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INVESTIGATIONS IN THE AETIOLOGY AND PATHOPHYSIOLOGY OF SAGO HAEMOLYTIC DISEASE

Thesis submitted by
Numaya RAI (Gurung) BSc (MLS), TU, IOM (NEPAL)
in
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in partial fulfilment of the requirements
for the degree of Master of Biomedical Sciences (Medical Lab Science)
at the School of Veterinary & Biomedical Sciences
James Cook University, Townsville, Australia

STATEMENT OF SOURCES

DECLARATION

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references given.

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ABSTRACT

Sago haemolytic disease (SHD) is a suspected mycotoxocosis that causes acute and sometime fatal intravascular haemolysis and has only been described in Papua New Guinea (Taufa, 1974; Donovan *et al.*, 1977). Although much is yet to be revealed about this condition, the main risk factor is the consumption of stale sago starch, a food stuff which is the staple carbohydrate for rural Papua New Guineans. It is thought that fungi that colonise the sago are responsible for the haemolytic compound that cleaves red cell membrane proteins. The condition often manifests in family clusters and a genetic predisposition has been postulated. Melanesian Ovalocytosis (MO), also known as South East Asian Ovalocytosis (SAO), is a autosomal recessive genetic disorder expressing a red cell membrane band 3 deletion in individuals who have inherited the gene responsible. The condition is subclinical but common in rural PNG where it is thought to offer some malaria protection (Bruce *et al.*, 2000). It is reasonable to assume that as this condition is widespread in rural PNG where SHD is endemic, it may a confounding predisposing factor to the manifestation of the acute haemoltyic crisis.

A number of organisms implicated in SHD have been isolated and crude haemolytic compounds were isolated as per methods of Bernheimer (Bernheimer, 1988). Red cell membrane ghosts where prepared from individuals with and without the MO band 3 deletion and exposed to the toxins. Proteins were separated using Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and the activity of the toxin was demonstrated by the cleaving of band known to be present through comparison with untreated controls. Although cleavage of bands was demonstrated by various toxin compounds no discernable difference could be identified between the two groups. This may suggest that MO is not involved as a host factor which predisposes SHD, although further studies are required to elucidate this finding.

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ABBREVIATIONS

B. cereus	*Bacillus cereus*
BA	blood agar
C. perfringens	*Clostridium perfringens*
C.albicans	*Candida albicans*
CPA	carrot potato agar
DDT	diothiothreitol
DLF	direct lytic factor
E. coli	*Escherichia coli*
EDTA	Ethylene diamine tetra acetic acid
gm	gram
g	gravity
G6PD	glucose 6 phosphate dehydrogenase
GSH	glutathione
H. influenzae	*Haemophilus influenzae*
H_2O_2	hydrogen peroxide
Hb	haemoglobin
HIV	human immuno deficiency virus
HMP	hexose mono phosphate shunt
hr	hour
HUS	haemolytic uremic syndrome
IV	intravenous
K^+	potassium
kDa	kilo dalton
l	litre
L. monocytogenes	*Listeria monocytogenes*
M	molar
min	minute
ml	milli litre
mM	milli molar
MO	Melanesian ovalocytosis
µl	micro litre
µm	micro metre
Na^+	sodium
NADPH	nicotinamide adenine dinucleotide phosphate
nm	nano metre
^oC	degree Celcius
OMPLA	outer membrane phospholipase A
P. steckii	*Penicillium steckii*
P. citrinin	*Penicillium citrinin*
P. sizovae	*Penicillium sizovae*
P.aeruginosa	*Pseudomonas aeruginosa*
PAT	platelets activating factor
PBS	phosphate buffered saline
PC	phosphotidylcholine
PCR	polymerase chain reaction

PE	phosphatidylethanolamine
PG	phosphotidylglycerine
PI	phosphotidylinositol
PNG	Papua New Guinea
PS	phosphatidylserine
RBC	red blood cell
RBCM	red blood cell membrane
rpm	revolution per minute
S. aureus	*Staphylococcus aureus*
S. typhimurium	*Salmonella typhimurium*
S. pneumoniae	*Streptococcus pneumoniae*
SC	subcutaneous
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
spp	species
TDS	toxin diluting solution
Y. enterocolitica	*Yersenia enterocolitica*