE OF LOW MOLECULAR WEIGHT PROTEINS IN THE DIAGNOSIS OF
RENAL TUBULAR DYSFUNCTION IN CHILDREN

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thesis submitted to the University of Auckland for the degree of Doctor of Medicine

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Abstract

Low molecular weight (LMW) proteins pass easily through the glomerular filter and are almost completely reabsorbed by the proximal renal tubule in health. An increase in their urinary excretion implies failure of reabsorption and may signal tubular dysfunction. \( \beta_2 \)-microglobulin (B2M) is a sensitive marker of tubular dysfunction, but is unstable in acid urine, whilst only limited data are available for other LMW proteins.

The aim of these studies was to determine the prevalence of elevated LMW protein excretion in children with renal disease and to identify factors influencing LMW protein excretion. Secondly, these studies sought to determine conditions where individual proteins might best indicate tubular dysfunction.

Enzyme-linked immunosorbent assays were used to measure B2M, \( \alpha_1 \)-microglobulin (A1M) and urine protein 1 (UP1) in plasma and urine, and retinol-binding protein (RBP) in urine. Albumin, the lysosomal enzyme N-acetyl-\( \beta \)-D-glucosaminidase (NAG), creatinine and pH were also measured in urine. Each protein excretion was expressed as a ratio to creatinine concentration.

B2M showed instability in urine with pH below 6.0, RBP was unstable in urine below pH 5.0 following frozen storage whilst A1M and UP1 were stable at physiological pH. Two groups of apparently healthy children were studied, and normal ranges were established for protein excretion in random and overnight samples. A comparison of B2M, A1M, UP1, RBP and NAG excretion was undertaken in tubular disease and in glomerular disease.
AIM, UP1 and NAG were correlated with increasing albumin excretion in steroid-sensitive nephrotic syndrome, in contrast to B2M and RBP. Compared with AIM, UP1 and NAG, RBP was more closely associated with tubulo-interstitial involvement histologically.

There was abnormal RBP excretion in reflux nephropathy, with levels increasing according to the degree of scarring. Increased RBP excretion was also seen in cystic disease, neurogenic bladder, allograft rejection and chronic glomerular disease. RBP excretion was inversely correlated with glomerular filtration rate in reflux nephropathy and in glomerular disease. RBP excretion was increased in diabetic children and was correlated with albumin excretion and with metabolic control.

LMW protein excretion is common in children with renal problems, and may be a marker of disease severity. RBP is the most suitable marker of tubular dysfunction.
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The following abbreviations have been used within this thesis
(standard biochemical symbols and abbreviations have not been
included):

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AIM</td>
<td>α₁-microglobulin</td>
</tr>
<tr>
<td>B2M</td>
<td>β₂-microglobulin</td>
</tr>
<tr>
<td>Cr-EDTA</td>
<td>⁵¹chromium edetic acid</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>CyA</td>
<td>Cyclosporin A</td>
</tr>
<tr>
<td>Da</td>
<td>Daltons</td>
</tr>
<tr>
<td>DMSA</td>
<td>⁹⁹ᵐtéchneetium dimercaptosuccinic acid</td>
</tr>
<tr>
<td>DTPA</td>
<td>⁹⁹ᵐtéchneetium diaminotetraethylpentacetic acid</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>FE</td>
<td>Fractional excretion</td>
</tr>
<tr>
<td>FSGS</td>
<td>Focal and segmental glomerulosclerosis</td>
</tr>
<tr>
<td>GFR</td>
<td>Glomerular filtration rate</td>
</tr>
<tr>
<td>GSC</td>
<td>Glomerular sieving coefficient</td>
</tr>
<tr>
<td>HLA</td>
<td>Human leucocyte antigen</td>
</tr>
<tr>
<td>HRP</td>
<td>Horse-radish peroxidase</td>
</tr>
<tr>
<td>HSP</td>
<td>Henoch-Schonlein purpura</td>
</tr>
<tr>
<td>IgA</td>
<td>Immunoglobulin A</td>
</tr>
<tr>
<td>IgG</td>
<td>Immunoglobulin G</td>
</tr>
<tr>
<td>LMW</td>
<td>Low molecular weight</td>
</tr>
<tr>
<td>Max</td>
<td>Maximum</td>
</tr>
<tr>
<td>MCGN</td>
<td>Mesangio-capillary glomerulonephritis</td>
</tr>
<tr>
<td>Min</td>
<td>Minimum</td>
</tr>
<tr>
<td>min</td>
<td>minute</td>
</tr>
<tr>
<td>NAG</td>
<td>N-acetyl-β-D-glucosaminidase</td>
</tr>
<tr>
<td>OPD</td>
<td>o-phenylene diamine</td>
</tr>
<tr>
<td>Pcreat</td>
<td>Plasma creatinine concentration</td>
</tr>
<tr>
<td>pI</td>
<td>Isoelectric point</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>PNP</td>
<td>Para nitro phenol</td>
</tr>
<tr>
<td>RBP</td>
<td>Retinol-binding protein</td>
</tr>
<tr>
<td>RIA</td>
<td>Radioimmunoassay</td>
</tr>
<tr>
<td>RID</td>
<td>Radial immunodiffusion</td>
</tr>
<tr>
<td>RN</td>
<td>Reflux nephropathy</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SDS-PAGE</td>
<td>Sodium dodecyl sulphate polyacrylamide gel electrophoresis</td>
</tr>
<tr>
<td>SSNS</td>
<td>Steroid-sensitive nephrotic syndrome</td>
</tr>
<tr>
<td>UK</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>UP1</td>
<td>Urine protein 1</td>
</tr>
<tr>
<td>VUR</td>
<td>Vesicoureteric reflux</td>
</tr>
</tbody>
</table>
The studies outlined in this thesis are the result of work undertaken as a Research Fellow within the department of paediatric nephrology at Guy's hospital, London, UK. I developed the laboratory assays for measurement of low molecular weight proteins and performed the majority of the assays myself, assisted at times by my laboratory colleagues. In addition, I undertook much of the specimen collection at Guy's hospital, and collected the clinical data. The data collation and analysis are the result of my endeavour.

I gratefully acknowledge below the contribution of the many people who have assisted me with this work.

Dr Diana Gibb provided urine samples from diabetic subjects and from healthy schoolchildren. Dr Godfrey Clark arranged for the collection and storage of plasma and urine samples from renal transplant recipients. Dr William van't Hoff collected plasma and urine samples on my behalf from some of the patients with cystinosis. Dr Jean Smellie and her assistant Nina Prescod collected many of the urine samples from children with urinary tract infection or vesicoureteric reflux. Professor Cyril Chantler, Professor George Haycock and Dr Sue Rigden kindly allowed me access to their patients for the purposes of these studies. The outpatient nurses in the children's renal unit and the nursing and junior medical staff from Dickens ward helped with specimen collection also.

The laboratory work was undertaken in the Children Nationwide Kidney Research Laboratory at Guy's hospital. I am grateful to Dr Neil Dalton and Mr Charles Turner for their constant help and encouragement with the development of laboratory assays and for assistance with laboratory techniques. Charles Turner was responsible for my introduction to the Personal Computer, which was to become an invaluable aid to data collection, analysis and subsequent preparation of manuscripts, and for an introduction to statistical analysis. I am especially grateful to Neil Dalton for his constant support, sharing of ideas and willingness to criticise these studies at all stages of development. My thanks extend to Ms Jeannette Gorst from Dako Limited, High Wycombe, UK who provided advice and samples of antibodies for use in the assays described herein.

The clinical data were obtained through the medical records department at Guy's hospital by chart review. In addition, Dr Diana Gibb provided the clinical data for the study on diabetes. Clinical data for the study of children with vesicoureteric reflux were also provided by Dr Jean Smellie and her research assistant Nina Prescod. Dr Smellie reviewed the imaging material and performed the scar typing on all intravenous urography studies.

There were many other staff at Guy's hospital who have assisted me at times with this work, including the radiology department staff, the medical illustration department, the Will's library, the nuclear medicine department and the clinical chemistry laboratory. Professor Stewart Cameron and Dr Geoff Frampton allowed me access to the ELISA plate-reader in the Clinical
Sciences laboratory. Dr Barry Hartley, Consultant Pathologist, assisted me with the interpretation of renal biopsy material.

Dr Elizabeth Wells from the Christchurch School of Medicine advised me on statistical matters relating to reflux nephropathy. Francis Harrington, librarian for Southern Health, helped me to obtain references, often from foreign journals at short notice. Dr Kate Bayston has assisted in proof-reading this manuscript.

Finally, I wish to record my special thanks to my supervisor, Professor Cyril Chantler, who arranged for the funding through the Special Trustees of Guy’s hospital that enabled me to undertake this work, and who provided me with the initial idea of studying low molecular weight protein excretion in children.

A portion of the chapter involving children with diabetes is also included in the MD thesis of Dr Gibb, submitted to the University of London.
I dedicate this thesis to my wife Kate, who has constantly encouraged me to continue with this project, and to my children Christopher, Hamish and Sarah who have displayed patience and understanding beyond their years.