

---

MACROGLUBULINS IN NORMAL

PIG SERUM

---



MACROGLOBULINS IN NORMAL

PIG SERUM

A thesis submitted to the University of Adelaide for the degree of Master of Science.

by

WARWICK SOUTER, B.Sc., A.U.A.

\* \* \*

Department of Microbiology,

University of Adelaide.

\* \* \*

TABLE OF CONTENTS

	Page
CHAPTER I. INTRODUCTION AND REVIEW OF LITERATURE	1
CHAPTER II. MATERIALS AND METHODS	27
Collection of pig serum .....	27
Gel Filtration Chromatography .....	27
Immuno - electrophoresis .....	29
Ion Exchange Chromatography .....	30
Density gradient ultracentrifugation ....	31
Preparative Zonal gel electrophoresis ...	32
Quantitative assay of Protein content ...	33
Production of antisera .....	33
Iodination of Pig Macroglobulin .....	34
CHAPTER III. ISOLATION OF IMMUNOGLOBULINS AND PREPARATION OF THEIR HEAVY CHAINS .....	35
Preparation of normal Pig gamma globulin (IgG) .....	35
Preparation of Pig Macroglobulin (IgM) ..	37
Preparation of chains by reduction of Pig Gamma globulin .....	40
Reduction of IgM to its polypeptide chains .....	42
Preparation of high molecular weight (29 S) component of normal pig serum ....	43

	Page
CHAPTER IV. STRUCTURAL STUDIES ON THE 29 S MACROGLOBULIN .....	49
Introduction .....	49
Studies on the effects of mercapto- ethanol on the 29 S component .....	49
The Effect of Potassium Borohydride on the 29 S component of partially purified normal pig serum .....	51
Antigenic Relationships between the 29 S component of Normal Pig Serum and other proteins present in Normal Pig Serum .....	54
CHAPTER V. DISCUSSION .....	58
BIBLIOGRAPHY .....	71
ADDENDUM .....	83
BIBLIOGRAPHY (ADDENDUM) .....	96

This thesis contains no material which has been accepted for the award of any other degree or diploma in any University and to the best of my knowledge and belief it contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

(W. SOUTER)

ABSTRACT

The sera of most mammals contain, in addition to the by now well established 19 S macroglobulins, IgM and  $\alpha_2$ M, globulins of even higher sedimentation coefficient such as 22 S, 29 S, 34 S and 42 S. These components have been reported in particular in the serum of patients with Waldenstrom's macroglobulinemia, a pathological condition associated with an elevation in concentration of serum macroglobulins. The exact chemical composition of these macroglobulins with sedimentation greater than 19 S remains obscure. Pig serum contains relatively high concentrations of a 29 S macroglobulin and therefore provides suitable material for an examination of the structural relationships between this component and other proteins present in porcine serum. The 29 S macroglobulin has been shown to be an immunoglobulin according to the following criteria: 1. It is a protein. 2. It has a  $\beta$  mobility by electrophoretic analysis. 3. It is labile to reduction with sulfhydryl reagents (e.g. 2 mercapto ethanol); and 4. It shares antigenic similarity with IgG globulin. The latter property is the strongest evidence that the 29 S macroglobulin is an immunoglobulin, particularly as the antigenic similarity appears to lie in the light polypeptide chains of both molecules.

A unique feature of the 29 S macroglobulin is its

lability towards reduction with potassium borohydride under controlled experimental conditions when the molecule dissociates into 7 S subunits. Under identical conditions of treatment 19 S macroglobulins (IgM) do not dissociate as has been clearly established by the use of radio-labelled preparations of IgM. However, both the 29 S and IgM macroglobulin dissociate into 7 S subunits upon reduction with 2-mercapto ethanol.

Homogeneous preparations of porcine IgG and IgM have been isolated as have the light and heavy chains from both of these globulins. Antisera have been prepared in rabbits directed against porcine IgG and specifically against the  $\gamma$  and  $\mu$  chains of these preparations.

Methods have been developed for the isolation of limited quantities of the 29 S macroglobulin but the difficulties encountered have prohibited its preparation in sufficient amounts to allow for a comprehensive study of the physical and chemical characteristics of the material. However, immunoelectrophoretic studies have shown that 29 S macroglobulin cross reacts with rabbit anti-porcine IgG but does not precipitate with rabbit anti-porcine  $\gamma$  or  $\mu$  chains. In similar manner a mixture of 29 S + 19 S macroglobulins develops only one precipitin line on immunoelectrophoresis

against rabbit antisera to porcine  $\mu$  chains. These results together with those of potassium borohydride reduction, clearly indicate that the 29 S macroglobulin is a unique entity and is not a polymeric form of either IgG or IgM immunoglobulins.

Preliminary evidence is provided to suggest that the 29 S macroglobulin may be endowed with antibody activity and that it appears early in the immune response of rabbits following injection with T4 bacteriophage and of mice with Salmonella typhimurium M206.