

THE RELATIONSHIP OF SERUM IMMUNOGLOBULIN LEVELS WITH AGE,
SERUM TOTAL PROTEIN AND LIPEMIA IN THE CANINE NEONATE

by

ANNE L. RAY

D.V.M., Auburn University, 1978

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

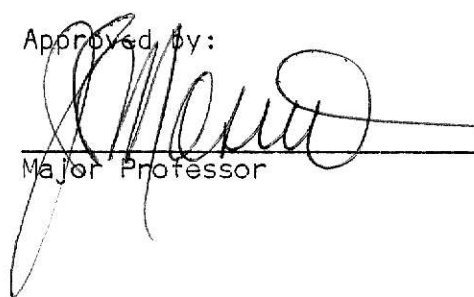
MASTER OF SCIENCE

Department of Surgery & Medicine

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1981

Approved by:


Major Professor

SPEC
COLL
LD
2668
T4
1981
R39
C.2

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	iii
LIST OF TABLES AND FIGURES	iv
INTRODUCTION	1
LITERATURE REVIEW	2
Origins of the immune system	2
Acquisition of humoral immunity	5
Immune competence in the fetus and neonate	5
Passive transfer of immunity	6
Closure of the gut	14
Neonatal passive immunity and susceptibility to disease	16
MATERIALS AND METHODS	18
Research animals	18
Methods	19
RESULTS	25
DISCUSSION	43
CONCLUSIONS	48
BIBLIOGRAPHY	49

ACKNOWLEDGEMENTS

I would like to give my sincerest thanks to Dr. Jacob E. Mosier, my major professor, and the other members of my committee, Dr. Neil V. Anderson, Dr. William E. Moore and Dr. Embert H. Coles, all of whom never failed in giving me their time, ideas and valuable guidance.

I am most appreciative of the help given by Dr. George Milliken, Department of Statistics, in the evaluation of my data.

A special thanks must go to Dr. Alain Voisin, Becky Brownson, Aggie Moll and Cindy Novy for their technical advice and assistance; and to Mallory Rooks and Linda Johnson for their artistic help.

Lastly, I want to thank Jean Becker for a job well done in the typing of this paper.

**THIS BOOK
CONTAINS
NUMEROUS PAGES
WITH THE ORIGINAL
PRINTING BEING
SKEWED
DIFFERENTLY FROM
THE TOP OF THE
PAGE TO THE
BOTTOM.**

**THIS IS AS RECEIVED
FROM THE
CUSTOMER.**

LIST OF TABLES AND FIGURES

Tables		Page
1	Distribution of pups per litter	28
2	Deaths that occurred and their causes	28
3	Comparison of IgG levels of pups that died to levels in pups from the same litter	29
4	Comparison of IgM levels of pups that died to levels in pups from the same litter	29
5	Comparison of IgA levels of pups that died to levels in pups from the same litter	30
6	Range of immunoglobulin values of pups at end of 16 week study and range of immunoglobulin values for adult dogs as reported in the literature	30
Figures		
1	Growth graph for litter 1	31
2	Growth graph for litter 2	32
3	Growth graph for litter 3	33
4	Growth graph for litter 4	34
5	The mean value and absolute range of serum IgG and their relationship to age	35
6	The mean value and absolute range of serum IgA and their relationship to age	36
7	The mean value and absolute range of serum IgM and their relationship to age	37
8	The mean value and absolute range of serum total protein and their relationship to age	38
9	Serum total protein and its relationship to IgG	39
10	Serum total protein and its relationship to IgM	40
11	Degrees of hemolysis and their relationship to serum total protein	41
12	Degrees of lipemia and their relationship to serum total protein	42

INTRODUCTION

The study of the immune system and how it relates to health and disease has become a very important area of study in veterinary medicine. Much of the information available concerning the interrelationship between passive immunity and diseases of neonatal animals is concerned with the bovine, equine and porcine species. Death losses due to inadequate passive immunity or insufficient immune competence of the individual in these species can result in great economic loss to the breeder. Information concerning the level of passively received immunity and early immune competence in the canine is limited.

The purpose of this investigation was to gain information concerning serum immunoglobulin levels and their relationship to age in the puppy. The relationship of serum immunoglobulin levels to other factors such as serum total protein and the error in measurement caused by lipemia was also studied. The relationship of serum total protein and serum hemolysis was investigated.

Carnivores possess an endotheliochorial placenta whereby the canine fetus receives some degree of passive immunity before birth. Serum immunoglobulin levels of pups at birth consist of mainly IgG. The transmission of passive immunity by way of colostrum to the canine neonate is much greater than that transmitted across the placenta. In pups maximal absorption of colostrum immunoglobulins occurs approximately 8 hours after birth. No appreciable absorption occurs 24 hours after birth.

The developing canine fetus has been shown to have some level of immune competence. The fetus is able to respond to certain antigens only at a fixed stage of gestation. It has been shown that neonates are able to respond to certain antigens within 2 hours after birth.

REVIEW OF LITERATURE

ORIGINS OF THE IMMUNE SYSTEM

The normal animal when exposed to a foreign antigen responds with the production of immunoglobulins and specific effector cells. Immunoglobulin production is characteristic of the humoral immune system while specific effector cell production is characteristic of the cell mediated immune system.⁴³

Mounting an immune response is a function of lymphocytes. Lymphoid stem cells initially arise from the yolk sac in the young fetus and later from the fetal liver. In the near term fetus and adult, the lymphoid stem cells arise from the bone marrow. These lymphoid stem cells are carried in the blood to central lymphoid organs, which are sites of production and differentiation of lymphocytes. The primary lymphoid organs are the thymus, found in mammals and birds, and the bursa of Fabricius, found only in birds. It has been suggested that the bone marrow and/or gut associated lymphoid tissue (GALT) have taken over the functions of the bursa in mammals.⁴³

Lymphocytes released by the thymus are called thymus-dependent lymphocytes or T-cells. T-cells make up most of the circulating lymphocytes although some find their way to secondary lymphoid organs. T-cells mediate the cell mediated immune response.⁴³

Lymphocytes released by the bursa of Fabricius or bone marrow are called bursa-dependent lymphocytes or B-cells. B-cells also seed the secondary lymphoid organs and are responsible for the humoral immune response.⁴³

Immunoglobulins, or antibodies, are proteins produced by plasma cells in response to interaction between antigen-sensitive B-cells and specific antigen. Immunoglobulins are capable of specifically binding to antigen resulting in the enhanced clearance of antigen from the body.⁴³

Following initial exposure to an antigen there is a lag period of several days where there is no detectable antibody response. After approximately 1 week, antibody levels start appearing and reach a peak at 10-14 days. This is known as the primary immune response. IgM is the first immunoglobulin class seen. IgG follows and reaches higher levels than IgM. Following the peak period these immunoglobulin levels start to decline rapidly.⁴³

After the second exposure to the antigen there is a shorter lag period before a higher and more pronounced antibody response. This is called the secondary immune response. Again IgM is seen first followed quickly by a much greater IgG response.⁴³

The structure of the IgG molecule has been extensively investigated and serves as a model for other immunoglobulins. The IgG molecule can be split into 3 equal sized fragments by papain. Two of these fragments correspond to the "arms" of the Y-shaped molecule and possess the ability to bind antigen. These are termed the Fab fragments. The third fragment corresponds to the "tail" of the Y-shaped molecule and cannot bind antigens. This portion of the molecule is called the Fc fragment.⁴³

The regions on the immunoglobulin molecule responsible for antigen binding are called variable regions and are found on the N-terminal portion of the Fab fragment. Other regions on the immunoglobulin molecule responsible for such functions as: initiating the complement cascade, binding of immune complexes to phagocytic cells, control of the fractional catabolic

rate of the immunoglobulin molecule, placental transfer, and antibody-mediated cell mediated cytotoxicity are termed constant regions and are found on the C-terminal half of the Fab fragment and throughout the Fc fragment. Constant regions are made up of subunits called C_L , C_{H1} , C_{H2} , C_{H3} . IgM and IgE possess an additional subunit called C_{H4} .⁴³

Immunoglobulins are classified according to their solubility in strong salt solutions, their electrostatic charge, their molecular weight, and their molecular structure. There are four major immunoglobulin classes found in domestic animals: IgG, IgM, IgA and IgE. In addition, man has an immunoglobulin class, IgD.⁴³

Immunoglobulin classes are further divided into subclasses based on antigenicity, electrophoretic mobility and biologic activity. Subclasses vary in functional characteristics among the domestic species.⁴³

ACQUISITION OF HUMORAL IMMUNITY

Humoral immunity is acquired in 2 ways: passively and actively. Passive immunity includes maternal immunoglobulins passed to the offspring by way of placental transfer and colostrum and milk secretions. Passive immunity may also be acquired through the administration of antiserum. Active immunity is acquired when the host mounts an immune response after coming in contact with an antigen it recognizes as foreign. Although the neonate acquires the majority of its humoral immunity from the passive transfer of maternal immunoglobulin, it has been shown that the fetus and newborn are capable of mounting an active humoral response.

Immune Competence in the Fetus and Neonate

There is an apparent inability of the developing fetus to respond to certain antigens until a fixed and critical stage of gestation is reached. This critical age of development appears to be different for each antigen in the given species. Prior to this age, the fetus fails to recognize the substance as foreign and does not respond to it.³⁹ One reason for the differing immunologic responses to different antigens may be attributed to the fact that the fetuses may have received passive immunity by way of the placenta against some of the antigens tested.

Canine fetuses from the 40th day of gestation to adults produced antibody after stimulation with bacteriophage ØX-174 and responded promptly to challenge. Serum antibody activity increased in proportion to the age of the dog at the time of stimulation.¹⁹

Slight reactivity to sheep RBC's (SRBC) developed in the canine fetus at around the 48th day of gestation.¹⁹

A higher dose of bovine gamma globulin (BGG) was required to elicit an immune response in the newborn pup than the dose required by adult dogs.²⁴ None of the neonates responded significantly to a primary or challenge dose of bovine serum albumin (BSA) although adult dogs produced antibody after the first inoculation and had good responses after challenge. The neonates did, however, respond after a single dose of BSA-adjuvant emulsion.¹⁹

In the adult dog, the primary immune response to SRBC and BGG was characterized by the production of equal amounts of IgG and IgM. The secondary immune response consisted mainly of IgG.²¹ In the newborn pup, the primary immune response to SRBC and BGG was characterized by the production of IgM, whereas the secondary immune response yielded both IgM and IgG, with IgG making up the major portion of the immunoglobulins produced. Although the newborn pup is immunoresponsive at birth, full immunologic maturity is not achieved until the 2nd or 3rd week of life where the primary and secondary immune responses match those seen in adult dogs.²⁴

Passive Transfer of Immunity

In animals there are 3 routes of passive transfer of immunity from mother to offspring: 1) absorption of immunoglobulins through the endodermal cells of the yolk sac, 2) absorption of maternal immunoglobulins through the placenta, and 3) postnatal absorption through the intestine.^{20,49}

Prenatal Transfer. Absorption through the yolk sac occurs in rabbits, birds and to some extent in rodents. IgG is the predominant immunoglobulin transferred. It has been shown that there are specific receptor sites on the yolk sac endothelium for IgG. These receptor sites recognize the Fc region, more specifically the C_H2 domain of the heavy chain on the IgG molecule.^{44,49}

Placental transfer of maternal immunity is the major route of immunoglobulin transfer in primates and occurs during the last 1/2 to 2/3 of gestation.²⁰ This route of transfer occurs to a much lesser degree in carnivores, such as dogs and cats.^{44,49}

Man and monkey possess a hemochorial placenta where the maternal blood bathes the trophoblast directly.³⁶ In the newborn infant, serum IgG levels are variable sometimes reaching a level twice that found in the mother. IgA and IgM levels in the newborn infant are always extremely low.⁴⁸ There appear to be placental receptors for only one class of immunoglobulins in the human: IgG. These receptor sites have a varying affinity for the IgG subclasses. The affinity for the subclass IgG₁ is greatest.²⁸ Binding to the placental Fc receptor takes place through interaction of the C_H2 and C_H3 regions on the IgG molecule.^{28,47}

In 1970, Brambell⁴ proposed his hypothesis on how proteins, including immunoglobulins, are transported across the human placenta to the fetus. He stated that proteins are thought to enter the endodermal cell of the placenta non-selectively by endocytosis. Within the vesicles thus formed, proteins which are ultimately transported from the cell attach themselves to receptors lining the vesicles. These receptors may be present on the surface of endodermal cell microvilli before the vesicles are formed. It was thought that such receptors provide protection from digestion by proteolytic enzymes when fusion of vesicles with lysosomes occurs and if the receptors are saturated, any free protein is broken down. The amount of IgG transmitted is dependent upon how well the attachment site on the Fc region fits the receptor. After movement of the phagolysosome through the cell, the attached proteins leave the receptor when the vesicle

contents are discharged from the cell into the intercellular space. In 1974, Gitlin and Gitlin¹³ supported Brambell's hypothesis and stated that the rate of immunoglobulin transport is dependent upon the degree of binding to specific receptors.

The absorptive cells of the yolk sac and intestine are morphologically similar to those of the placenta in that they have microvilli on their surfaces and pinocytosis can be demonstrated on the villi.⁴⁹

Carnivores possess an endotheliochorial placenta. In this type of placenta the fetal chorion is in contact with the maternal capillary endothelium.^{4,36,43} There is transmission of passive immunity before birth by way of the placenta but the transmission after birth by way of the colostrum is greater.^{4,43} Precolostral immunoglobulin content in serum of puppies and kittens consists mainly of IgG.^{14,15}

Ungulates have an epitheliochorial placenta where the chorion is separated from the maternal blood by 3 maternal tissues: the maternal capillary endothelium, connective tissue and the uterine surface epithelium.³⁶ Virtually no placental transfer of maternal immunoglobulins occurs in these species, therefore, passive immunity is acquired through the ingestion of colostrum and milk.

In all species except ungulates, materno-fetal and materno-neonatal transfer of IgG is an Fc region-governed specific process. In ungulates, the uptake of immunoglobulins through the intestinal wall is a non-specific process.⁴⁴ IgG from species with prenatal transport will bind to the Fc receptor of human placental tissue. This is not so of IgG from animals with no prenatal transfer.⁴⁷

Postnatal Transfer. The transfer of IgG from maternal circulation to the colostrum is a peculiar feature of early lactation in those species

which do not transfer IgG to the fetus through the placenta. Species which are able to transfer IgG to the fetus through the placenta have colostrum containing mostly locally produced secretory IgA.²⁵

Although very little absorption of immunoglobulins occurs from the gut of the human infant, colostrum and milk play an important role in protecting the newborn from certain disease conditions. Human mammary secretions contain, along with immunoglobulins, T and B lymphocytes, macrophages and neutrophils that possess phagocytic activity, lysozyme, lactoferrin and certain resistance factors all of which have antimicrobial properties.^{15,30} These constituents of mammary secretion help to prevent infection in the maternal mammary gland as well as in the infant's gastrointestinal tract.¹⁵

Human mammary secretions contain immunoglobulins of all classes in appreciable amounts although secretory IgA is the predominant type.^{15,30} It has been shown that small amounts of colostrum immunoglobulins are absorbed from the neonatal intestine during the first 18-24 hours after birth, however, the precise mechanism of intestinal absorption is not known.^{18,30}

Secretory IgA found in mammary secretions is more resistant to pH changes and proteolytic enzyme digestion than are the other immunoglobulin classes, including serum IgA, found in colostrum and milk. Therefore, it is thought that secretory IgA plays an important role in conferring resistance to infection to the infant's gastrointestinal tract.^{15,41} With the decreased incidence of breast feeding, some have reported an increased frequency of colonization and infection caused by gram negative bacteria in the infant's gastrointestinal tract. A rapid improvement of diarrhea caused by Escherichia coli was observed after the feeding of breast milk.^{5,15,41} As well as protecting the infant from the hazards of microbial infections, IgA may limit the absorption of dietary antigens, thereby reducing the risk of allergic reactions mediated immunologically.⁵

Rodents, carnivores and ungulates primarily depend on post-natal gut absorption of colostral and milk immunoglobulins for their acquisition of passive immunity.

In rodents, such as mice and rats, postnatal absorption of immunoglobulins occurs in the proximal small intestine^{14,21,29,38} for about 2-3 weeks.^{2,21,29} During this time, IgG and IgA from the maternal milk bind selectively to specific separate receptors on the villi. Receptor-bound IgG is transferred through the enterocytes into the blood stream. Receptor-bound IgA is not absorbed but remains on the cell surfaces.²⁹ Binding occurs on the apical surfaces of the villi and is virtually absent in the cryptal regions.² Binding is specific for the Fc region of the immunoglobulin.^{14,32} The mechanism of transport of IgG from the gut epithelium to the circulation is presumed to be the same as that theorized for the transport of immunoglobulins across the placenta.⁴ Since serum IgA and IgM levels are present in the neonatal rat, it is likely that the small amounts of IgA and IgM transported represent a background level of non-specific absorption not involving the immunoglobulin receptor system.¹⁴

In the distal half of the small intestine, during the first 3 weeks, IgA and IgG are non-specifically absorbed into larger vesicles. Unlike the smaller vesicles found in the proximal small intestine, the function of these large vesicles is to digest the endocytosed immunoglobulins.^{29,38}

Precolostral piglet serum is deficient of immunoglobulins, however, traces of serum protein antigenically related to IgG, have been shown.^{9,32}

In the sow, colostral immunoglobulin levels exceed those found in maternal serum. Sow serum IgG levels decrease 10-24 days prepartum, are minimal at parturition, and increase postpartum.¹⁰

All colostral IgG and a high proportion of IgM are transported from maternal serum. Forty per cent of colostral IgA is from the serum. The majority of colostral IgA is in the form of secretory IgA (sIgA) produced locally in the mammary gland. sIgA is characterized by a secretory component attached to the immunoglobulin molecule as it is produced in the secretory gland.^{3,37}

IgG is the predominant colostral immunoglobulin. As colostrum turns to milk, IgA becomes the predominant immunoglobulin. IgM makes up a very small percentage of the colostral immunoglobulins and increases slightly in milk.⁹ In milk, 90% of IgA and IgM and 70% of IgG is locally produced.³

At 24 hours of age, IgG makes up the majority of piglet serum immunoglobulins. IgG then begins to fall slowly reaching a minimum at approximately 38 days. Evidence has been presented indicating that there is selective intestinal absorption of IgA from colostrum and that intestinal absorption in the neonatal piglet seems to differentiate against sIgA.³³ After the first 24 hours, serum IgA concentrations in the piglet serum begin to fall rapidly reaching a minimum in about 20 days. The same phenomenon is seen with IgM with this immunoglobulin reaching a minimum level at approximately 11 days. The decrease in immunoglobulin levels in serum at such an early age is a function of protein catabolism and dilution due to increased body size and therefore increased blood volume.⁹ After the piglet serum immunoglobulins bottom out, they begin to slowly rise due to acquired immunoglobulin production.

Calves receive no precolostral immunoglobulins from the cow. At parturition colostral levels of IgG₁, IgA and IgM are higher than those levels found in the maternal serum indicating a selective transfer. However, as colostrum turns to milk, IgG₁ and IgM levels are below those found in serum. Milk IgA has been found to occur in both greater or lesser concentrations than serum IgA.^{6,34}

It has been shown that IgG₁ in the cow is selectively transported from the maternal serum into lacteal secretions before and after parturition.^{6,8,10,25} Maximum transfer of IgG₁ occurs 1-3 days before calving¹⁰ and is the most predominant immunoglobulin of bovine colostrum.³⁴ The control of such transport may be regulated by the estrogen and progesterone levels of the cow.^{8,40}

While immunoglobulin levels are high in colostrum, they start falling rapidly within 24 hours so that only low levels of immunoglobulins are assayed on the 3rd day.³⁴ After calving, the selective transfer of serum proteins into the mammary gland decreases while the local synthesis of milk proteins increases.¹⁰ The mammary gland of the cow shows mainly IgG producing cells although IgA and IgM producing cells are present as well.^{7,8,50} IgG₁ remains to be the most predominant milk immunoglobulin. At no time does IgA play a dominant secretory role in bovine milk although the levels in early lactation may exceed the levels found in maternal serum suggesting a selective secretory process.³⁴ As compared to other species, bovine milk contains low levels of immunoglobulins.^{7,34,35}

There seems to be a lack of selectivity in the calf's intestinal absorption process. Immunoglobulin profiles of calf serum resemble very closely the immunoglobulin profiles of bovine colostrum.³⁴ Uptake of colostrum protein occurs primarily in the ileum of the calf.⁹

Foals are born with a virtual absence of circulating immunoglobulins. Mare's serum and pre-suckle colostrum has quantitatively and qualitatively similar immunoglobulin composition.²⁶ After suckling there is a marked decrease in total colostrum immunoglobulin values.²⁰ IgG₂ is the predominant immunoglobulin.^{20,26,27}

In the foal, uptake of colostrum protein appears to take place throughout the small intestine.²⁰ Absorption seems to be maximum during the first 24 hours after birth and then declines rapidly until there is no demonstrable absorption at 5 days of age.²⁶ Immunoglobulin absorption is less selective than that of the rat, however, IgG seems to be absorbed in disproportionately greater amounts than are other immunoglobulin classes.^{20,26}

Both total serum immunoglobulin concentrations and specific antibody titers found in foals approximate those found in mare's serum within 24 hours after the foal is born and permitted to suckle.²⁶ Foal serum immunoglobulins begin to decrease by 16 days of age reaching a minimum value at 1-2 months of age.^{20,21,24,25,26} At this time, immunoglobulin synthesis is just beginning. In foals that receive little passive immunity, IgG, IgA, and IgG(T) production begins at 16-24 days of age. It appears that IgM production in these foals begins much earlier since serum IgM levels continued to climb after 3 days of age when the first serum sample was tested.^{24,37}

Several days before whelping, the bitch has a sharp decrease in serum IgG with a concurrent decrease in total serum immunoglobulin concentrations.¹⁶ This trend follows that seen in cows and sows which have selective transfer of immunoglobulins from maternal plasma to colostrum prior to parturition.

Canine colostrum is rich in IgG and IgA with IgG being the predominant immunoglobulin. Low levels of IgM and IgE are also present.⁴³ Colostrum IgG is slightly higher in concentration than that found in the bitch's serum. Colostrum IgA is significantly higher than that of dam's serum. Colostrum IgM is lower than that of dam's serum.^{37,46}

As canine colostrum changes to milk, the concentration of all immunoglobulin classes decreases, with the exception of IgM. IgA becomes the predominant immunoglobulin of milk. Milk IgA values are higher than maternal serum levels throughout lactation. IgG levels are initially higher but with time drop to below maternal serum values. IgM in milk remains relatively constant and is always less than maternal serum levels.^{37,46}

The majority of milk IgA is produced in the mammary gland as is milk IgM. These two immunoglobulins, as they appear in colostrum and milk, are designated sIgA and sIgM.^{42,46}

In pups, maximum absorption of immunoglobulins occurs when they are ingested approximately 8 hours after birth. Absorption is complete 15 hours after feeding. No detectable immunoglobulin absorption occurs 24 hours after birth.¹¹

Closure of the Gut

Closure of the gut to absorption of immunoglobulins is not clearly understood especially since closure occurs at different times in different species. Closure doesn't seem to be due to the cessation of pinocytosis by the intestinal epithelial cell, but seems to be either a failure of intracellular processing or a failure of release from the cell.²⁰

Many factors seem to be able to influence closure of the neonatal gut. There is good evidence that certain endocrine secretions can change the permeability of the small intestinal cells after birth.⁵ Premature closure occurred in rats after giving certain doses of exogenous corticosteroids.^{27,31} Pups from bitches that were treated prepartum with ACTH or hydrocortisone had significantly lesser immunoglobulin absorption than pups from untreated

bitches, suggesting that steroids may influence immunoglobulin absorption in pups.¹¹ It may be that there is a change of adrenal output in neonates that induces closure.

There seem to be factors present in milk that can induce closure. These factors are heat stable, non-protein and non-fat components of low molecular weight.^{20,23}

In piglets, but not in pups or calves, starvation can postpone closure for several hours. Consumption of food may be necessary before closure can occur.^{27,31}

NEONATAL PASSIVE IMMUNITY AND SUSCEPTIBILITY TO DISEASE

An individual's immune system is the mechanism whereby one fights off the threat of disease caused by foreign agents. The acquisition of passive immunity is important for protection of the newborn until the newborn's own immune system is mature enough to take over the role of protection. For this reason the mechanisms concerning the acquisition of humoral immunity in the fetus and neonate should be understood. With failure of these mechanisms the neonate is susceptible to various diseases.

In the dog, high levels of antibody to the canine distemper virus in maternal plasma results in placental transfer of anti-distemper antibodies to the offspring.²² Gillespie et. al.¹² state that approximately 3 per cent of the dam's titer is transferred placentally to the fetus. Thus a dam with a high titer transfers more antibodies in utero. Pups receiving this immunity but not colostrum, become susceptible to challenge at approximately 2 weeks of age.

Although placentally derived antibodies play an important role in the canine neonate, colostrum and milk in various domestic species provide most, if not all, of the passively acquired humoral immunity in the newborn. The colostrum and milk of these animals contain antibacterial and antiviral immunoglobulins.^{1,15,17,26}

Pups and kittens deficient in maternal antibodies are susceptible to various viral diseases such as canine distemper, infectious canine hepatitis, herpes virus infection, and feline panleukopenia. The combined placental and colostrum transfer of anti-distemper antibodies to the newborn pup results in a neonatal serum titer that averages approximately 77 per cent of the dam's titer. At around 1½ weeks of age the pup's titer is reduced by 50 per cent. At 5-6 weeks of age approximately 50 per cent of the pups

are susceptible to challenge of the virulent virus (their titers have dropped to below 20-30). Pups cannot respond properly to a distemper vaccine until they have lost colostral protection and become susceptible to the disease.¹²

Piglets not receiving colostrum rich in anti-corona virus IgA are susceptible to TGE. Calves failing to receive or absorb colostrum are prone to certain bacterial and viral diarrheas with Escherichia coli being an important pathogen. Foals with reduced immunoglobulin levels in serum may develop bacterial septicemias.⁴⁵

MATERIALS AND METHODS

RESEARCH ANIMALS

Four pregnant beagle bitches were obtained from Theracon, Inc., Topeka, Kansas. The bitches were in the last 1-2 weeks of gestation at the time of purchase. They had been vaccinated for distemper, hepatitis, parainfluenza, leptospirosis and rabies several months prior to breeding.

The pregnant bitches were held in individual runs where they were observed through a closed circuit television monitor. As the bitch showed signs of impending parturition she was taken to a separate cage where whelping could be personally observed.

At birth each pup was identified and 2 ml of blood collected from a jugular vein. The pup was then returned to its dam where nursing was observed for the first 24 hours. Nursing time for each pup was recorded. At the end of the first 24 hours of life, 2 ml of blood were again drawn from each pup.

The bitch and her litter of pups were returned to the holding runs 24 hours after parturition where they were maintained for approximately 3 weeks. The bitches were fed a mixture of canned and dry food^{a,b} twice a day. The pups were allowed to nurse ad libitum.

At 3 weeks of age the pups, along with their dams, were moved to runs in an area where other research animals were kept. The pups nourishment consisted of bitches milk. The bitches were fed a dry food^c free choice and were given one multiple vitamin tablet^d each day.

^aKennel Ration meat flavored canned food. The Quaker Oats Co., Chicago, IL 60654.

^bWayne's Dry dog food^R. Allied Mills, Inc., Chicago, IL 60606.

^cM-260. Grain Science & Industry, Kansas State University.

^dVisorbites^R. Norden, Lincoln, NE 68501.

At four weeks of age the pups were introduced to a mixture of dry puppy food^e mixed with warm water and were allowed to eat this ad libitum. During this time the pups still were nursing from the bitch.

At 6 weeks of age the pups were weaned and divided into groups of 2 or 3 per run.

From 8 weeks of age until the end of the experiment the pups were maintained on the same dry puppy food. Each pup was weighed periodically and the weights recorded.

At 4 and 6 weeks of age each pup was given a dose of 2.5mg/lb. body wt. pyrantel pamoate for treatment of Ancylostoma and Toxacara species. Each bitch was treated at the same time as the pups.

Each pup was vaccinated at 8, 10 and 12 weeks of age for distemper, hepatitis and parainfluenza.^f Vaccination was considered necessary since new dogs coming into the research facility could be a source of infection for these diseases.

METHODS

Following the collection of blood samples at birth and at 24 hours of age, 2 ml of blood were drawn from each pup at weekly intervals throughout the experiment. The blood was collected from the jugular vein into individual 4 ml volume glass tubes. These blood samples were allowed to sit at room temperature for approximately 45-60 minutes to permit time for clot retraction to occur. The blood samples were then centrifuged at 500g for 5 minutes. The serum was removed using a Pasteur pipette. The amount of lipemia and/or hemolysis of each serum sample was recorded. Each serum

^ePurina Puppy Chow^R. Balston Purina Co., St. Louis, MO 63188.
^fNordens Vanguard DA₂^R. Norden, Lincoln, NE 68501.

sample was divided between 2 labeled 1.5 ml capacity crytotubes and frozen at -30°C until the immunoglobulin evaluation was done.

An immunoglobulin standard serum was prepared as follows: 100 ml of whole blood was collected from a greyhound bitch. The blood was divided among 3-35 ml round bottomed screw-cap centrifuge tubes and allowed to stand at room temperature for approximately 90 minutes until clotting was complete. The clotted blood was centrifuged at 1,000g for 30 minutes at 4°C . The serum was collected and mixed with sufficient 10% sodium azide to make a 0.1% sodium azide solution. The standard serum was stored at 3°C until ready for use.

Standard serum values of IgG, IgM and IgA were determined by using commercially available radial immunodiffusion (RID) kits.^g Serial dilutions of the standard serum were made with sterile 0.9% saline and, where necessary, serial concentrations were performed using the Minicon^{®h} method. The diluting and concentrating procedures allowed for the correct range of immunoglobulin class values in the standard serum, and thus for readable-sized rings on the RID plates. The individual immunoglobulin class values of the standard serum were determined on the commercial RID plates.

RID slides were prepared to test different immunoglobulin class antisera concentrations in order to find the ideal concentration to be used in the production of the RID plates. The procedure was as follows:

Step 1. Tris-HCl buffer was made by putting 12.11 gm hydroxymethyl aminomethaneⁱ in a 1L beaker and adding distilled water q.s. to 1L. The mixture was then titrated using a pH meter and adding 10N HCl to bring

^gMiles Laboratories, Inc., Elkhart, IN 46515.

^hAmicon Corp., Lexington, MA 02173.

ⁱTrizma Base reagent grade, Sigma Chemical Co., St. Louis, MO 63178.

the pH of the Tris solution to 7.4. The 0.1M Tris - HCl buffer solution was then poured into 10-100 ml capped vials and sterilized by heating to 120°C for 15 minutes. After cooling, it was stored at 3°C until used.

Step 2. 2.5 gm agarose^j was put into a 250 ml Erlenmyer flask. Tris-HCl buffer q.s. to 100 ml was added. The agarose was dissolved in the Tris-HCl buffer by placing the Erlenmyer flask in a 99°C boiling water bath. When all of the agarose had dissolved, 2 ml of warmed 1% sodium azide was added to the mixture. The agarose mixture was then placed in a 55°C water bath.

Step 3. Each glass slide was pre-coated with a thin film of agarose solution to facilitate adherence of the agarose-antiserum mixture to the slide. This was done by placing several drops of the 55°C 2.5% agarose at one end of the slide. The agarose was then quickly spread the entire length of the slide by using a second slide at a 45° angle to the first, much as in the technique of making a blood smear.

Step 4. Vials of commercial lyophilized canine antisera^k were reconstituted according to the manufacturer's directions. When reconstituted, the antisera concentrations were: anti-canine IgG: 2 mg/ml, anti-canine IgM: 1mg/ml, and anti-canine IgA: 1.2 mg/ml. Dilutions of 1:10, 1:15, and 1:20 were made for each immunoglobulin class using Tris-HCl buffer solution.

Step 5. 1.5 ml aliquots of each antiserum dilution were placed into individual 5 ml capped plastic vials.^l These vials were placed in the 55°C water bath to equilibrate the antisera temperature with that of the agarose solution.

^jType 1: low EEO, Sigma Chemical Co., St. Louis, MO 63178.

^kMiles Biochemicals, Elkhart, IN 46515.

^lFalcon tubes 12 X 75 mm style, Falcon & Division Becton, Dickinson & Co., Oxnard, CA 93030.

Step 6. An agarose-antiserum mixture was prepared by transferring 1.5 ml of the warmed 2.5% agarose with a warmed pipette into a vial containing 1.5 ml of dilute antiserum. Mixing was obtained by slowly inverting the capped vials 5 times. The vials were returned to the 55°C water bath until the mixture was needed to be spread on the coated slides.

Step 7. The pre-coated glass slides were placed on a leveling table. The 3 ml contents of each vial containing the heated agarose-antiserum mixture were quickly poured onto a coated glass slide being careful not to produce bubbles. The mixture was allowed to harden at room temperature for 5 minutes. The slides were then placed in the refrigerator at 3°C for 10 minutes to finish hardening.

Step 8. Four equally spaced wells were punched in the hardened agarose-antiserum on each slide by using a cutter and template.^m The agar was carefully aspirated from each well by attaching a suction tube to the end of the cutter and applying gentle suction. Using the serial dilutions of standard serum, each well was filled with 10 ul of serum.

Step 9. Plastic petri dishes were used as incubating chambers for the RID slides. Each petri dish bottom was fitted with a circle of moistened paper towel. A wooden applicator stick was broken into 3 equal lengths. These pieces were placed on top of the paper as a support for the slide. An RID slide was placed on top of the applicator sticks and then the petri dish was covered. The petri dishes containing the slides were placed in a refrigerator at 3°C. The precipitin ring diameters on the RID slides were measured at 24 hours and again at 3 days.

From these slides the optimal dilution of each immunoglobulin class for use in making the RID plates was determined.

^mCutter and Template Kit, Miles Biochemicals, Elkhart, IN 46515.

The antiserum dilutions used for each immunoglobulin class were: IgG - 1:10, IgM - 1:15, and IgA - 1:10. The procedure for making the RID plates for each immunoglobulin class was as follows:

Step 1. Five milliliters of dilute antiserum were dispensed into sterile 10 ml capped plastic vialsⁿ using a sterile pipette. The tubes were placed in a 55°C water bath.

Step 2. Using a heated glass pipette, 5 ml of warmed 2.5% agarose was added to each of the tubes containing warmed antiserum. The contents of each tube were mixed by inverting the capped tubes 5-7 times slowly, avoiding bubble formation. The tubes were returned to the 55°C water bath after mixing.

Step 3. The empty RID plates^o were placed on a leveling table. The contents of each tube were quickly poured into individual RID plates starting at one corner. The agarose-antiserum mixture was allowed to spread evenly over the entire plate surface. Any bubbles formed during the pouring process were quickly chased to the corner of the plate using the tip of a pasteur pipette. The poured plates were left undisturbed at room temperature for 5 minutes. The plates were then placed in a 3°C refrigerator for 5-10 minutes to enhance the hardening process.

Step 4. Seventeen evenly spaced wells were punched in the hardened agarose-antiserum medium in each plate using the technique used for the RID slides.

Step 5. The plates were covered and stored gel-side down in a sealed plastic bag at 3°C until ready for use.

The frozen puppy serum samples were allowed to thaw at room temperature for 20 minutes. The cryotube was inverted several times, thoroughly

ⁿFalcon tubes 17 X 100 mm style, Falcon: Div. Becton, Dickinson & Co., Oxnard, CA 93030.

^oMiles Laboratories, Elkhart, IN 46515.

mixing the sample before dispensing 10 ul of serum into a well of the RID plate. Five wells on each RID plate were reserved for the serial dilutions of the standard serum.

The IgG plates were covered and stored at 3°C for 24 hours before reading the precipitin rings. The IgM and IgA plates were stored, covered, at room temperature and read at 7 days.

The IgG plates were read before the precipitin rings reached end point to avoid the encroachment of expanding rings on one another. A linear equation used for determining the unknown serum IgG values was established by plotting the log of concentration versus the diameter (log C vs d) of the standards.

RID plates used for measuring IgM and IgA were read at end point. A linear equation used for determining the unknown serum IgM and IgA values was established by plotting the concentration versus the diameter squared (C vs d^2) of the standards.

The total protein of each serum sample was determined using 2 methods: refractometer^P readings and a spectrophotometric procedure using biuret reagent.^Q

The data collected in this study was analyzed using the Statistical Analysis System. When determining the relationships of immunoglobulin values to age, lipemia analysis of variance was used. When determining the relationships of immunoglobulin values to weight and total protein, analysis of covariance was used.

Comparisons found statistically significant were significant at the 0.05 level.

^PAmerican Optical, Scientific Instrument Division, Buffalo, NY 14215.

^QTechnicon, Tarrytown, NY 10591.

RESULTS

The study was initiated using 24 beagle pups from 4 litters. The distribution of pups per litter is shown on table 1. Blood samples were collected from the pups at time of birth (before nursing), at 24 hours after birth, and at weekly intervals for 16 weeks.

Weight gain is considered a good indicator of overall health in young puppies, therefore, weight was monitored regularly during the first 9 weeks. Weight gain for most of the puppies was dramatic during the first 9 weeks (figures 1-4). The pups in litter 4 had low birth weights when compared to the birth weights of pups in the first 3 litters. With the exception of 3 pups, all pups in litters 1-3 had birth weights of 250 gms or more. All 8 pups in litter 4 had birth weights under 250 gms. Litter 4 had the greatest number of pups of the 4 litters studied. Pups #3 and #4 of litter 4 were the only pups that failed to gain weight over the first 24 hours. These 2 pups died during the first week of life.

The relationship between age and serum immunoglobulin values in the pups was statistically significant for all immunoglobulin classes tested. At birth IgG and IgM immunoglobulins are present in very low concentrations, whereas IgA levels are zero. At 24 hours of age, after the pups had suckled colostrum, the values of all immunoglobulin classes markedly increased. After this initial surge, IgG and IgA levels dropped dramatically (figures 5&6). The $1/2$ life for the disappearance of IgG was found to be 7.3 days. The $1/2$ life of IgA was 3.2 days. After approximately 4 weeks, the trend reversed and serum IgG and IgA levels started to climb. Serum IgM values slowly increased after the first 24 hours. (figure 7).

The colormetric method used in measuring serum total protein gave consistently higher values than the refractometer method, however, when plotted against age both methods resulted in similar graphs (figure 8). The relationship of total protein and age was statistically significant. The overall trend was an increase in total protein with an increase in age.

Regardless of method used to determine total protein it was found that the relationship of serum total protein and serum IgG or IgM values was statistically significant. As total protein values rose so did IgG and IgM values. This trend is illustrated in figures 9 and 10. The relationship between total protein and IgA proved not to be statistically significant.

Each serum sample was observed for hemolysis and each sample was given a rating between 0 and +++. It was found that the relationship of hemolysis and total protein (found by either of the 2 methods) was statistically significant (figure 11). As the degree of hemolysis increased the serum total protein values increased.

Each serum sample was observed for lipemia and was given a rating between 0 and +++. It was found that the relationship of lipemia to serum total protein determined by the colormetric method was statistically significant, whereas the relationship of lipemia to serum total protein determined by the refractometer method was not. The more lipemic a serum sample was the higher the total protein when using the colormetric method (figure 12). When lipemia was compared to serum IgG, IgM and IgA values there was no statistically significant relationship.

During the latter part of the study the kennel became infected with parvovirus. How the kennel became infected is unknown. The virus could

have been introduced by asymptomatic carrier dogs being introduced to the kennel during the study or the virus could have been introduced on contaminated shoes.

The beagle pups of this study were the youngest dogs in the kennel and the first to show clinical signs of parvovirus gastroenteritis. Only one other dog in the kennel became ill. This dog had a severe bloody diarrhea that resolved spontaneously after 24 hours. This dog recovered. The diagnosis of parvovirus gastroenteritis was based on clinical signs, hematologic findings, positive hemagglutination test of feces, significant serum parvovirus titers, and necropsy findings. The mortality rate approached 50% in the beagle pups. Deaths due to the parvovirus occurred between 11 and 14 weeks of age. Table 2 illustrates the deaths and their causes that occurred throughout the study.

It seemed logical to assume that pups with the lowest immunoglobulin levels would be the ones most susceptible to disease and would be the ones more prone to death. However, the results revealed that the immunoglobulin levels of the pups that died were not necessarily the lowest within the litter (tables 3-5).

**THIS BOOK
CONTAINS
NUMEROUS PAGES
WITH DIAGRAMS
THAT ARE CROOKED
COMPARED TO THE
REST OF THE
INFORMATION ON
THE PAGE.**

**THIS IS AS
RECEIVED FROM
CUSTOMER.**

TABLE 1

Litter #	Number of Pups in Litter
1	7
2	5
3	4
4	8

Distribution of pups per litter.

TABLE 2

Puppy Identification	Age at Death	Cause of Death
Litter 1 #1	14 wks	Parvovirus
Litter 1 #4	14 wks	Parvovirus
Litter 1 #5	14 wks	Parvovirus
Litter 1 #6	14 wks	Parvovirus
Litter 2 #4	12 wks	Parvovirus
Litter 3 #2	13 wks	Parvovirus
Litter 3 #3	13 wks	Parvovirus
Litter 3 #4	13 wks	Parvovirus
Litter 4 #3	4 days	Weak Puppy
Litter 4 #4	4 days	Weak Puppy
Litter 4 #5	9th wk	Unknown
Litter 4 #7	11th wk	Parvovirus
Litter 4 #8	11th wk	Parvovirus

Deaths that occurred and their causes.

TABLE 3

Litter	Age	Puppy Number							
		1	2	3	4	5	6	7	8
1	13 wks	465	409	281	370	346*	323*	597	
	14 wks	174*	245	281	323*			487	
2	12 wks	319	405	319	202*	376			
3	13 wks	520	330*	520*	263*				
4	24 hr	453	989	1559*	762*	327	550	926	128
	9 wks	401	346			346*	514	284	32
	11 wks	396	319				276	297*	458*
		IgG mg/dl							

Comparison of IgG levels (mg/dl) among pups in the same litter. Starred numbers indicate immunoglobulin values of pups that died. Values are from last blood samples taken previous to death.

TABLE 4

Litter	Age	Puppy Number							
		1	2	3	4	5	6	7	8
1	13 wks	153	126	80	94	125*	105*	200	
	14 wks	202*	212	155	90*			218	
2	12 wks	280	167	134	257*	102			
3	13 wks	120	161*	109*	112*				
4	24 hr	9	11	11*	9*	9	9	13	18
	9 wks	126	88			92*	88	54	79
	11 wks	142	88				69	69*	202*
		IgM mg/dl							

Comparison of IgM levels (mg/dl) among pups in the same litter. Starred numbers indicate immunoglobulin values of pups that died. Values are from last blood samples taken previous to death.

TABLE 5

Litter	Age	Puppy Number							
		1	2	3	4	5	6	7	8
1	13 wks 14 wks	26 3*	25 2	15 4	28 7*	18*	24*	15 6	
2	12 wks	23	25	47	2*	13			
3	13 wks	21	1*	2*	10*				
4	24 hr 9 wks 11 wks	76 18 6	165 9 trace	165*	139*	83 21*	123 13 trace	172 7 3*	165 9 trace*
		IgA mg/dl							

Comparison of IgA levels (mg/dl) among pups in the same litter. Starred numbers indicate immunoglobulin values of pups that died. Values are from last blood samples taken previous to death.

TABLE 6

	Immunoglobulin (mg/dl)		
	IgG	IgM	IgA
16 wk. mean values	531	138	22
16 wk. range	367 - 831	68 - 203	7 - 34
adult range (Tizard)	500 - 1700	70 - 270	20 - 120
adult range (Reynolds & Johnson)	925 - 1445	145 - 156	79 - 83
adult range (Heddle & Rowley)	740 - 1020	125 - 148	38 - 52

Range of immunoglobulin values of pups at end of 16 week study. Range of immunoglobulin values for adult dogs as reported in the literature.

FIGURE 1. Growth graph for Litter 1

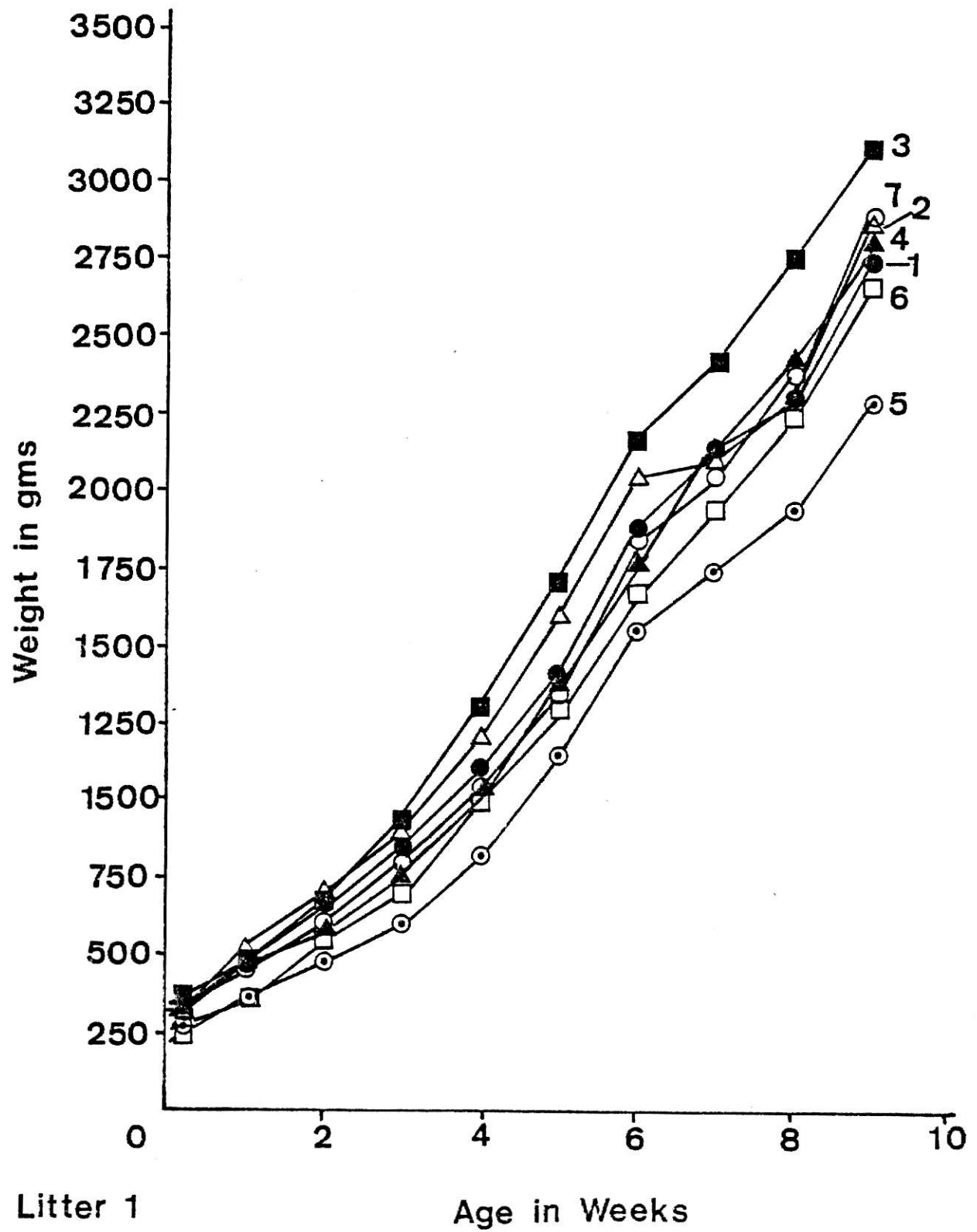


FIGURE 2. Growth graph for Litter 2

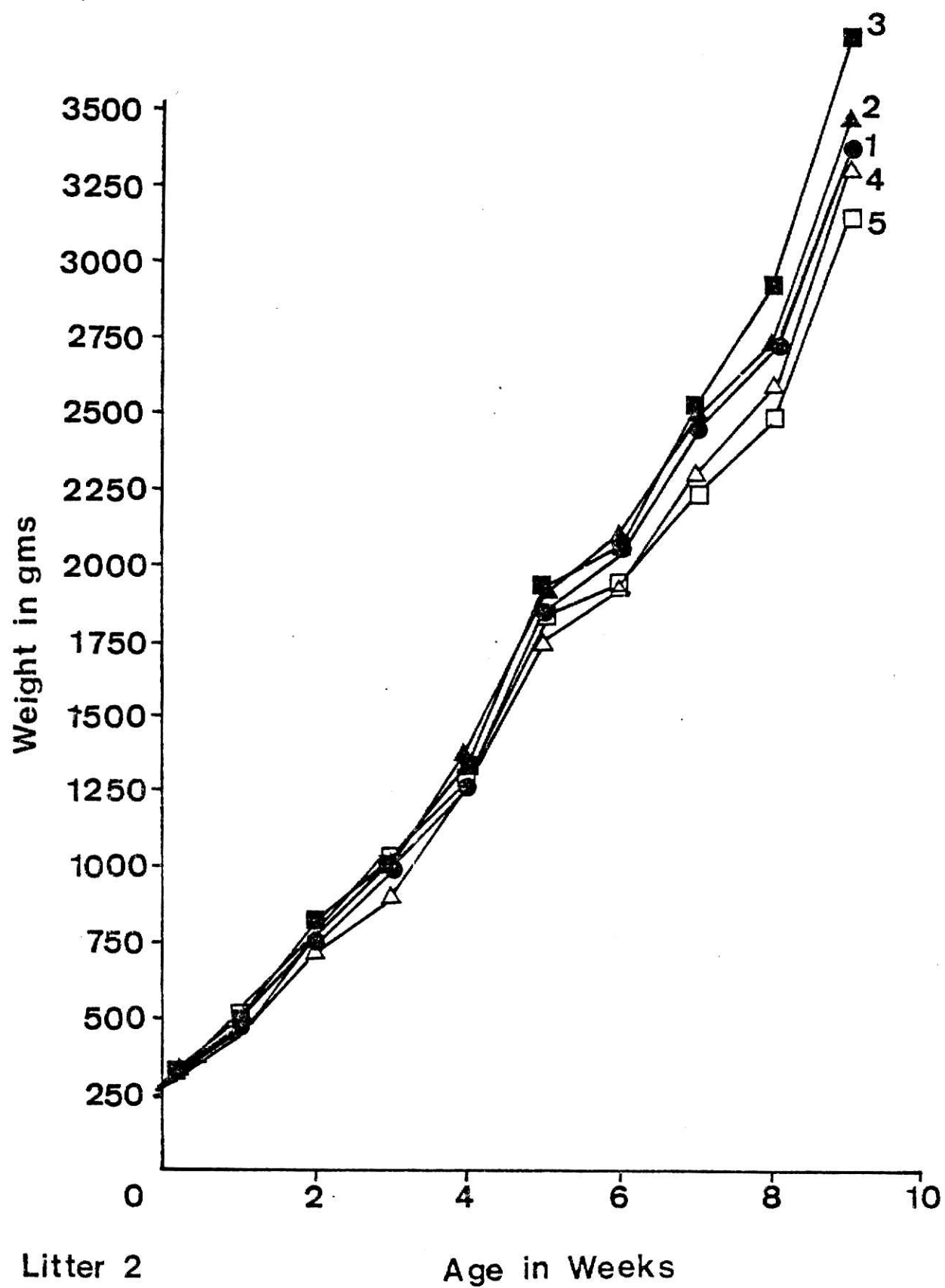


FIGURE 3. Growth graph for Litter 3

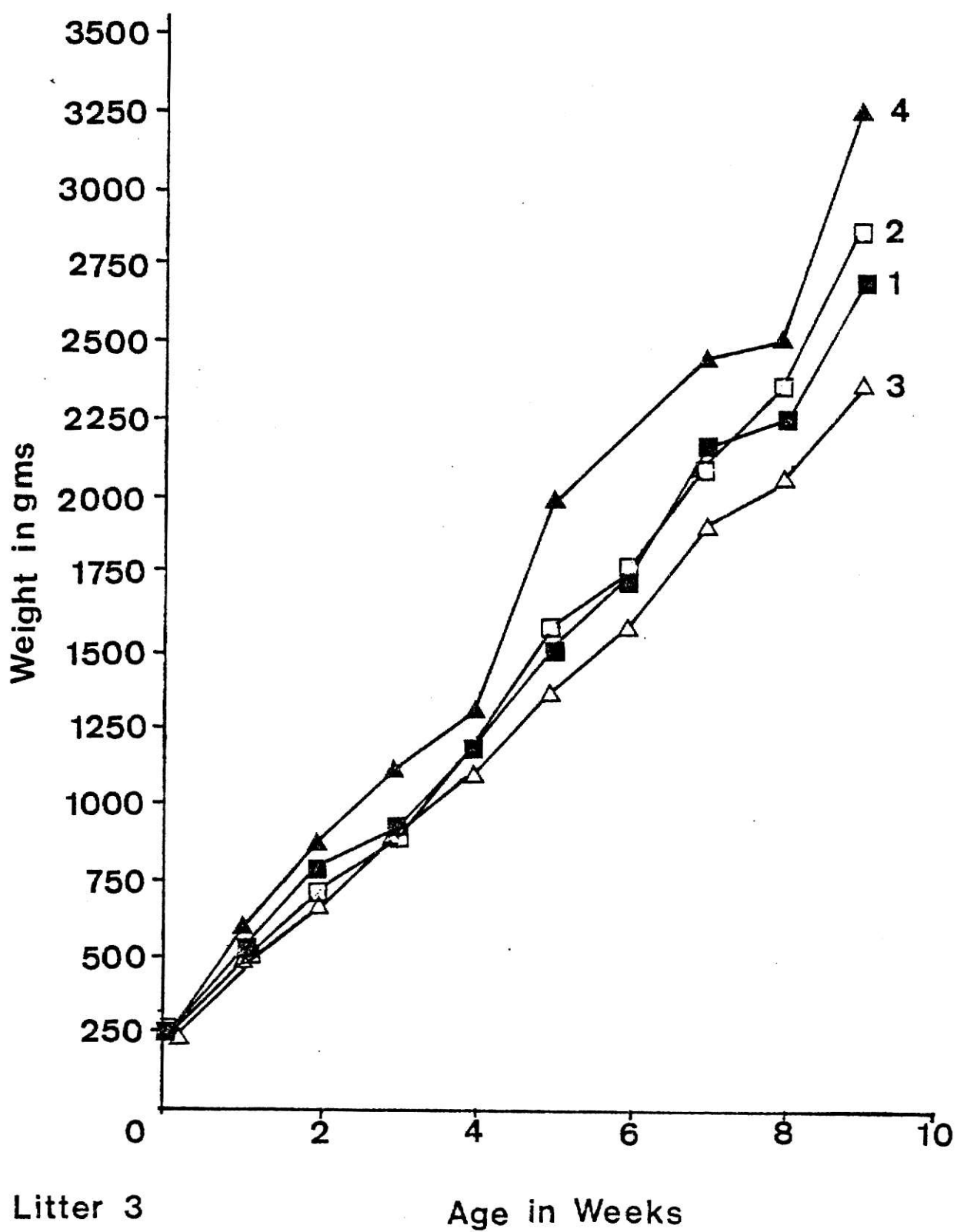


FIGURE 4. Growth graph for Litter 4

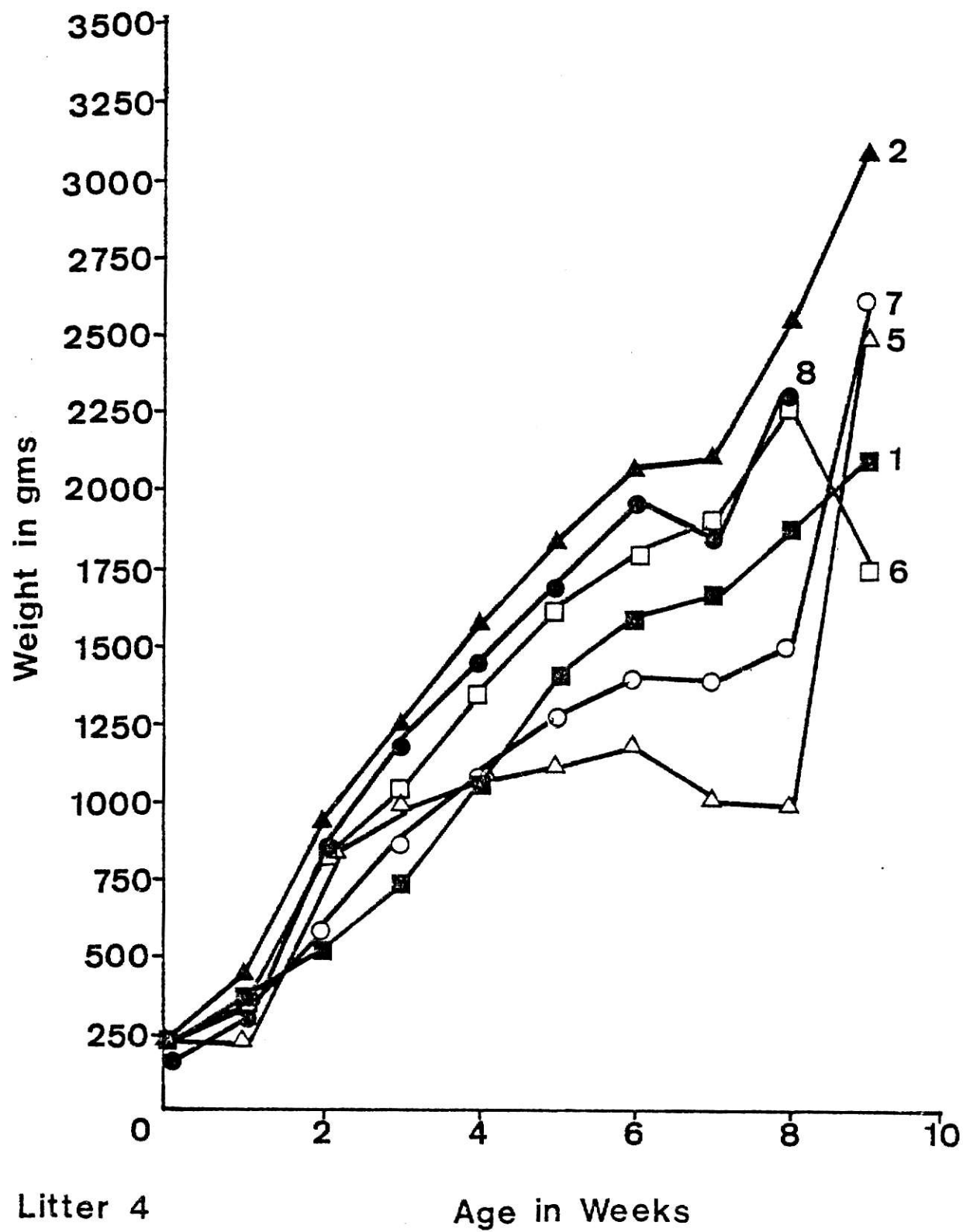


FIGURE 5. The mean value and absolute range of Serum IgG and their relationship to age.

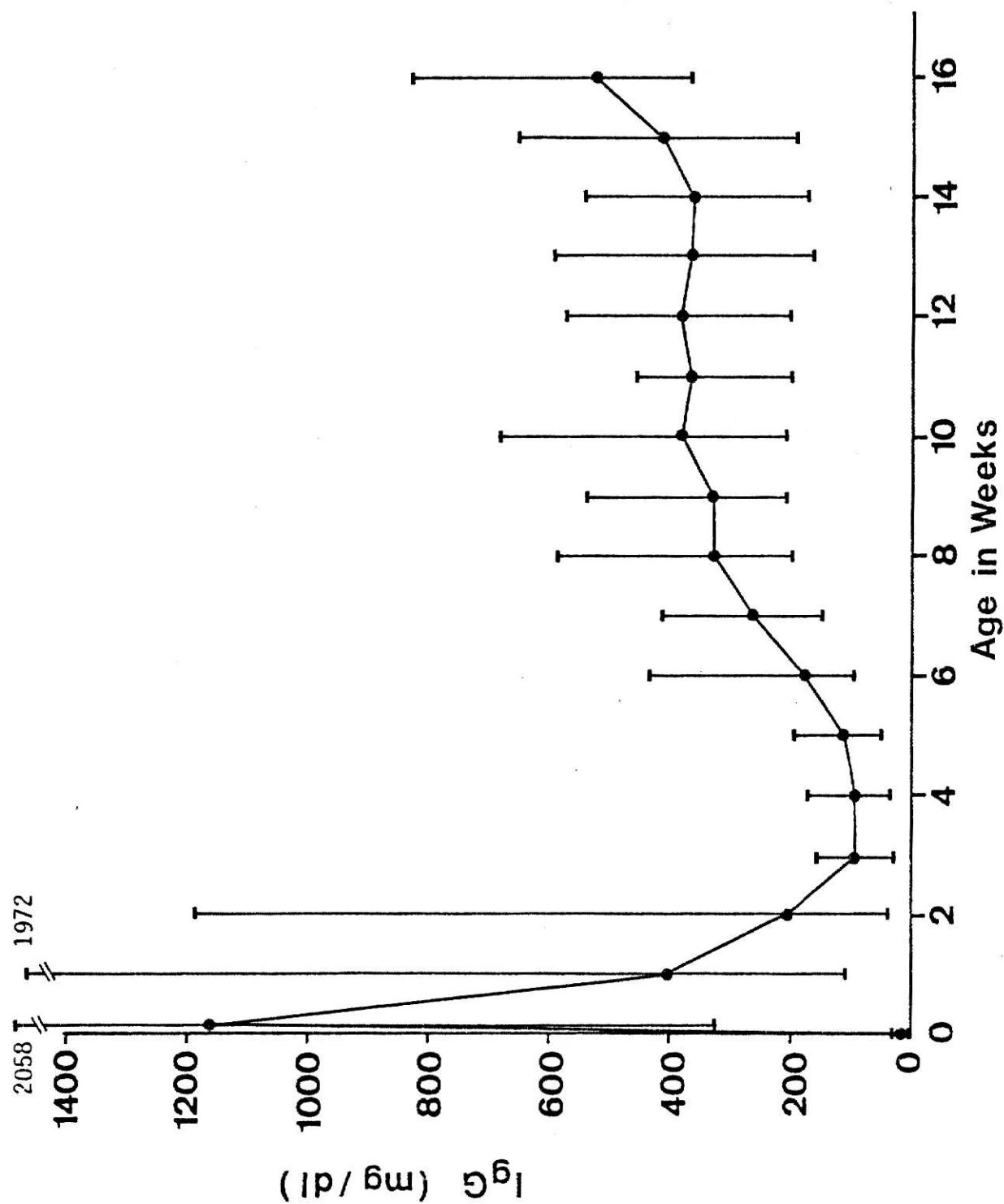


FIGURE 6. The mean value and absolute range of Serum IgA and their relationship to age.

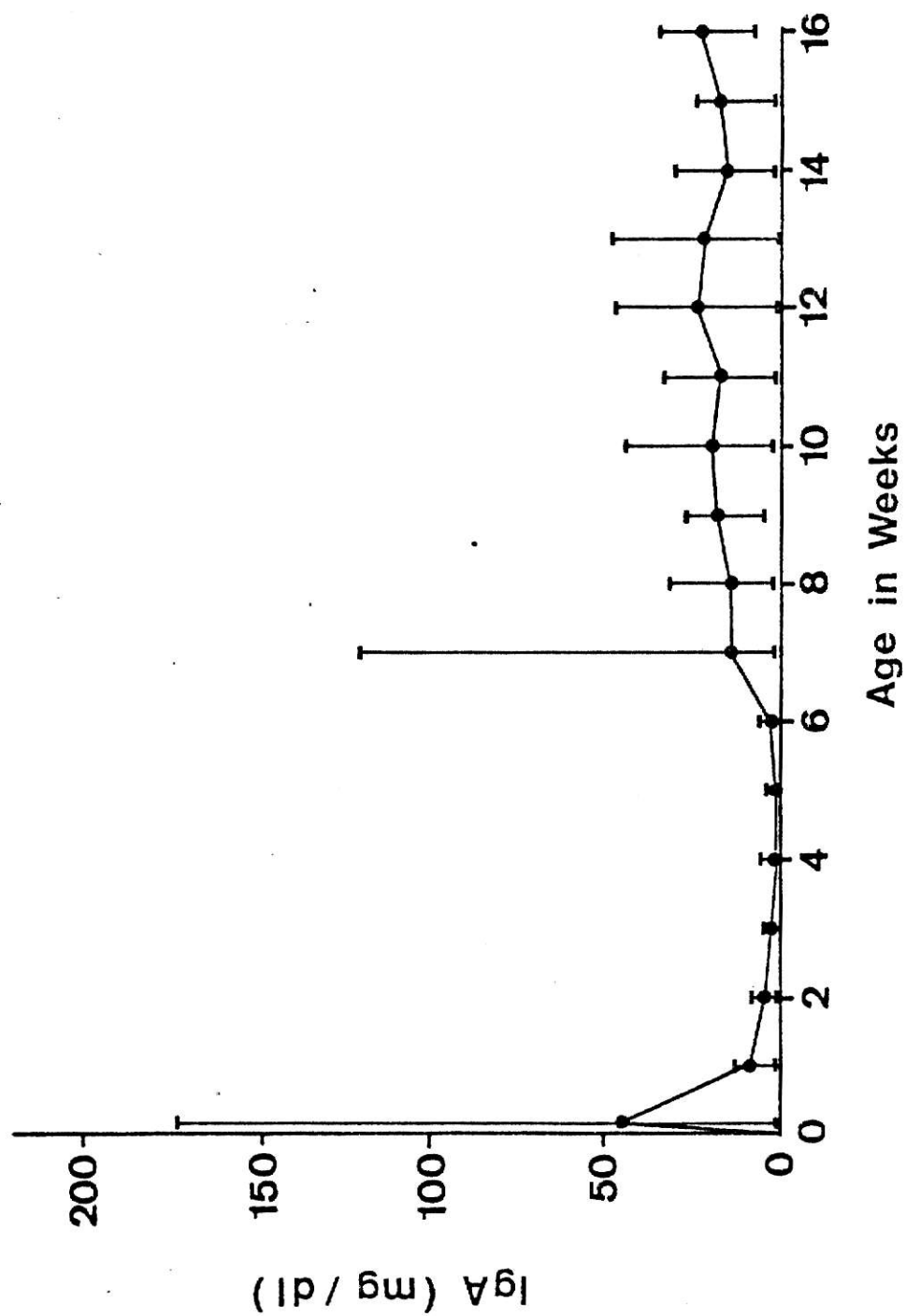


FIGURE 7. The mean value and absolute range of Serum IgM and their relationship to age.

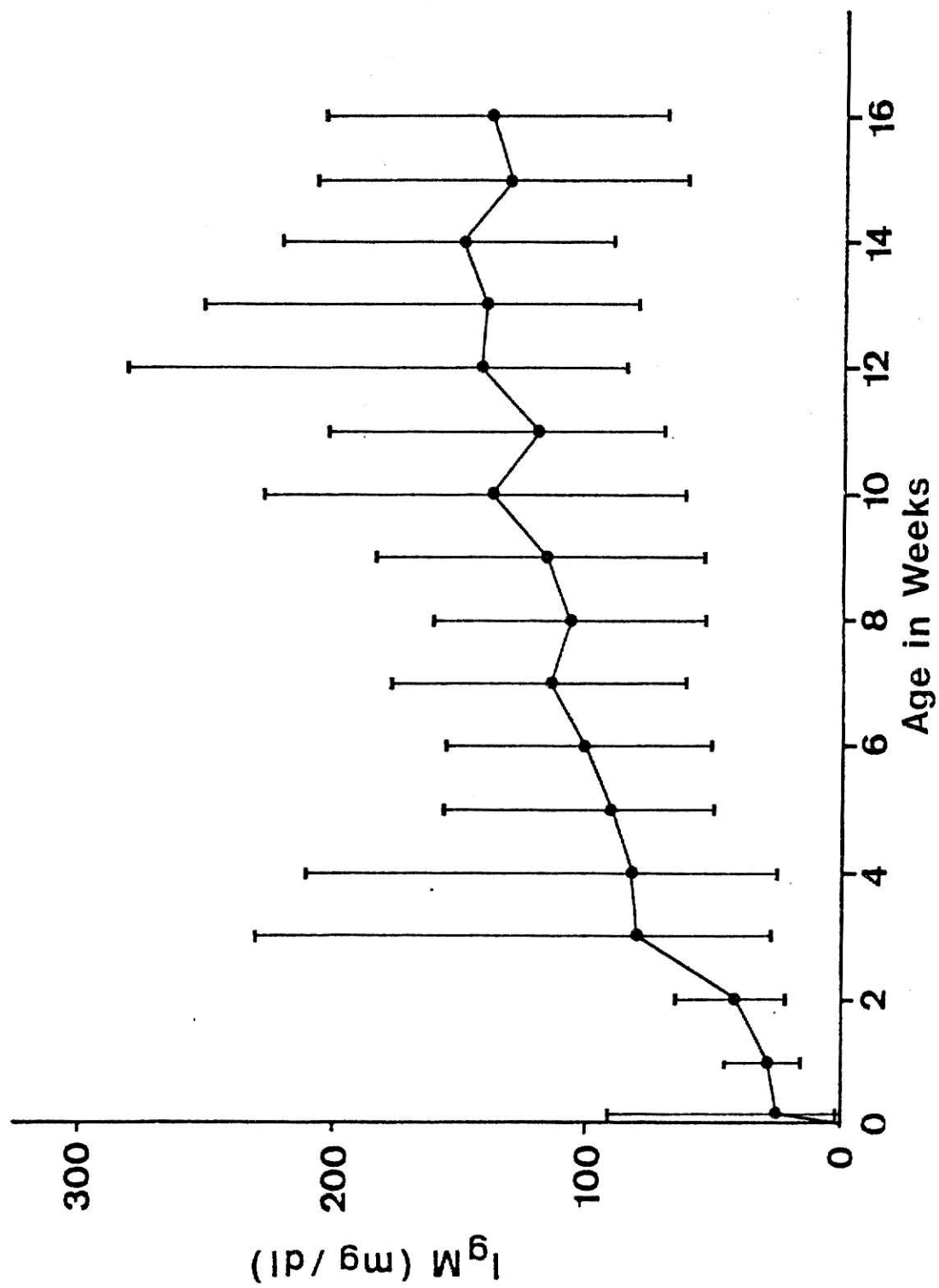


FIGURE 8. The mean value and absolute range of Serum Total Protein and their relationship to age.
 *Indicates Biuret range - Indicates Refractometer range

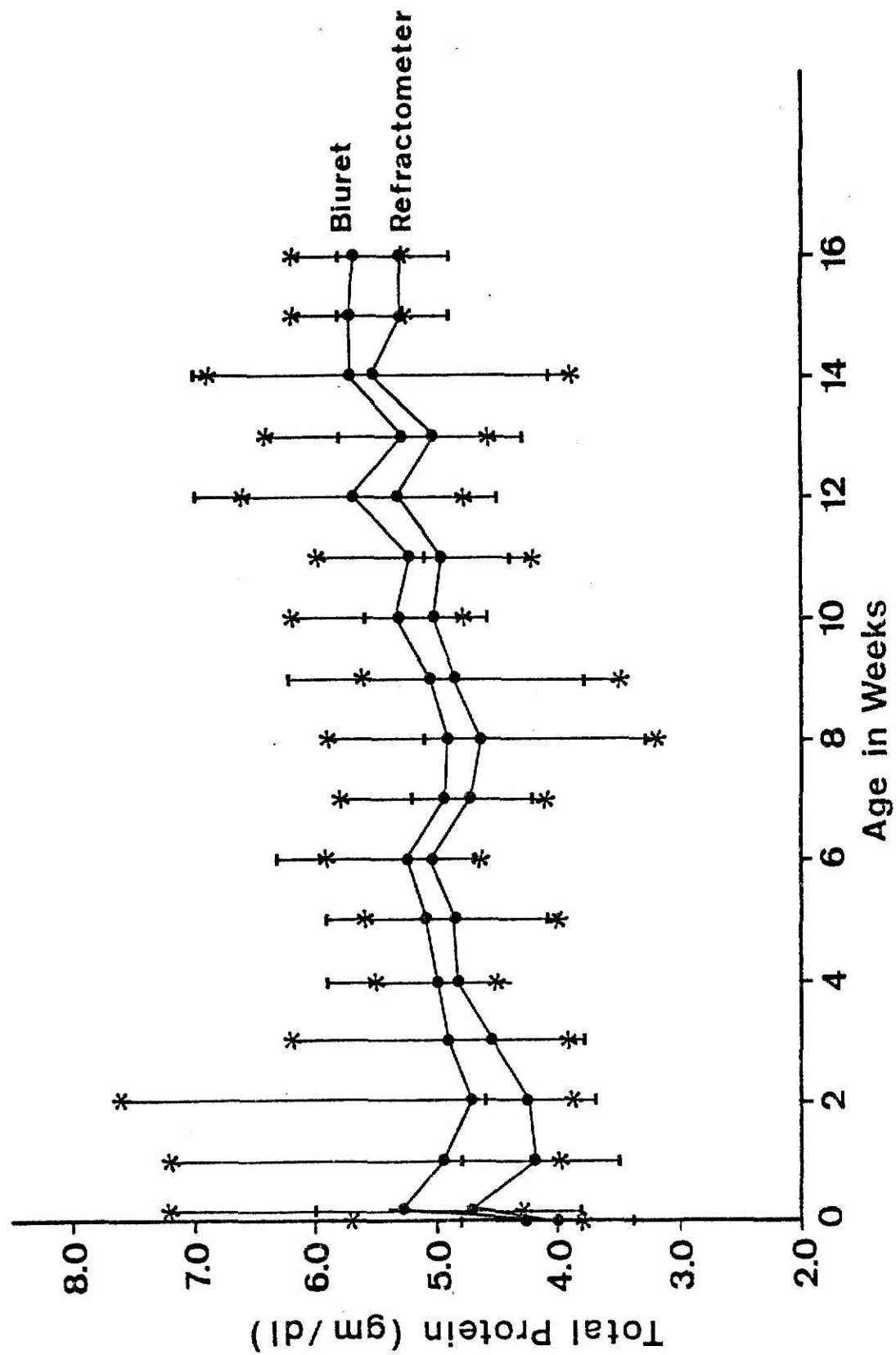


FIGURE 9. Serum Total Protein and its relationship to serum IgG levels

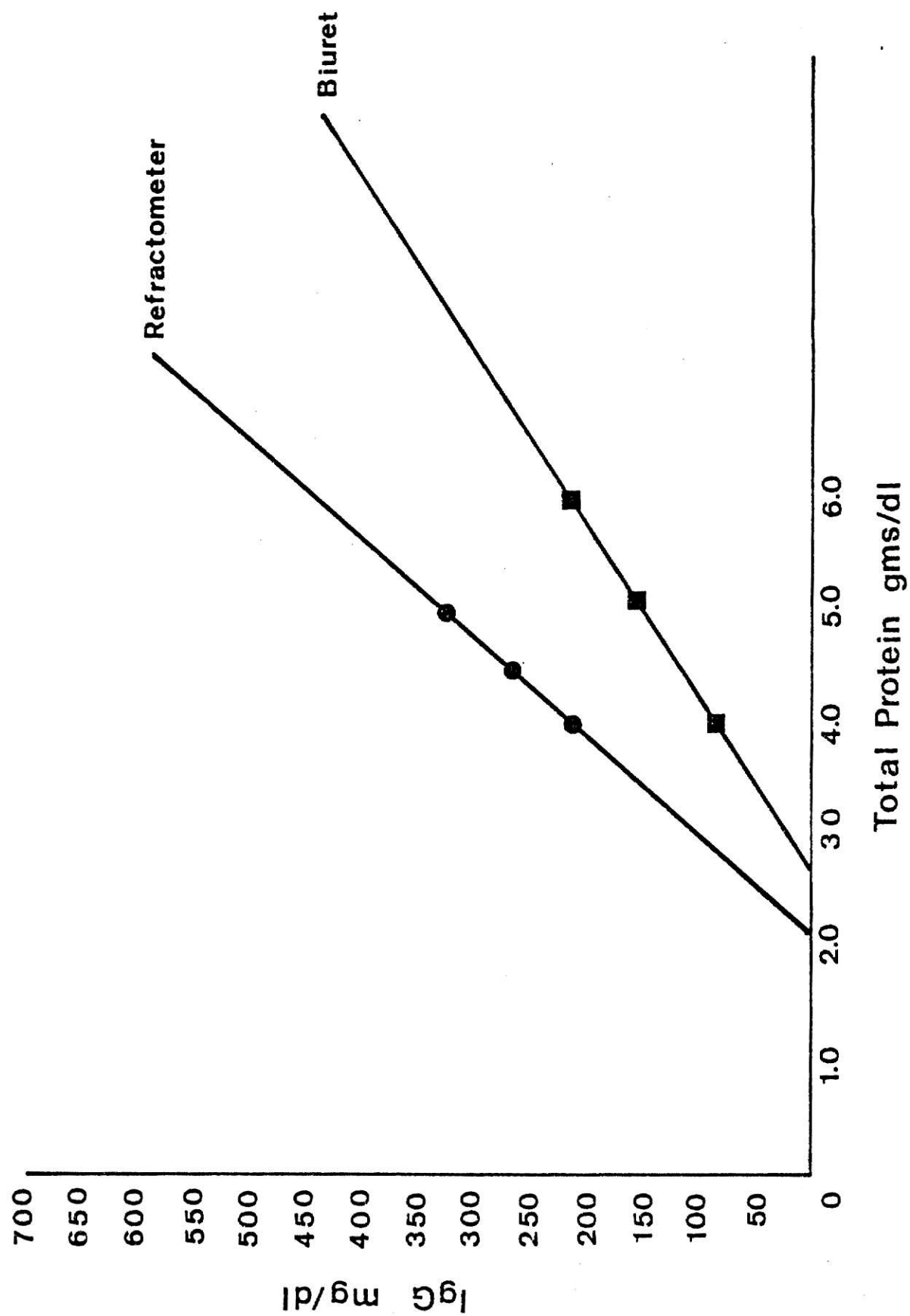


FIGURE 10. Serum Total Protein and its relationship to serum IgM levels

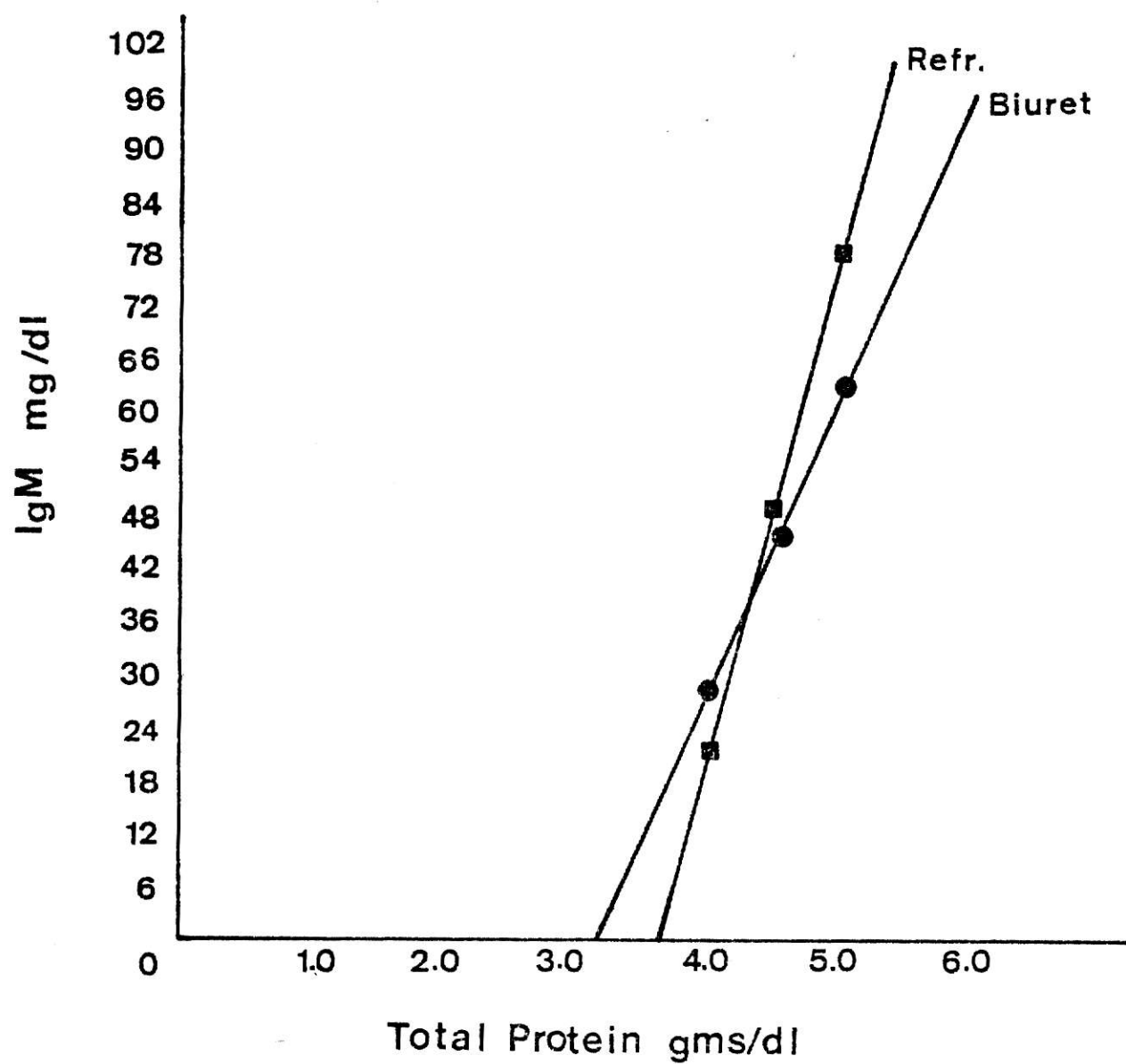


FIGURE 11. Degrees of Hemolysis and their relationship to Serum Total Protein

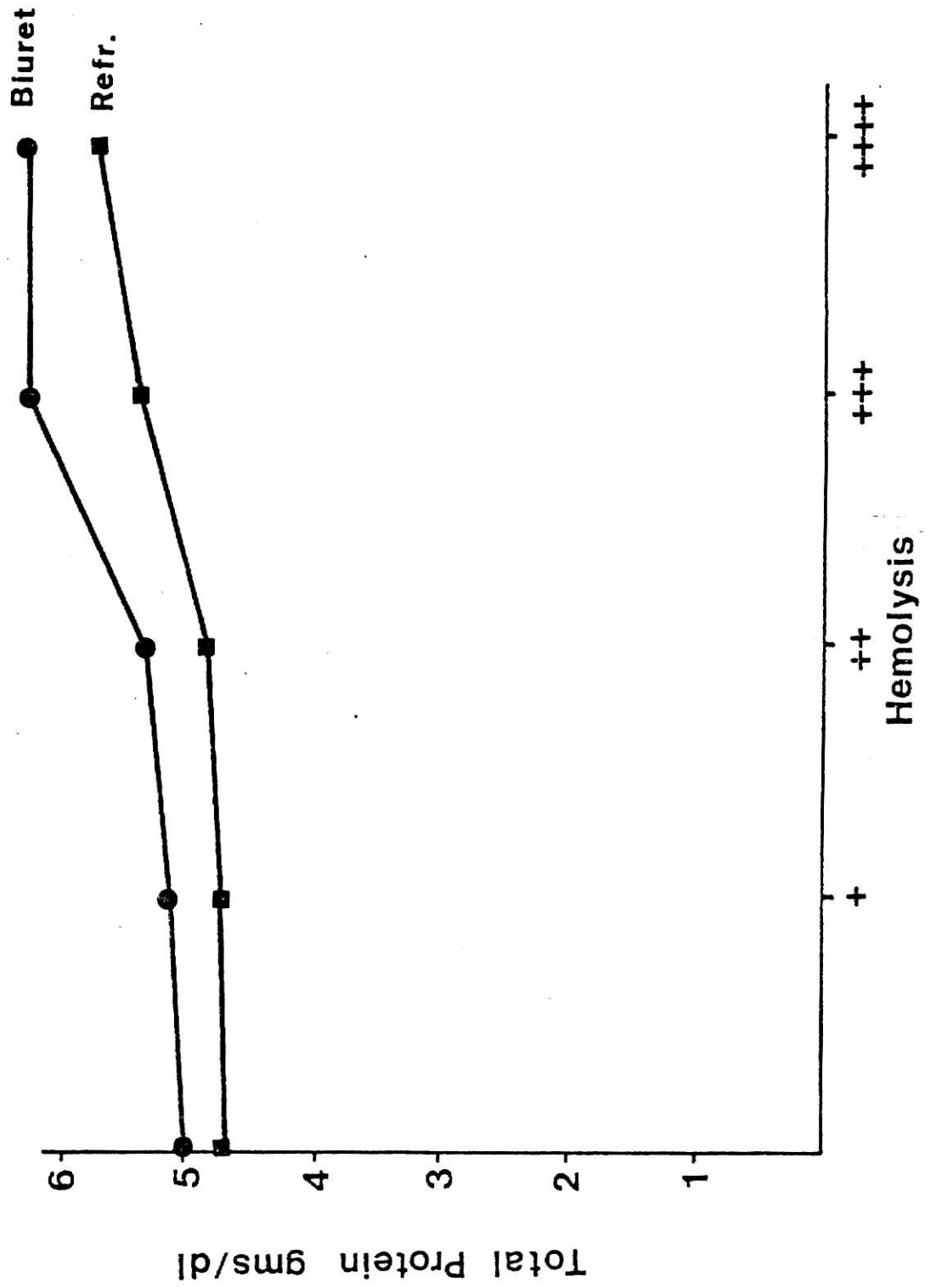
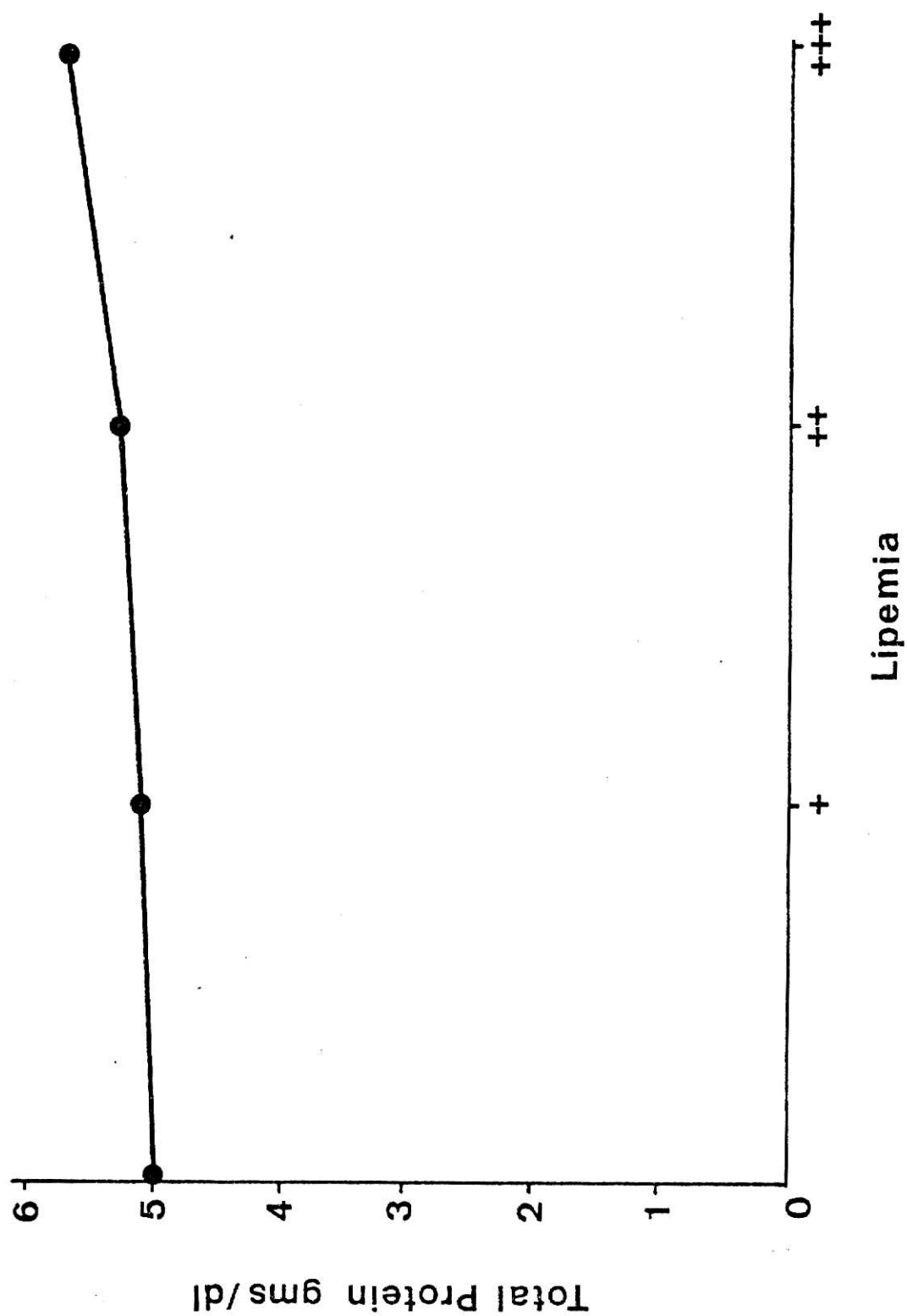


FIGURE 12. Degrees of Lipemia and their relationship to Serum Total Protein



DISCUSSION

At birth the serum immunoglobulin concentrations of the pups were very low. IgG values ranged from 1-31 mg/dl with the mean being 16 mg/dl. IgM values ranged from trace-9 mg/dl with the mean being trace. Trace amount was considered to be the measurement in those samples that showed a precipitin ring on the RID plate but whose value was found to be <1 mg/dl using the equation $C \text{ vs } d^2$. In all pups the IgA values at birth were 0 mg/dl.

After the pups had nursed, serum samples from blood drawn at 24 hours of age showed a remarkable rise in all immunoglobulin class concentrations. Serum IgG values ranged from 237-2042 mg/dl with the mean being 1161 mg/dl. Serum IgM values ranged from trace - 82 mg/dl with 26 mg/dl being the mean. Serum IgA concentrations at 24 hours showed tremendous variation between litters. Litter 1 had IgA values that ranged from trace - 8 mg/dl. All pups in litters 2 and 3 had IgA values of 0 mg/dl. Litter 4 had IgA values ranging from 72-172 mg/dl. The IgA mean of all pups at 24 hours was 46 mg/dl. The reason for the variation of 24 hour serum IgA values among the litters is not clear. The precipitin rings of the 24 hour serum samples from litter 4 were very faint and not entirely circular raising the question of whether or not these precipitin rings were due to artifact. These findings were reproduced when the 24 hour samples from litter 4 were tested again on the RID plates for IgA. Unlike litter 4 whose IgA values peaked at 24 hours, litters 1-3 IgA values peaked at 1 week. The range of this peak was 1-14 mg/dl.

During the first week the serum IgG $1/2$ life was 4.1 days. This finding was based on the drop in serum IgG levels seen at that time when presumably very little IgG was being produced. When corrections were made for the volume of blood removed at sampling and for the expanded

plasma volume at 1 week of age, as compared to the plasma volume at 1 day of age, the 1/2 life was 7.8 days. After the first week the IgG 1/2 life became longer due to IgG production adding to the total serum IgG pool. Waldman and Strober⁴⁹ reported the IgG 1/2 life to be 8 days. The 1/2 life of serum IgA during the first week was found to be 2.5 days. When corrections were made for the volume of blood removed and for the expanded plasma volume the IgA 1/2 life was 3.2 days. As seen with IgG, the serum IgA 1/2 life became longer after the first week. No 1/2 life was determined for serum IgM since no drop in IgM values was observed.

The rise in serum IgG and IgA seen at approximately 4-6 weeks of age probably reflected the immunoglobulin synthesis that exceeded the catabolism of the passively received immunoglobulins. The failure of serum IgM to drop could be due to a slower catabolic rate and an earlier production of IgM by the pups than that seen for IgG and IgA.

At the end of the present 16 week study, the mean serum immunoglobulin values were: IgG - 531 mg/dl, IgM - 138 mg/dl and IgA - 22 mg/dl. The serum IgG levels in the pups at 16 weeks of age were lower than most serum IgG levels established for adult dogs^{16,37,43} probably indicating that the 16 week old pups had not received the antigenic exposure necessary for normal adult IgG levels. The same was found to be true for IgA. Most puppy serum IgM levels fell within the normal serum IgM ranges established for adult dogs, which may be due to the earlier IgM production observed. These findings are illustrated in table 6.

Since commercial RID plates are relatively expensive, serum total protein was measured to see if it could be used as an indicator of serum immunoglobulin levels. If so it would provide a more practical test for

practitioners to use when trying to screen out pups with abnormally low immunoglobulin levels. The colormetric method of measurement gave consistently higher serum total protein values, although when plotted against age the graphs for both methods were similar.

Serum total protein fluctuated with respect to age but the overall trend was an increase as the pups got older. There was a decrease in serum total protein between the ages of 24 hours and 1-2 weeks and appeared to be related to the decrease in serum immunoglobulins seen at this time. Another decrease in serum total protein occurred between the ages of 6 and 8 weeks and may reflect the change of diet as the pups were weaned at 6 weeks of age. Weaning resulted in less protein ingestion since the pups no longer had access to bitch's milk. The fluctuations in serum total protein seen between 10 and 16 weeks of age could have been due to illness among some of the pups at this time.

The serum total protein directly correlated with serum IgG and IgM levels in these pups, therefore, serum total protein could be used as a rough indicator to screen out groups of pups with potentially low immunoglobulin levels. However, on an individual basis there was quite a bit of variation between serum total protein values and serum IgG and IgM indicating that one should be careful when trying to correlate serum total protein and IgG or IgM levels in individual pups. It would not be of any use when trying to screen out pups with low IgA values since serum total protein did not correlate well with serum IgA.

It is sometimes difficult to collect blood samples from neonatal puppies without some hemolysis. Thus the effect of hemolysis on serum total protein was examined.

There was a direct correlation between the degree of hemolysis and serum total protein. Hemolysis causes an increase when using the colormetric method because of the increased pigment and increased protein in the serum sample. This in turn causes a decreased percent transmittance and a higher total protein reading.

The refractometer is based on refraction of light caused by molecules in serum. In the normal animal serum electrolytes and certain enzymes remain relatively constant, however, the amount of serum protein can vary. With hemolysis there is a release of intracellular protein causing an increase in serum protein that in turn causes increased refraction of light and a higher total protein reading.

Blood samples collected from non-fasted pups are often lipemic. Of 336 samples in this study 127 had some degree of lipemia. The possibility of a relationship between degree of lipemia and serum total protein and serum immunoglobulin levels was examined. As the degree of lipemia increased total protein determined by the colormetric method increased. There was no correlation between degree of lipemia and serum total protein determined with the refractometer. The difference between the two methods might be explained by assuming that lipemia has more of an affect on serum turbidity and hence transmittance than on light refraction.

When the degree of lipemia was compared to serum IgG, IgM and IgA values there was no correlation. Thus it is apparent that lipemia does not affect serum immunoglobulin levels.

By the end of the study 13 of the original 24 pups had died. The serum immunoglobulin levels of the pups that died were reviewed to see if they were lower than the levels of their litter mates. The last samples that were taken prior to death were examined.

For IgG, 4 out of the 13 pups had lower levels when compared to litter mates. Six of the 13 had lower IgM values. Only 2 out of 13 had the lowest IgA values.

This is contrary to what is generally thought - pups with the lowest immunoglobulin levels are most susceptible to disease and death. However, one must keep in mind the nature of the disease process.

The 2 pups in litter 4 that died during the first week had not been nursing and were dehydrated. Dehydration will cause serum protein concentration to increase. This phenomenon could mask an abnormally low serum immunoglobulin values.

The pups that developed parvovirus experienced vomiting and/or diarrhea. The pups that died as a result of the infection showed severe vomiting and diarrhea with dehydration that developed rapidly. Serum immunoglobulin levels measured during periods of dehydration would be expected to be abnormally high. However, protein loss into the gut lumen as a result of the breakdown of the epithelial barrier of the intestines (the intestinal lesions of parvovirus) would result in a lowered serum immunoglobulin concentration.

CONCLUSIONS

The highest values for serum IgG were shown at 24 hours of age. Three out of the 4 litters showed a peak of serum IgA at 1 week of age. The fourth litter showed an IgA peak at 24 hours of age. IgM did not experience a drop in serum levels but continued to rise after the first 24 hours.

The 1/2 life of serum IgG during the first week of life, after correcting for expanded plasma volume due to growth and for the volume of blood removed at sampling, was 7.8 days. The 1/2 life of serum IgA after correction was 3.2 days.

Synthesis of IgG and IgA was significant at 4-6 weeks of age. Serum IgG and IgA had not reached normal adult levels by 16 weeks of age. Serum IgM was within normal adult ranges by this time.

Serum total protein showed a positive correlation with serum IgG and IgM levels when looking at the group as a whole. However, on an individual basis it was harder to show a positive correlation due to the variation between total protein and IgG and IgM values. Degree of hemolysis showed a positive correlation with serum total protein.

Lipemia showed a positive correlation with serum total protein determined by the colormetric method. There was no correlation between degrees of lipemia and serum immunoglobulin values.

There was no correlation between serum immunoglobulin levels and puppy deaths.

BIBLIOGRAPHY

1. Bohl, E.H. and Salf, L.J. 1975. Passive immunity in transmissible gastroenteritis of Swine: Immunoglobulin characteristics of antibodies in milk after inoculating virus by different routes. *Infect. and Immun.* 11, no. 1: 23.
2. Borthistle, B.K., Kubo, R.T., Brown, W.R., and Grey, H.M. 1977. Studies on receptors of IgG on epithelial cells of rat intestine. *J. of Immunol.* 119, no. 2: 471.
3. Bourne, F.J. and Curtis, Jill. 1973. The transfer of immunoglobulins IgG, IgA and IgM from serum to colostrum and milk in the sow. *Immunol.* 24: 157.
4. Brambell, F.W. Rogers. 1970. The transmission of passive immunity from mother to young. In *Frontiers of Biology*. Vol. 18, North Holland Publishing Co., Amsterdam.
5. Breast-feeding: the immunological argument. *British Medical J*, I, May 1976: 1167.
6. Butler, J.E. 1971. Review of the bovine immunoglobulins. In session I. Characteristics of bovine immunoglobulins and related molecules. *J. of Dairy Science* 54: 1315.
7. Butler, J.E., Kiddy, C.A., Maxwell, C, Hylton, M.D., and Asofsky, R. 1971. Synthesis of Immunoglobulins by various tissues of the cow. From session I. Characteristics of bovine immunoglobulins and related molecules. *J. of Dairy Science* 54: 1324.
8. Butler, J.E., Winter, A.J., and Wagner, G.G. 1971. Symposium: Bovine immune system. *J. of Dairy Science* 54: 1309.
9. Curtis, J., and Bourne, F.J. 1971. Immunoglobulin quantitation in sow serum, colostrum, and milk and the serum of young pigs. *Biochem. Biophys. Acta.* 236: 319.
10. Delouis, C. 1978. Physiology of colostrum production. *An. Rech. Vet.* 9, no. 2: 193.
11. Gillette, D.D. and Filkins, M. 1966. Factors affecting antibody transfer in the newborn puppy. *Am. J. Physio.* 210: 419.
12. Gillespie, J.H., Baker, J.A., Burgher, J., Robson, D., and Gilman, B. 1958. The immune response of dogs to distemper virus. *The Cornell Veterinarian* 48: 103.
13. Gitlin, J.D. and Gitlin, D. 1974. Protein binding by specific receptors on human placenta, murine placenta and suckling murine intestine in relation to protein transport across these tissues. *J. of Clin. Invest.* 54: 1155.

27. McGuire, T.C., Crawford, T.B., and Henson, J.B. 1972. The isolation characterization and functional properties of equine immunoglobulin classes and subclasses. Proc. 3rd Int. Conf. Equine Infectious Diseases, Paris: 364.
28. McNabb, T., Koh, T.Y., Dorrington, K.J., and Painter, R.H. 1976. Structure and function of immunoglobulin domains V. Binding of immunoglobulin G and fragments to placental membrane preparations. J. of Immunol. 117, no. 3: 882.
29. Nagura, H., Nakane, P.K., and Brown, W.R. 1978. Breast milk IgA binds to jejunal epithelium in suckling rats. J. of Immunol. 120, no. 4: 1333.
30. Ogra, S.S., Weintraub, D., and Ogra, P.L. 1977. Immunologic aspects of human colostrum and milk. III Fate and absorption of cellular and soluble components in the gastrointestinal tract of the newborn. J. of Immunol. 119, no. 1: 245.
31. Patt, J.A. 1977. Factors affecting the duration of intestinal permeability to macromolecules in newborn animals. Bio. Rev. 52: 411.
32. Porter, P. 1969. Transfer of immunoglobulins IgG, IgA and IgM to lacteal secretions in the parturient sow and their absorption by the neonatal piglet. Biochem. Biophys. Acta. 181: 381.
33. Porter, P. 1969. Porcine colostrum IgA and IgM antibodies to Escherichia coli and their intestinal absorption by the neonatal piglet. Immunol. 17: 617.
34. Porter, P. 1971. Immunoglobulin IgA in bovine mammary secretions and serum of the neonatal calf. Biochem. Biophys. Acta. 236: 664.
35. Porter, P. 1976. Intestinal absorption of colostrum IgA anti-E. coli antibodies by the neonatal piglet and calf. Maternofetal transmission of immunoglobulins: The proceedings of a symposium on transmission of immunoglobulins from mother to young. (Ed) W.A. Hemmings, Cambridge Univ. Press, Cambridge.
36. Ramsey, E.M. 1975. The placenta of laboratory animals and man. Developmental Biology Series (Ed) James D. Ebert. Holt, Rinehart and Winston, U.S.A.: 55.
37. Reynolds, H.Y., Johnson, J.S. 1970. Quantitation of canine immunoglobulins. J. of Immunol. 105, no. 3: 698.
38. Rodewald, R. 1973. Intestinal transport of antibodies in the newborn cat. J. Cell Biol. 58: 189.
39. Silverstein, A.M., Parshall, C.J., and Pendergast, R.A. 1967. Studies in the nature of immunologic immaturity. Ontogeny of Immunity (Ed) R.T. Smith, R.A. Good and P.A. Miescher, Univ. of Fla. Press, Gainesville, FL: 143.

14. Guyer, R.L., Koshland, M.E., and Knopf, P.M. 1976. Immunoglobulin binding by mouse intestinal epithelial cell receptors. *J. of Immunol.* 117, no. 2: 587.
15. Hanson, L.A. and Winberg, J. 1972. Breast milk and defense against infection in the newborn. *Archives of Disease in Childhood.* 47: 845.
16. Heddle, R.J. and Rowley, D. 1975. Dog immunoglobulins I. Immunochemical characterization of dog serum, parotid saliva, colostrum, milk and small bowel fluid. *Immunol.* 29: 185.
17. Hill, I.R. and Porter, P. 1974. Studies of bactericidal activity to *E. coli* of porcine serum and colostrum immunoglobulins and the role of lysozyme with secretory IgA. *Immunol.* 26: 1239.
18. Iyengar, L. and Selvaraj, R.J. 1972. Intestinal absorption of immunoglobulins by newborn infants. *Archives of Disease in Childhood.* Vol. 47: 411.
19. Jacoby, R.O., Dennis, R.A., and Griesemer, R.A. 1969. Development of immunity in fetal dogs: Humoral responses. *Am. J. of Vet. Res.* 30, no. 9: 1503.
20. Jeffcott, L.B. 1972. Passive immunity and its transfer with special reference to the horse. *Biol. Rev.* 47: 439.
21. Jones, R.E. 1976. The selective uptake and transmission of proteins to the circulation from the small intestine of the suckling rat. *Biochimica Biophysica Acta.* 451: 151.
22. Krakowka, S. and Long, D. 1978. Influence of transplacentally acquired antibody on neonatal susceptibility to canine distemper virus in gnotobiotic dogs. *J. of Infect. Diseases.* 137, no. 5: 605.
23. Lecce, J.G., Morgan, D.O., and Matrone, G. 1964. Effect of feeding colostrum and milk components on the cessation of intestinal absorption of large molecules (closure) in neonatal pigs. *J. Nutr.* 84: 43.
24. Lewis, R.M., Smith, C.A., and Garfield, L. 1973. Kinetics of antibody synthesis to particulate and soluble antigen in newborn pups and adult dogs. *Am. J. of Vet. Res.* 34: 235.
25. Mach, Jean-Pierre and Pahud, Jean-Jacques 1971. Bovine Secretory Immune System. In session I. Characteristics of bovine immunoglobulins and related molecules. *J. of Dairy Sci.* 54: 1327.
26. McGuire, T.C. and Crawford, T.B. 1973. Passive immunity in the foal: Measurement of immunoglobulin classes and specific antibody. *Am. J. of Vet. Res.* 34, no. 10: 1299.

40. Smith, K.L., Muir, L.A., Ferguson, L.C., and Conrad, H.R. 1971. Selective transport of IgG1 into the mammary gland: role of estrogen and progesterone. *J. of Dairy Sci.* 54, no. 12: 1886.
41. Steven, D.H. and Samvel, C.A. 1979. The anatomy of placental transfer: Ch. 1. Placental transfer (Ed) G. Chamberlain, A.W. Wilkinson. Pitman Medical Publishing Co. Ltd., Kent, Eng.
42. Thompson, R.E. and Reynolds, H.Y. 1977. Isolation and characterization of canine secretory immunoglobulin M. *J. of Immunol.* 118, no. 1: 323.
43. Tizard, I.R. 1977. An introduction to veterinary immunology. W. B. Saunders Company, Philadelphia, PA.
44. Tsay, D.D., Ogden, D., and Schlamowitz, M. 1980. Binding of homologous and heterologous IgG to Fc receptors on the fetal rabbit yolk sac membrane. *J. of Immuno.* 124, no. 4: 1562.
45. Turner, A.S. 1978. Diseases of foals - perinatal problems. Class supplement. Ohio State University.
46. Vaerman, J.P., and Heremans, J.F. 1969. The immunoglobulins of the dog - III. The immunoglobulins of canine secretions. *Immunochem.* 6: 779.
47. Van Der Meulen, J.A., McNabb, T.C., Haeffner-Cavaillon, N., Klein, M., and Dorrington, K.J. 1980. The Fc_γ receptor on human placental plasma membrane, I. Studies on the binding of homologous and heterologous immunoglobulin. *G. J. of Immunol.* 124, no. 2: 500.
48. Virella, G., Amelia Silveira Nunes, M. and Tamagnini, G. 1972. Placental transfer of human IgG subclasses. *Clin. Exp. Immunol.* 10: 475.
49. Waldman, T.A. and Strober, W. 1969. Metabolism of Immunoglobulins. *Prog. Allergy.* 13: 1.
50. Yurchak, A.M., Butler, J.E., and Tomasi, T.B. 1971. Fluorescent localization of immunoglobulins in the tissues of the cow. In Session I Characteristics of bovine immunoglobulins and related molecules. *J. of Dairy Sci.* 54: 1324.

THE RELATIONSHIP OF SERUM IMMUNOGLOBULIN LEVELS WITH
AGE, SERUM TOTAL PROTEIN, HEMOLYSIS, LIPEMIA
AND NURSING TIME IN THE CANINE
NEONATE

BY

ANNE L. RAY

D.V.M., Auburn University, 1978

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Surgery and Medicine

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1981

ABSTRACT

There is limited information concerning the level of passively received immunity and early competence in the canine. The purpose of this investigation was to gain information concerning serum immunoglobulin levels and their relationship to age in the puppy. Also, investigated was the relationship of serum immunoglobulin levels to other factors such as serum total protein, nursing time, and serum hemolysis and lipemia.

In this investigation blood samples were drawn at regular intervals from birth to 16 weeks of age. Serum IgG, IgM and IgA levels were determined using the radial immuno-diffusion technique. Serum total protein was measured using the colormetric method and the refractometer method.

At 24 hours of age, after all pups had been allowed to nurse, all serum immunoglobulin classes tested (IgG, IgM, IgA) showed a remarkable increase, after which IgG and IgA levels started to decrease. At approximately 4 weeks of age the pups started their own significant production of serum IgG and IgA. Serum IgM levels never dropped due to significant neonatal IgM production throughout the study.

Serum total protein determined by both methods showed a positive correlation with serum IgG and IgM levels.

The degree of hemolysis showed a positive correlation with serum total protein. The degree of lipemia showed a positive correlation with serum total protein determined by the colormetric method.

During the course of study, 13 of the original 24 pups died: 3 due to unknown causes, 10 due to parvovirus gastroenteritis. It could not be shown from this investigation that the serum immunoglobulin levels were directly related to most deaths.