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LEPTOSPIROSIS: PATHOGENESIS AND RED CELL DESTRUCTION

A thesis presented in partial fulfilment of the requirements for the Degree of Doctor of Philosophy at Massey University Palmerston North New Zealand

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1983
ABSTRACT

A study was made of the morphological changes in red blood cells (RBC's) from hamsters and calves during the development of haemoglobinaemia following infection with Leptospira interrogans serovars ballum and pomona respectively.

The major changes seen by scanning electron microscopy of RBC's from the haemoglobinaemic animals were spherocytosis and surface pitting. The major change seen by transmission electron microscopy was vacuolation of abnormally shaped RBC's with some vacuoles containing a small amount of a fine granular material. Few RBC's showed evidence of haemoglobin loss even though the animals from which the RBC's came were severely haemoglobinaemic. Those RBC's which did show haemoglobin loss contained membrane-bound dense granular inclusions in addition to the vacuoles observed in the fully haemoglobinated RBC's. The spherocytes from the haemoglobinaemic animals probably arose from echinocytes which were seen in prehaemoglobinaemic hamsters. Echinocytes seen in calves injected with 'toxin' can probably be considered as equivalent to echinocytes seen in the prehaemoglobinaemic hamsters. These echinocytes had membrane-bound portions of cytoplasm segregated from the remainder of the cytoplasm. It is thought that these portions of cytoplasm are defective and subsequently become digested in autophagocytic vacuoles with complete digestion resulting in the empty vacuoles or those containing a small amount of fine granular material as seen in the fully haemoglobinated RBC's. Inability of the cell to either fully digest or expel material within autophagocytic vacuoles may explain dense granular inclusions seen within partially haemoglobinated RBC's which are considered the most severely affected RBC's.

Present studies support other work that a 'toxin' elaborated by the organisms rather than mechanical damage is responsible for the
lesions observed. The original lesion is thought to be biochemical although biochemical studies were beyond the scope of the present work. This biochemical lesion is likely to be similar in all affected tissues. Sufficient biochemical and physiological differences exist between adults and neonates and between individuals of similar age of the same species, and between different animal species to explain the differences in susceptibility of RBC's to leptospiiral 'toxins'.

RBC's from cattle, hamsters and humans suspended in non-immune plasma and incubated with ballum and pmona were never haemolysed while those suspended in saline were always haemolysed. Normal plasma thus has a protective effect. The protective action of plasma demonstrated in vitro required reconciliation with some conflicting findings of parallel studies in vivo in which RBC's were destroyed resulting in haemoglobinemia. It therefore appears that another mechanism may be responsible for RBC destruction in vivo. Because RBC sequestration resulting in lowering of the PCV and haemoglobin occurred in the prehaemoglobinemia animals, involvement of the reticulomacrophage system appeared likely. Other workers have suggested that RBC's which already have an abnormality may be further damaged or lysed within the splenic circulation. Thus in leptospiiral infections, leptospiiral 'toxins' may induce changes in RBC's leading to their sequestration within the spleen resulting in further damage and ultimately lysis and haemoglobinemia.

The ground is now set for further studies to identify the putative biochemical lesions which would pave the way for development of new therapeutic regimes to prevent the more severe clinical features of the disease.
The opportunity to work for this thesis was provided by the Department of Veterinary Pathology and Public Health and made possible by the support of the Phyllis Irene Grey Fellowship.

I would like to thank my initial chief supervisor Dr R.H. Sutton for his assistance, interest and encouragement in the early stages of this research. I would also like to thank his successor, Dr A.C. Johnstone for his assistance in later experiments and for reading the drafts of this thesis. Professor B.W. Manktelow gave valuable advice and encouragement in completing the final copy of this thesis. In addition I would like to express my gratitude to Dr R.B. Marshall who was always willing to discuss experiments and results with me and provide help and encouragement.

Funds and facilities for the research were provided by the Department of Veterinary Pathology and Public Health, Veterinary Research Fund and Glaxo Laboratories (NZ) Ltd. The electron microscopy was carried out with the help and advice of members of the Electron Microscopy Unit, DSIR, Palmerston North and in particular, Mr D. Hopcroft. The Radiotherapy Department of the Palmerston North Public Hospital and Mr Trott provided facilities for the irradiation of hamsters. I would also like to thank the staff of the Clinical Pathology Department of the Palmerston North Hospital for the use of their autoanalyser and Mr B. Riddler of the Department of Agricultural Economics and the staff of the No 4 Dairy Unit for providing neonatal calves and some older cattle for blood sampling.

The hamsters were provided by the National Health Institute and maintained by Dr L.M. Schollum. In addition Dr Schollum provided the cultures of leptospires used in these experiments and assisted with the microscopic agglutination titres. Histological slides were prepared by Mrs P.M. Slack and Miss S.M. Malloch and culture media were prepared by Mrs J.L. Schramer. Preparation of photographs was done with the assistance of Mr T. Law.
I also wish to thank the staff of the Massey University Library for their prompt and efficient assistance in obtaining references. Mrs F.S. Wicherts typed this thesis and I would like to thank her for her excellent advice on thesis preparation.

Finally I would like to thank my husband, Ghee Yong, for his support and understanding over the years of working for this thesis.
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