STUDIES ON THE EFFECTS OF FEEDING COLOSTRUM TO NEWBORN CALVES

by

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Part IV

Investigations into Certain Aspects of the Mechanism of Absorption of Globulins from Colostrum

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GENERAL INTRODUCTION

"..... it must be appreciated that most present day methods of rearing calves are harshly artificial and the question that may rightly be asked is not why do calves so reared become ill, but rather how do most of them survive." H. W. Smith (1962).

Considerable losses of neonatal calves are experienced annually in this country. The extent of these losses has been repeatedly estimated in surveys which have been carried out during the past 40 years. Jordan (1933), Smith (1934), Lovell and Bradford Hill (1940), Hector and Rowatt (1948), Withers (1952, 1953). The most recent estimates available have both come from the Animal Health Division of the Ministry of Agriculture. In an analysis of post mortem examinations carried out at Veterinary Investigation Centres during 1959 - 61 on calves of up to 6 months of age, almost 60% were on calves under 4 weeks of age, Veterinary Investigation Service (1964). Within this 4 week period, septicaemia and gastroenteritis were responsible for 66% of the mortalities. A preliminary report of the National Survey of Calf Wastage and Husbandry, Ministry of Agriculture (1966), suggested that there is a wastage of 5 - 6% of calves born which take their first feed. In absolute terms, this represents an annual loss of about 183,000 calves. Although the age range of the calves surveyed was not given, septicaemia and gastrointestinal disorders were responsible for 70% of the mortalities. In dairy herds, 50% of the mortalities occurred within 10 days of birth, and in beef herds the figure of 38% was found for the same period.

The accuracy of such surveys is open to question in respect of how representative they are of the overall picture, and how accurate are the diagnoses, especially when an inevitable delay occurs between

the time of death and necropsy, however, septicaemias and gastro-enteritis appear to be the most important causes of death in young animals. Bearing in mind the work of Smith (1962) who demonstrated that after death there occurs a proliferation of E. coli within the alimentary tract and that the post mortem changes which occur in the gut may simulate the macroscopic appearance of inflammation, the diagnosis of gastroenteritis may not be valid in every case. In addition, it is possible that this diagnosis may be based upon the presence of diarrhoea. Whereas gastroenteritis is almost invariably accompanied by diarrhoea, the converse is not necessarily true, and in calves a gastroenteritis is not a constant feature in the pathology of neonatal diarrhoea. Allowing for these discrepancies in these surveys, a picture emerges of the pattern of losses during the neonatal period. During the first week of life, septicaemia is relatively more important than gastroenteritis, but this position is reversed in the following weeks.

In the aforementioned surveys variations have been observed in the mortality rates of calves born and reared under different systems of management, in different parts of the country and at different times of the year. Significant differences have been shown to exist between the mortality rates in dairy and in beef herds, Withers (1953), Ministry of Agriculture (1966). It has also been shown that the method of feeding colostrum may influence subsequent survival, Withers (1953). Regional variations have been shown to exist between herds of similar type, Withers (1953), Ministry of Agriculture (1966).

A very marked seasonal incidence has been noted in overall mortality. In the National Survey (1966) 50% of the losses occurred

within the period January to April, and in the earlier survey of Withers (1952 - 53), over 40% of the losses occurred within the 3 month period, February to April. Reasons for this variation include the fact that during this period a larger population of calves is at risk, due to the economic advantages obtained by the production of winter milk. Secondly, the mortality rate increases possible as a result of the interaction of many factors such as the build up of infection during the prolonged use of calf pens, Wood (1955), an increase in the virulence of pathogenic strains of infectious agents due to continuous passage in susceptible animals and adverse environmental conditions such as the cold, damp and excessive temperature variation present at this time of year.

Of the infectious agents which have been associated with neonatal disease in the calf, Escherichia coli stands out as the commonest pathogen associated with septicaemia and by virtue of its natural habitat in the intestines, it has often been implicated as a causal agent of neonatal diarrhoea. A review of the role of E. coli in neonatal diseases of calves has been made by Gay (1965), in which the syndromes associated with E. coli (colibacillosis) have been classified into 3 forms on clinical, bacteriological and on possible pathological grounds. These syndromes were described as follows:

a) Colisepticaemia

A syndrome resulting in the rapid death of a calf and associated with an E. coli bacteraemia. Bacteriological isolations from the internal organs of a given case are of a single strain in pure culture.

b) Enteric toxaemia

A syndrome also resulting in the rapid collapse and death of a calf but associated with a massive proliferation of certain specific strains of E. coli in the small intestine. There is no bacteraemia and death is presumed to be due to a toxaemia.

c) Enteric form

This is a syndrome associated with diarrhoea. Death depends on the severity of the physiological disturbances induced.

It would appear that colisepticaemia is caused by certain invasive strains of E. coli. The susceptibility to this disease is much influenced by the degree of passive immunity which has been obtained by the calf. Calves which have acquired antibodies from the cow are resistant to "septicaemic invasion" by E. coli. The specific nature of the protective antibody is as yet unknown as the several possibilities which have been suggested have not been shown conclusively to be important, Briggs (1951), Briggs, Lovell, Aschaffenburg, Bartlett, Kon, Roy, Thompson and Walker (1951), Smith (1962). Experimentally the condition of colisepticaemia can only be produced in colostrum deprived calves of 2 to 3 days of age, Glantz, Dunne, Holst and Hokanson (1959), Smith (1962), Penhale (1965).

The enteric toxaemia form is generally associated with strains of mucoid E. coli which possess A type K antigens and belong to O groups 8, 9 and 101. This syndrome, originally described in Canada by Gay, McKay and Darnum (1964b) has not yet been described in the United Kingdom. Since the publication of this description, observations have been carried out by Smith and Halls (1967a and b)

on the ability of certain strains of E. coli to produce dilatation in ligated segments of small intestine. It was shown that a dilating substance (enterotoxin) was produced by certain pathogenic strains. This enterotoxin was recovered from strains of E. coli which had the antigenic structure 08:K?; 09:K9; and 0101:K?, and which had been recovered from calves with diarrhoea. A strain with an antigenic structure 078:K80, which is one of the strains commonly associated with colisepticaemia did not produce any dilatation. It is interesting to note that an untypable strain investigated by Smith and Halls (1967a) was isolated from an outbreak of disease in very young calves in the United Kingdom. The clinical description, the failure to isolate E. coli from the internal organs and the high level of passive immunity are all factors which might make one suspect that this was an outbreak of enteric toxaemia. It is possible that this syndrome does exist in this country but is at present being misdiagnosed.

The importance of E. coli in the pathogenesis of the enteric form of colibacillosis is still in doubt. This is largely due to the inability of most workers to reproduce the syndrome under carefully controlled conditions. Smith (1962) listed a series of points which in addition to the one just mentioned, suggested to him that there was no bacterial factor involved in the causation of diarrhoea. These points included the absence of incriminating serological findings, the similarity of phage types of E. coli found in the small intestine of diarrhoeic and healthy calves, and the changes in the predominant phage type of E. coli during a diarrhoeic episode. The same author did suggest that the primary condition may

be aggravated by bacteria, a fact which might explain the beneficial results obtained by the use of certain antibiotics either in prophylaxis or treatment.

In the description of colisepticaemia it was mentioned that susceptibility to this disease was very much dependent on the absence of protective antibodies acquired by the calf from its dam. These antibodies are present in colostrum and are absorbed intact from the intestine by a mechanism which is active for the first 24 - 36 hours of life. The relationship between the ingestion of colostrum and the development of septicaemia was demonstrated first by Smith and Little (1922). Of 10 calves which were allowed to drink colostrum, all survived. In contrast, only 3 of a group of 12 colostrum deprived calves survived. Further confirmation of this relationship was presented by Aschaffenburg and co-workers (1949a,b; 1951a,b; 1953) and Ingram, Lovell, Wood, Aschaffenburg, Bartlett, Kon, Palmer, Roy and Shillam (1956). In a series of experiments conducted during 1950 to 1953 and involving 328 calves, 26% of the 225 calves which received colostrum died compared with 91% of the 103 calves which were colostrum deprived.

Until the work of Fey and Margadant (1961) in Switzerland, no investigation had been carried out into the immune globulin levels of neonatal calves born and reared under normal farm conditions and their relationship to disease. These workers found that 5 (10.9%) out of 46 "normal" calves at 1 week of age had a pronounced hypo-gammaglobulinaemia in spite of having received colostrum. They also found that 21 (95.5%) out of 22 calves which died of colisepticaemia were deficient in passively acquired globulins. In view of these

findings and the fact that disease in neonatal calves is a large problem in this region it was decided to investigate the problem of deaths in newborn calves in relation to their state of passive immunity.

The first problem encountered in this investigation was the choice of method of measuring the concentration of circulating immune globulin. Of necessity, the test chosen had to be relatively simple and quick to perform since it was hoped that it might be used as a method of screening large batches of calves. The test which appeared most suited for this purpose was the zinc sulphate turbidity test described by Aschaffenburg (1949). The test was originally designed by Kunkel (1947) to measure alterations in the concentration of gamma globulins in the serum of humans. This author and others, de la Huerga (1950), Adner (1957), Discombe (1959), Reinhold (1960) have investigated the correlation between the turbidity reaction developed by this test and changes in the serum protein profile consequent upon disease.

In the test described by Aschaffenburg (1949) certain modifications were made to the original technique, the major one being an increase in the concentration of the zinc sulphate reagent. This permitted the detection of the increases in the serum immune globulin concentration which occur after colostrum has been fed to newborn calves. No investigation was made by Aschaffenburg either into factors which might affect the test or into a correlation between the immune globulin present and the intensity of the turbidity reaction.

From the foregoing it is evident that there is a lack of information on factors which might affect the zinc sulphate turbidity test when applied to calf serum. Therefore, in the first part of this study

there is described an investigation using neonatal calf serum, into some of the factors which have been shown to influence the reaction with human serum, so that the relative importance of these factors might be assessed. In view of this assessment a standardised procedure has been adopted for the performance of the test. Using this procedure the zinc sulphate turbidity reaction has been calibrated in terms of specific immune globulin fractions.

In part II of this study, by means of the zinc sulphate turbidity test, observations have been made on the serum immune globulin concentrations of neonatal calves. The changes which occur in the concentrations of the immune globulins during early life have been followed by other workers, mostly by measurement of the concentrations of specific antibodies or electrophoretically determined globulin fractions. By use of the zinc sulphate turbidity test, these changes have been followed in calves during the first 3 weeks of life.

Secondly, in view of the relationship between colostrum deprivation and colisepticaemia, an investigation has been carried out into the correlation between the concentrations of immune globulins in the serum of market calves and neonatal mortality, and finally, evidence is presented in the form of a two year survey which demonstrates the relative immune globulin concentrations of market calves in relation to season.

As a result of the absorption of immune globulin molecules intact from colostrum, large quantities of protein enter the circulation. In addition to their immunological function, other physical characteristics of these proteins may exert effects which may aid the survival of the neonatal calf. The intravenous infusion of protein solutions has been

recognised as a method of expanding the plasma volume through an osmotic effect. Moreover, by virtue of their chemistry, proteins are known to exert a buffering action in solutions in which there is a tendency to changes in pH. Both of these aspects have been investigated in part III of this study.

In the ultimate part of this study, a review has been made of the literature relating to the absorption of immune globulins by domestic animals. In calves two particular aspects of the absorption mechanism have been investigated. The first aspect studied was the effect of the time of feeding on the final concentration of serum immune globulin. The other aspect investigated was an assessment of the efficiency of the absorption mechanism.

PART I

DESCRIPTION AND CALIBRATION OF THE ZINC SULPHATE TURBIDITY TEST

SECTION A

Description of Factors Influencing the Zinc Sulphate Turbidity Reaction and its Measurement

A method of estimating the alterations in the serum gamma globulin concentration by a turbidimetric technique was described by Kunkel (1947). This technique involved the use of a very dilute solution of zinc sulphate and its immediate application was in the measurement of serum gamma globulins in humans suffering from liver disease. The concentration of zinc sulphate used gave minimal precipitation with normal serum, but a readily detectable reaction was obtained with hyperglobulinaemic sera. A correlation was found between the turbidity and the gamma globulin concentration as calculated from the electrophoretic pattern as well as between the turbidity and the total globulin concentration as determined by the Howe (1921a and b) fractionation technique.

In his original work, Kunkel (1947), showed that by increasing the concentration of the reagent used a corresponding increase occurred in the percentage of protein precipitated. Aschaffenburg (1949) made use of this fact and adapted the original test of Kunkel (1947) for use in newborn calves. The test then provided a method of detecting the increases in the globulin fraction of serum proteins which occur after the absorption of immune globulins from colostrum. The main alteration in the original technique was that of increasing the concentration of zinc sulphate from 24 mgms/litre to 208 mgms/litre. This was necessary in order to obtain conditions under which no turbidity occurred with the serum of calves deprived of colostrum, whereas turbidities of an intensity reflecting the amount of protein ingested developed after the calves had been given colostrum. In the original

publication, Kunkel (1947) found a close correlation between zinc sulphate turbidity and gamma globulin as well as total globulin. This finding was supported by Discombe, Jones and Winstanley (1954), who, using zone electrophoresis found a coefficient of correlation of 0.91. Other workers, de la Huerga, Popper, Franklin and Routh (1950); Ricketts, Sterling, and Levine (1951) and Adner (1957) have found the relationship to be less close. Wilson, Brown and Hainline (1957) found a coefficient of correlation of 0.59 using moving boundary electrophoresis to measure the concentration of gamma globulin and Reinhold (1960) found a similar correlation.

In a series of calves fed different quantities of the non-fatty fraction of colostrum an increase in the zinc sulphate turbidity as measured in a photoelectric absorptiometer was found in those which received 200 ml. and over, Aschaffenburg (1949). The calves which received most had on average the highest turbidity. The increases in total serum and euglobulin nitrogen were not consistent and no attempt was made to correlate these increases more precisely with the zinc sulphate turbidity.

The conditions defined by Kunkel (1947) under which the test was performed were determined as a result of investigations into some of the factors which may influence the turbidity reaction. These factors included the effects of pH, ionic strength and albumin concentration. Later authors have repeated these investigations and have described other factors, de la Huerga et al. (1950), Discombe et al. (1954), Yonan and Reinhold (1957), Reinhold (1960), the most important of which would seem to be the influence of carbon dioxide and the temperature at which the test is performed. All of these investigations have been performed on human sera using the more dilute

reagent. Therefore, a study has been made of how these factors affect the zinc sulphate turbidity test performed on calf sera and using the procedure described by Aschaffenburg (1949). The factors investigated are the choice of filter, the effects of time and temperature, pH, ionic strength, albumin concentration, gamma globulin concentration, and the use of plasma or serum.

SUB-SECTION A

Determination of the Optimum Filter for Use in the Measurement of Turbidity

Introduction

The majority of workers (Aschaffenburg, 1949; de la Huerga, 1950; Discombe et al., 1954; Neimann-Sorensen, Konggaard and Kruse, 1966) have adopted the recommendation of Kunkel (1947) and have used light of 650 mμ wavelength or an Ilford No. 608 filter when measuring the zinc sulphate turbidity in a spectrophotometer or colorimeter. Smith (1962) performed the zinc sulphate test on calf sera using the filter described by Aschaffenburg, but in a later paper (Smith, O'Neill and Simmons, 1967) the test was modified by omitting the use of a filter. No reason was given for this change of technique. Shank and Hoagland (1946) determined the absorption spectra of barium sulphate, haemolysed red blood cells and a dilution of serum containing 14 mg% of bilirubin through the range of 400 to 700 mμ. At 650 mμ minimal interference from light absorption due to bilirubin or haemolysed red cells occurred. As the wavelength increased through the range, there was a progressive decrease in the percentage of light absorbed by the barium sulphate solution. Patterson (1967) compared three simple laboratory tests for gamma globulins in calf sera, one of which was the zinc sulphate turbidity test. He described how more precise measurements of the turbidity were possible by use of a colorimeter with a 625 (yellow-green) filter, or a spectrophotometer using light of 450 mμ wavelength. It has subsequently been confirmed by Patterson (personal communication, 1968) that higher "absorbance" is obtained with light of 400 - 550 mμ than with light of 650 mμ wavelength.

Because of the relatively poor light absorption obtained at 650 mμ wavelength it was decided to investigate the use of other wavelengths in measuring the zinc sulphate turbidity.

Experiment

To Determine the Absorption Spectra of Neonatal Calf Haemoglobin and the Turbidity Produced in Calf Serum by the Zinc Sulphate Reagent

The zinc sulphate turbidity test was performed on a non-haemolysed serum sample. The turbidity was measured on an EEL colorimeter using the series of Ilford spectrum filters Nos. 621 - 608. For each filter used, the colorimeter needle was adjusted to zero using a "blank" solution of distilled water.

A solution of haemolysed calf red cells was then prepared. This was done by centrifuging a heparinised blood sample taken from a calf. The supernatent plasma was poured off and the red cells were re-suspended in saline and then centrifuged again. This procedure was repeated twice in order to provide a suspension of red blood cells. These were then ruptured mechanically, re-suspended in saline and centrifuged in order to minimise the cellular content of the supernatent fluid which was then added to a colorimeter tube. The absorption spectrum of calf haemoglobin was then determined by a similar procedure to that described above.

The response curves of the Ilford spectrum filters are given in the handbook of "Operating Instructions" provided with the EEL colorimeter. From the figure therein, the wavelength corresponding to the maximum transmission for each filter was determined and is shown in Table 1.1.

Table 1.1

Filter No.	Colour	Wavelength (mμ)
621	Violet	450
622	Blue	460
623	Blue-green	490
624	Green	520
625	Yellow-green	540
626	Yellow	570
607	Orange	600
608	Red	680

The colorimeter reading for each filter tested was then plotted against this wavelength, and is shown in Figure No. 1.1 These results indicate that for both solutions greater "absorbance" and hence sensitivity is found when light of wavelengths shorter than 650 mμ are employed.

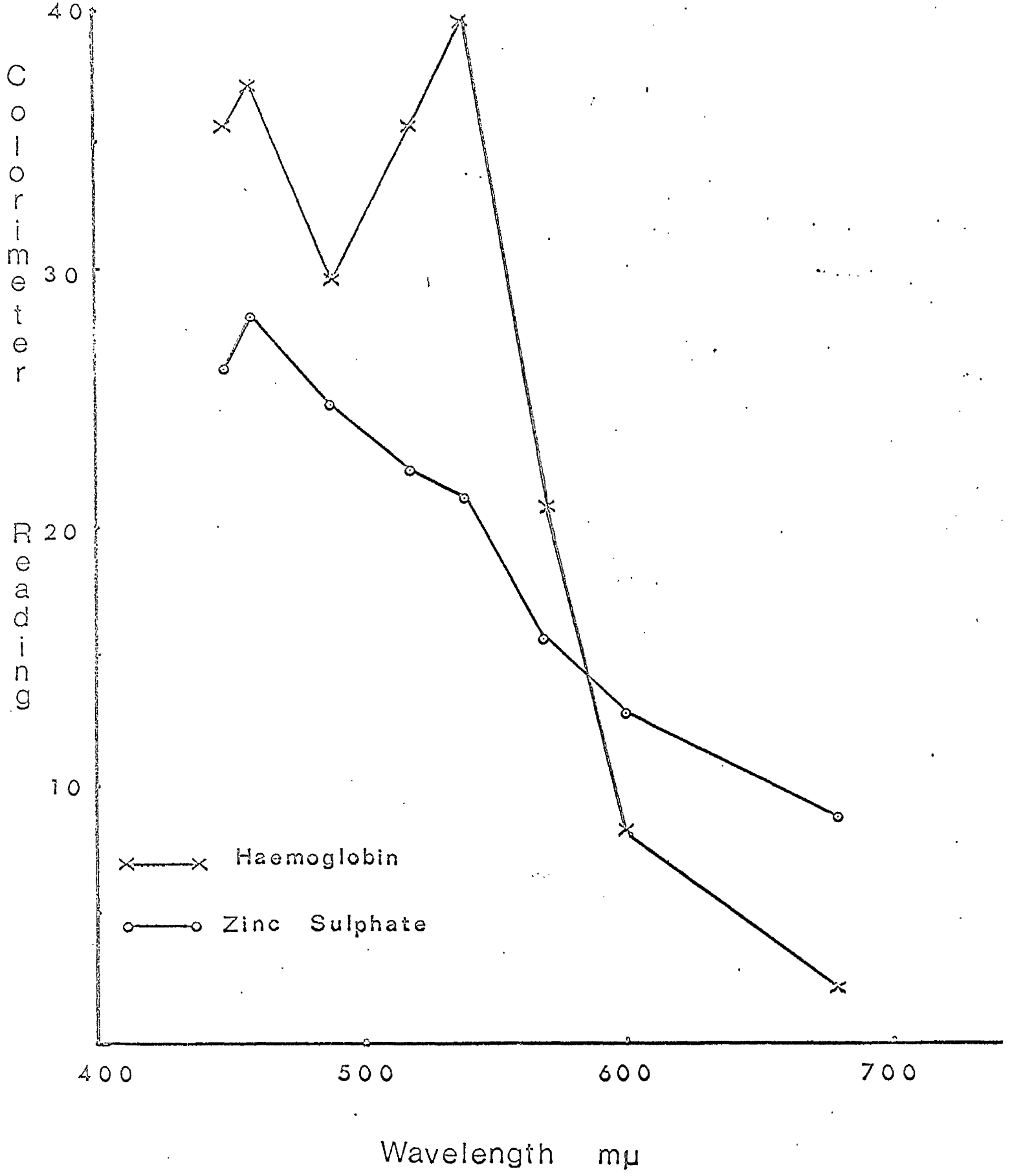
Discussion

It would appear that the absorption spectra obtained for the zinc sulphate turbidity test and haemolysed calf red blood cells is approximately similar to that obtained by Shank and Hoagland (1946). There is greater sensitivity to both zinc sulphate turbidity and haemoglobin at the shorter wavelengths i.e. filters Nos. 621 - 625. Within this range filter No. 623 would appear to be least sensitive to haemoglobin.

Conclusions

In the routine performance of the test it was decided therefore to use a No. 623 filter and to include a control tube containing 0.1 ml. serum and 6 ml. distilled water. This procedure minimises the effects of haemolysis while giving greater sensitivity to the measurement of turbidity.

Fig. 1.1. The absorption spectra of calf haemoglobin and the turbidity produced by the zinc sulphate turbidity reaction.



SUBSECTION B

The Effects of Time and Temperature on the Zinc Sulphate Turbidity Reaction

Introduction

As with any chemical reaction, the rate at which turbidity develops is influenced by the temperature of the reagents and the time allowed for the development of the reaction. Although most workers have adopted standard conditions of time and temperature for the zinc sulphate reaction relatively little investigation has been made of the influence of these factors on this test.

Kunkel (1947) allowed the reaction to proceed for 30 minutes before measuring the amount of turbidity. No mention was made of temperature control so presumably it was performed at room temperature. Discombe et al. (1954) allowed the reaction "more than 10 but less than 120 minutes", and made no specific mention of temperature. Yonan and Reinhold (1957) allowed the test to stand for 30 minutes and maintained it at 25°C ± 1°. Aschaffenburg (1949) allowed 1 hour to pass before measuring the intensity of the flocculum and noted that at this time the reaction was not quite complete.

De la Huerga and Popper (1950) using another turbidity test to estimate the amount of gamma globulin present in serum noted that turbidity increased rapidly during the first fifteen minutes and then became fairly constant for more than one hour. After twenty four hours the readings were greater by 10 - 15% than the 30 minute readings. This they attributed to hydration of the precipitate, since chemically both amounts were identical. They concluded that a period of 30 minutes was best. The same authors noted that there was about 30% less turbidity if the reaction was performed in the "ice box"

(actual temperature not given) and a 30% increase if performed at 37°C. At 56°C there was an increase in turbidity of up to 50%. In the range between 20°C and 30°C the differences did not exceed 1% per centigrade degree. These differences were ascribed to alterations in hydration and it was suggested that for precise determinations the test should be performed at 25°C. Nelmann-Sorensen, et al. (1966), using the zinc sulphate turbidity test on calf serum, noted that the reaction was very sensitive to temperatures over 20°C. They have performed this test at 22 - 23°C and have measured the turbidity after 60 - 70 minutes. Patterson (1967) assessed the turbidity 60 minutes after mixing, but no mention is made of temperature control.

Experiment

To Determine the Effects of Time and Temperature on the Development of the Zinc Sulphate Turbidity Reaction

In order to elucidate the effects that time and temperature have on the amount of turbidity developed, the following experiment was performed.

Ten serum samples from young calves were selected at random. The zinc sulphate turbidity test was performed as follows.

To a 10 ml. colorimeter tube containing 6 ml. of zinc sulphate solution (208 mg. per litre), 0.1 ml. serum was added (Test). An identical volume of serum was added to a matched colorimeter tube containing 6 ml. of distilled water (Control). The reagents were mixed by gentle shaking for a few seconds and then allowed to stand in a test tube rack until the required time had elapsed. The solutions were gently shaken again to ensure an even suspension. The turbidity was measured on an EEL colorimeter, using a Blue-green filter (Ilford No. 623) and having previously zeroed the instrument with a tube

containing distilled water. The difference between the reading obtained from the test and control tubes was taken as the turbidity reading. The test was read at four fixed time intervals after the initial mixing of serum and zinc sulphate solution, namely 15, 30, 60 and 120 minutes. Five different temperatures were chosen and the serum was added to the zinc sulphate solution after it had equilibrated with the ambient temperature. The lowest temperature ($6^{o}C$) was obtained by allowing the tubes to stand in a refrigerator. The upper three temperatures ($25^{o}C$, $31^{o}C$, $37^{o}C$) were obtained by standing the tubes in a water bath. The fifth temperature ($20^{o}C$) was the ambient temperature of the laboratory. The results of this experiment are shown in Figure 1.2.

From these results it can be seen that the reaction continues to develop throughout the period of 120 minutes and is influenced greatly by temperature. Table 1.2. shows the effect of temperature on the rate of development of the turbidity reaction.

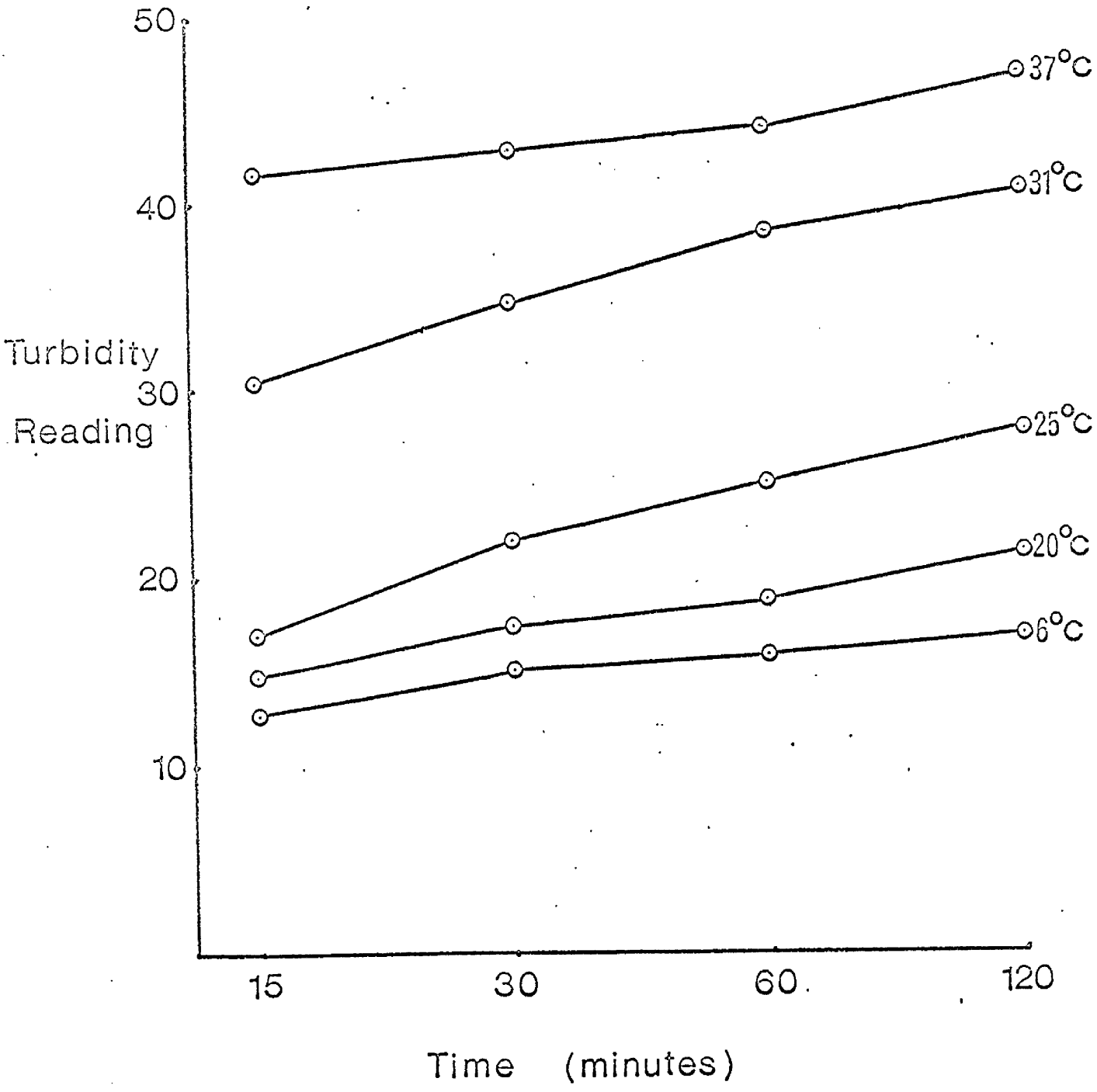
Table 1.2.

Temperature Range (^{o}C)	6 - 20	20 - 25	25 - 31	31 - 37
Time (mins)	Percentage Increase in Turbidity per Centigrade Degree			
15	1.05	2.7	13.5	6.2
30	1.08	5.1	9.6	3.9
60	1.26	6.7	9.0	2.4
120	1.97	6.0	7.8	2.5

Discussion

It would appear that for any time between 30 and 120 minutes the rates of development of the turbidity reaction are quite similar.

Fig. 1.2. The effects of time and temperature on the development of the zinc sulphate turbidity reaction in calf serum.



However, the rate of development is not uniform throughout the range of temperatures studied, the greatest rate being seen in the 25 - 31°C range. Compared with the rate of 1% per Centigrade degree described by de la Huerga and Popper (1950) using the ammonium sulphate-sodium chloride turbidity test, the percentage increases found here for the same range of temperatures are much higher.

Conclusion

In view of these findings it would appear that the reaction is particularly sensitive to temperatures above 20°C, whereas the effect of time is relatively constant. Ideally, the test should be performed at a constant temperature in the region of 20°C and should be read after a fixed time has elapsed from the start of the test, probably one hour. In the subsequent studies of factors influencing the zinc sulphate turbidity reaction and in the adoption of a standardised technique, the test has been performed at about 20°C (room temperature) and has been read after one hour.

SUB-SECTION C

The Influence of pH on the Development of the Zinc Sulphate Turbidity Reaction

The pH of the reagent is of importance. It was shown by Gurd and Goodman (1952) that the binding of zinc ions by human serum albumin was competitive with that of hydrogen ions and that a fall in pH occurred when zinc ions were added to a solution containing albumin. Tanford (1952) and Gurd and Goodman (1952) presented evidence which suggested that zinc ions combine with imidazole groups of histidine residues of proteins.

Kunkel (1947), using a copper sulphate solution, showed the effects of varying pH and ionic strength. He found that the best differentiation between normal and hepatitis serum occurred in the pH range 6.5 - 7.5 and in the presence of a low ionic strength (I = 0.01). In order to maintain a constant pH of 7.5, he used a barbiturate buffer containing 280 mg. barbituric acid and 210 mg. of sodium barbiturate per litre. His ultimate preference for zinc sulphate instead of copper sulphate was influenced by the reaction of copper with barbiturate, with which it forms a precipitate, if kept for any length of time.

Aschaffenburg (1949) found it unnecessary to use buffer and used only distilled water of neutral pH in making up the zinc sulphate solution. Where necessary, the pH of the water was adjusted to neutrality by the use of dilute acid or alkali. Other workers, however, have continued to use barbiturate buffer, Discombe (1954), Yonan and Reinhold (1957), although Reinhold (1960) had cause to alter the relative concentrations of the reagents to 302 mg. barbituric acid and 190 mg. sodium barbiturate per litre because the original buffer

concentrations provided an alkaline pH in his laboratory. He claimed that deviations of more than 0.05 from pH 7.5 would cause alterations in the turbidity produced.

It is interesting to note that Maclagan (1948) doubted that a buffer of ionic strength lower than 0.01 would be able to exert any decisive influence on the pH of the reaction mixture. The buffer proposed by Kunkel (1947) has an ionic strength of about 0.001 and therefore may not be effective in regulating pH.

Serious changes in the pH of the reagent were found to occur if the solution was allowed to absorb carbon dioxide, Discombe, Jones and Winstanley (1954). In order to overcome this source of error, Discombe (1959) kept the zinc sulphate reagent in an automatic burette protected by a soda lime tube. The serum sample was dispensed using a capillary pipette and a teat to prevent possible change of pH if expired air were to enter the fluid. Reinhold (1960), recommended that the distilled water used for making up the reagent should be boiled for 10 minutes in order to drive off dissolved carbon dioxide.

Yonan and Reinhold (1957) noted that zinc sulphate turbidity readings were lowered if the measurements were delayed by one or two hours after separating the serum from the clot. Although actual measurements were not made, it was assumed that this was due to a loss of carbon dioxide. A series of serum samples in which gaseous exchange with the atmosphere had been minimised by use of a mineral oil seal, gave unexpected results. The zinc sulphate turbidity rose significantly after storage. The reason for this was unexplained. Reinhold (1960) stated that by exposing serum which has equilibrated with the atmosphere to alveolar air or a gas mixture containing 5% carbon dioxide in

nitrogen for one to two minutes the original turbidity was almost restored. Reinhold and Yonan (1956) found that removal of free carbon dioxide by exposure of sera to a vacuum lowered the readings. Exposure to an atmosphere high in carbon dioxide caused an increase in the readings.

Possible explanations for this phenomenon were put forward by Reinhold (1960) who suggested that carbon dioxide may form carbamino derivatives which then react with zinc ions to form insoluble salts. Alternatively, the combination of carbon dioxide with gamma globulin may alter the configuration of the protein molecule so that the imadazolyl groups become more accessible to the zinc ions.

Experiment

To Determine the Influence of pH on the Development of the Zinc Sulphate Turbidity Reaction

The following experiment was performed to find out what effect pH had on the zinc sulphate reaction. Twenty calf sera with turbidity readings between 0 and 40 were selected. These sera were then tested using:

1) Unbuffered zinc sulphate solution, Aschaffenburg (1949).

2) Zinc sulphate solution to which had been added barbiturate buffer in the proportions described by Kunkel (1947), i.e. 280 mg. barbituric acid and 210 mg. sodium barbiturate per litre.

3) A zinc sulphate solution to which had been added barbiturate buffer in the proportions described by Reinhold (1960), i.e. 302 mg. barbituric acid and 190 mg. sodium barbitone per litre.

The test was allowed to develop for 60 minutes at room temperature before being read.

The measured pH of these solutions were respectively:

1) pH = 4.7

2) pH = 7.53

3) pH = 7.6

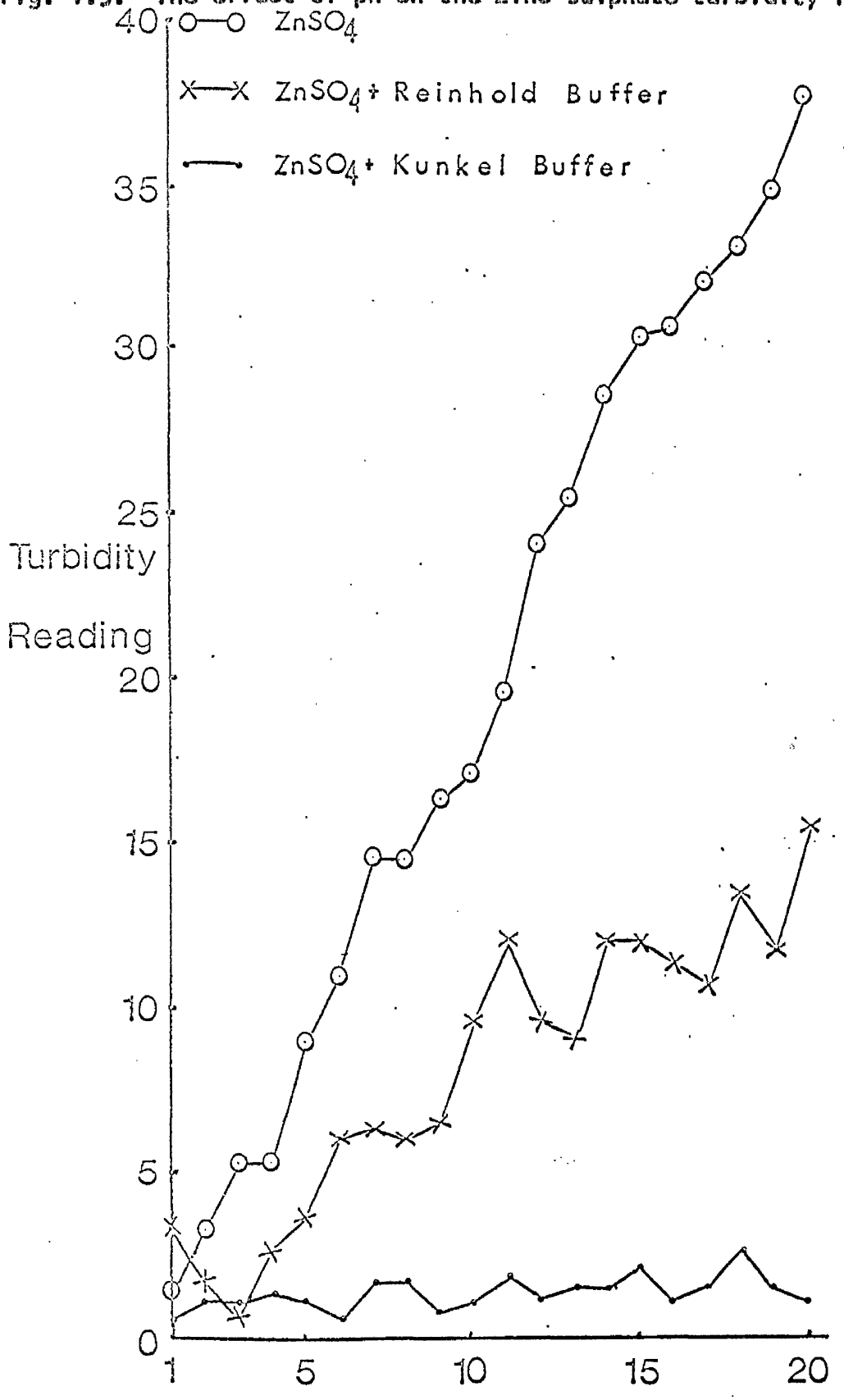
The results are shown in Figure 1.3.

It was found that the Kunkel buffer depressed the development of the reaction so that only one reading higher than 2.5 was obtained. Compared with the unbuffered solution the Reinhold buffer gave results which were consistently lower, the difference being most marked at the highest levels.

Discussion

These results would appear to confirm the importance of the pH of the reagents. The poor results obtained with the Kunkel buffer would agree with the findings of Reinhold (1960), who noted that it produced too alkaline conditions for the test. A possible reason for the difference existing between the unbuffered reagent and the one containing the Reinhold buffer may be the increased ionic strength of the buffered solution. However, the calculated ionic strength of the buffered zinc sulphate solution is I = 0.0039. (For buffer systems consisting of a weak monobasic acid and its alkali metal salt..... the ionic strength is equal to the concentration of the salt (Long, 1961). By reference to Figure 1.4. showing the effect of ionic strength on the development of turbidity, it can be seen that the depressant effect may be calculated from the regression equation, i.e. $y = 2.987 + 1.805$. For an ionic strength of 0.0039, the corresponding depression is 0.7 zinc sulphate units. It would appear, therefore, that the increased ionic strength of the buffered solutions is not the major factor causing the depression.

Fig. 1.3. The effect of pH on the zinc sulphate turbidity reaction.



Conclusions

It would appear that under the conditions existing in this laboratory higher turbidity readings may be obtained by the use of unbuffered zinc sulphate reagent. The buffers recommended by Kunkel (1947) and Reinhold (1960) cause a depression of the turbidity reaction. In the routine performance of the turbidity test, therefore, no buffers have been included.

SUB-SECTION D

The Influence of Ionic Strength on the Zinc Sulphate Turbidity Reaction

Introduction

Ionic strength is a measure of the electrical field existing in a solution and is calculated from the formula $I = \frac{1}{2}\sum [I] Z_I^2$ where I = ionic strength, I = concentration of the ion, Z_I = the net charge of the ion and $\sum$ implies a summation over all the ions in the solution. Use has been made of this fact in many of the flocculation tests used in human medicine. Maclagan (1948) reviewed 9 tests, including the zinc sulphate test of Kunkel (1947) and showed that the serum dilution factor ranged from 1 to 61 and the ionic strength of the various reagents varied from 0.15 to 0.002.

Kunkel (1947), using a copper sulphate solution, observed the turbidity produced under different conditions of pH and ionic strength. He found that greater turbidities occurred when a solution of ionic strength I = 0.01 was used rather than one of strength I = 0.1.

Experiment

To Determine the Effect of Ionic Strength on the Zinc Sulphate Turbidity Reaction

The method used to investigate the effect of ionic strength on the intensity of the turbidity reaction was as follows. A solution of zinc sulphate (250 mg./litre) was prepared using boiled distilled water. From 10 ml. of physiological saline (8.5 g. NaCl per litre) a series of 1:1 dilutions was prepared, i.e. 1/2, 1/4, 1/8, 1/16, again using distilled water as diluent. The ionic strengths of the original and the modified zinc sulphate solution are shown in Table 1.3.

Table 1.3.

	Original	1/16	1/8	1/4	1/2	1
Ionic Strength (1×10^{-3})	2.8	4.4	5.9	8.9	15	27.1

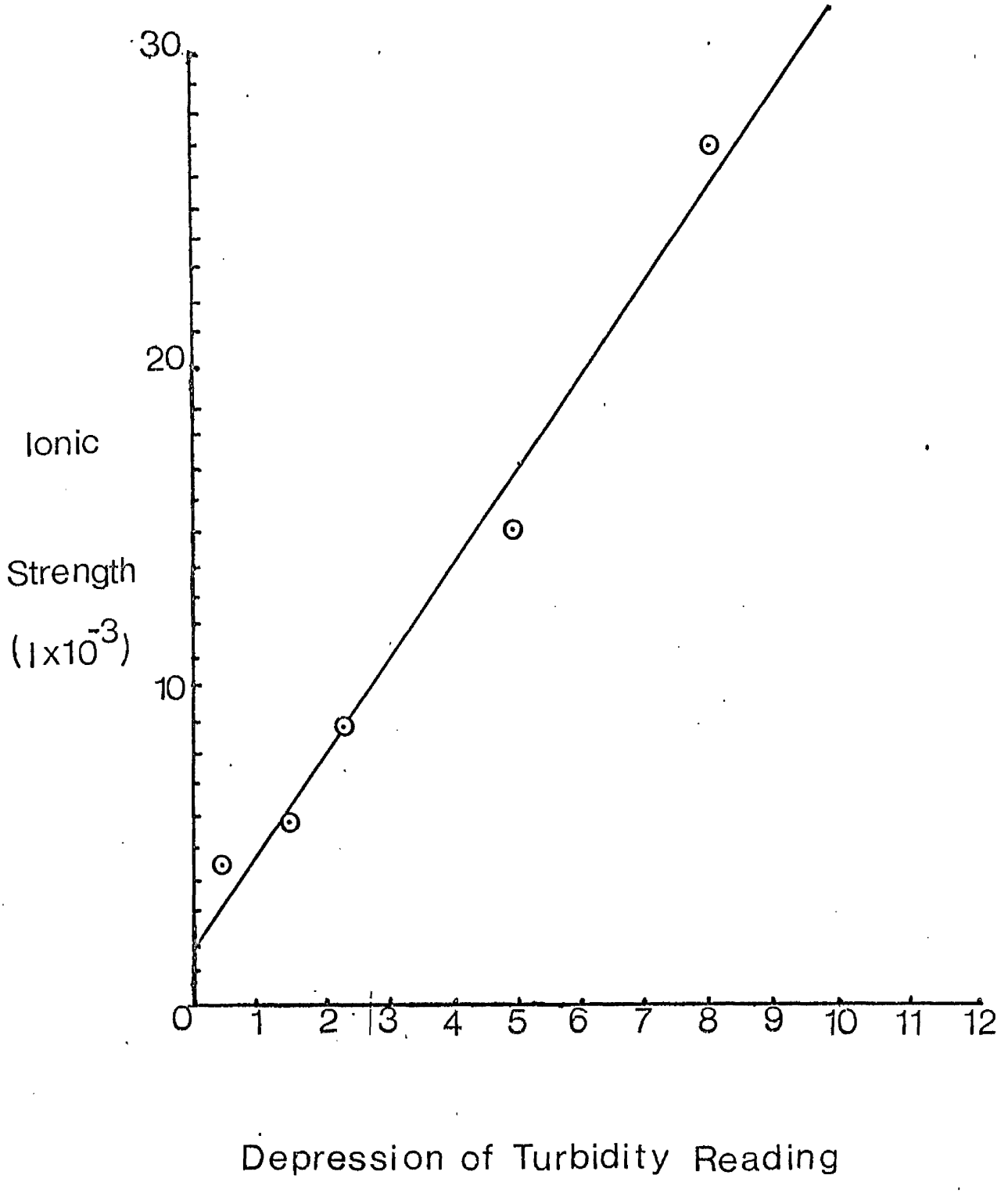
The test was carried out using 5 ml. of the zinc sulphate solution, 1 ml. of the saline or one of its dilutions and 0.1 ml. of serum. The effect of ionic strength on the intensity of the turbidity reaction was found in 10 different serum samples, the turbidities of which ranged from 15 to 50. The results obtained are shown in Figure No. 1.4.

The effect of increasing the ionic strength of the reagent is such as to cause a depression of the turbidity developed. The range of ionic strengths tested was from I = 0.0044 to I = 0.0271. It will be seen that the regression line intercepts the y axis at 0.0018, a value which is only 0.001 removed from the calculated ionic strength of the original zinc sulphate solution.

Discussion

The results obtained in this investigation confirm the importance of low ionic strength in this reaction. In order to obtain this low ionic strength a serum dilution factor of 61 has been employed. This is necessary to reduce interference from electrolytes present in the serum. In view of this it is interesting to speculate whether the alterations in the fluid and electrolyte concentrations seen as a result of certain diseases would be reflected by an alteration of the zinc sulphate turbidity. For example, in neonatal diarrhoea in calves, the loss of fluid and ultimate reduction of the plasma volume, Fisher (1965), might be expected to cause an increase in the turbidity reaction. However, there is a simultaneous metabolic acidosis, the

Fig. 1.4. The depressant effect of increasing ionic strength on the zinc sulphate turbidity reaction in calf serum.



effects of which might depress turbidity due to change of pH and the disturbance of the relative proportions of electrolytes and possibly ionic strength.

Conclusion

The results found here agree in principle with the findings of other workers. Factors which cause an increase in the ionic strength of serum or the zinc sulphate solution will tend to cause a depression of the turbidity reaction.

SUB-SECTION E

The Influence of Albumin Concentration on the Development of the Zinc Sulphate Turbidity Reaction

In patients with liver disease, Kunkel (1947) produced a slight fall in the intensity of the turbidity reaction by administering intravenously large amounts of human albumin. He also demonstrated by adding human albumin to positively reading serum taken from patients suffering from hepatitis, that there was a decrease in the amount of globulin precipitated. From a graph, it appeared that the depression did not become evident until the concentration of albumin exceeded 1 g. per 100 ml. above which concentration the depression increased until concentrations of about 20 g. of albumin per 100 ml. were obtained.

De la Huerga and Popper (1950) attempted to study the mechanism of zinc sulphate turbidity by adding human serum albumin to gamma globulin solutions and to sera. When albumin was added to 1.2% and 2.5% gamma globulin solutions, up to a concentration of 1 g. per 100 ml. the effect was to raise the intensity of the turbidity. Levels in excess of this, however, had a depressant effect. The result of adding albumin (1.5 g. per 100 ml.) to normal and pathological sera was to depress the turbidity by 20 - 50%, the percentage depression being lower in these sera with high turbidities.

Experiment

To Determine the Influence of Increasing Albumin Concentrations on the Turbidity Reaction

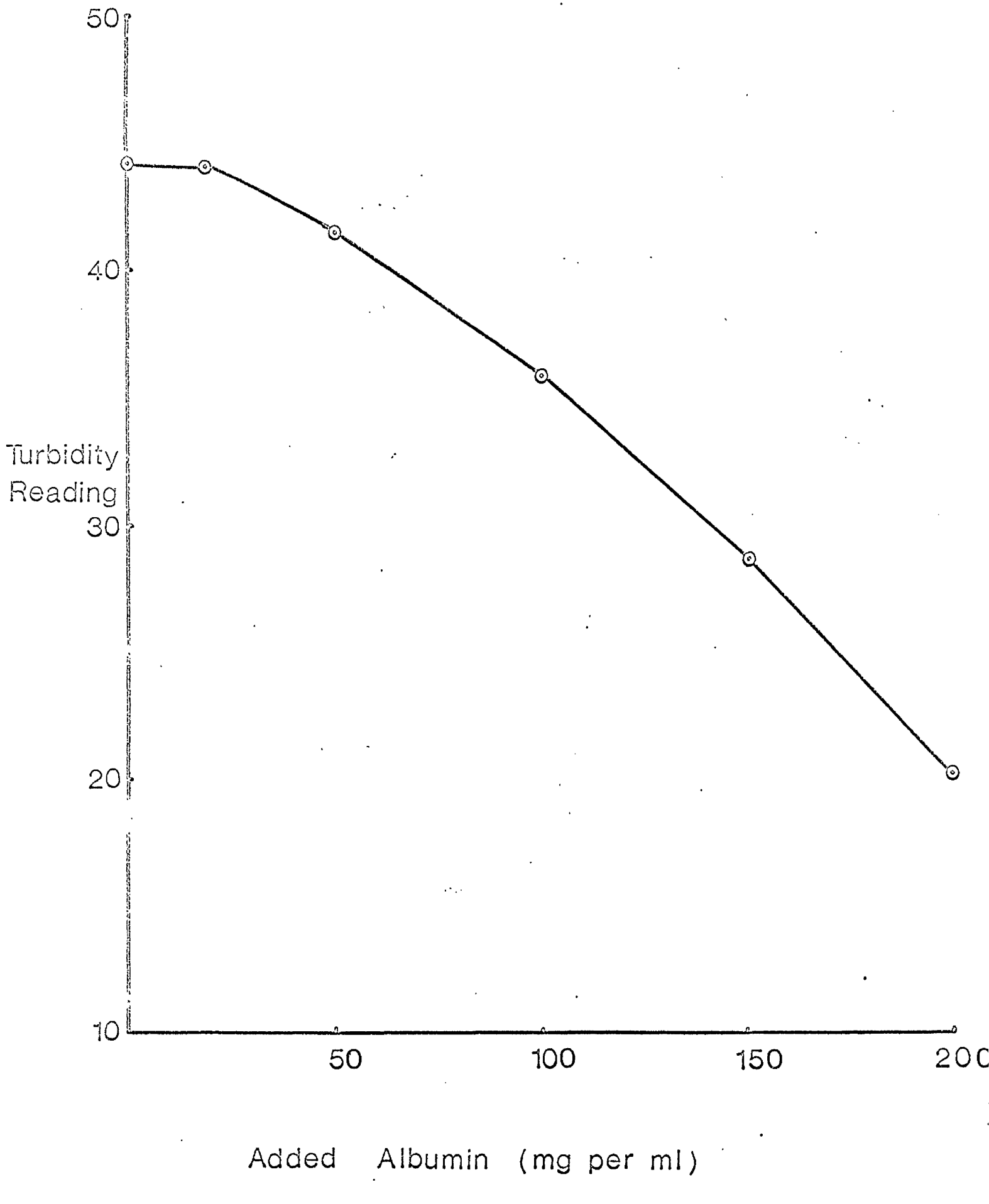
The method used to determine the influence of albumin on the zinc sulphate reaction was as follows. A range of concentrations (2.5, 5, 10, 15, 20 mg. per ml.) of bovine albumin solution was prepared by

dissolving crystallised bovine albumin (Armour Pharmaceutical Company Ltd., Eastbourne, England) in distilled water and making serial dilutions. A more concentrated solution of zinc sulphate (250 mg. per litre) than the one described by Aschaffenburg (1949) was prepared using boiled distilled water. This increased concentration is to allow for the dilution which occurs when the albumin solution is added. Five serum samples with turbidity readings ranging between 30 and 50 were selected and the test performed in the following manner. For each concentration of albumin solution 5 colorimeter tubes were set up. Into each was added 5 ml. of the zinc sulphate solution, 1 ml. of the appropriate albumin solution and 0.1 ml. of each serum sample. Thereafter, the performance of the test was as described previously. A depression of the turbidity reaction was noted only at albumin concentrations of greater than 25 mg. per ml. The results are shown in Figure No. 1.5.

Discussion

The range of albumin concentrations studied in this experiment is equivalent to the range 25 - 200 mgs. per ml. It was found that increasing concentrations of albumin had a depressant effect on the development of the turbidity reaction. The higher concentrations of albumin used by Kunkel (1947), i.e. up to 200 mgs. per ml. are outwith the values seen in normal or pathological sera. However, the depression of turbidity observed by this author began to be apparent at levels greater than 10 mg. per ml. De la Huerga et al. (1950) found that the depressant effect was obvious at this concentration. From the results obtained in the present work it would appear that there is very little depression of turbidity below concentrations of 25 mg. per ml. To confirm this point, a second series of albumin concentrations was

Fig. 1.5. The effect of albumin concentration on the zinc sulphate turbidity reaction.



prepared, equivalent to the range 0 to 40 mg. per ml. The results are shown in Table 1.4. and from these it would appear that a significant depression of turbidity does not occur within this range of concentrations.

Table 1.4.

The Effect of Albumin on the Zinc Sulphate Turbidity Reaction

Added Albumin mg./ml.	0	2.5	5.0	10.0	20.0	30.0	40.0
Average Turbidity	43.7	42.9	43.2	43.4	44.0	42.5	42.5
Difference	-	-0.8	-0.5	+0.1	-0.3	+0.3	-1.2

The normal concentration of albumin in the serum of newborn colostrum deprived calves has been measured by several workers. Roberts, Worden and Rees-Evans (1954) found concentrations of 3.15 ± 0.28 g. per 100 ml. serum, and Pierce (1955a) found 2.68 ± 0.1 g. per 100 ml. serum. It would appear, therefore, that the normal variations encountered in the serum albumin concentration are unlikely to affect the turbidity reaction.

Conclusions

From the results obtained, it would appear that within the range 2.5 - 30 mg per ml, the depressant effect of albumin is so slight as to be undetectable by the methods used. A significant depression would begin to be apparent only when albumin in concentrations of 40 mg per ml and over are added to the levels existing naturally in the serum.

SUB-SECTION F

To Determine the Effect of Adding Bovine Gamma Globulin to Hypogammaglobulinaemic Calf Serum

In view of the fact that the serum of newborn calves is markedly hypogammaglobulinaemic until lactoglobulins have been absorbed from the colostrum, the addition of gamma globulin to such sera would allow a method of quantitating the zinc sulphate turbidity test through the range of levels encountered naturally. A preparation of bovine gamma globulin is available commercially, (Armour Pharmaceutical Company Ltd., Eastbourne, England), which has been prepared by the Cohn fractionation technique and is the equivalent of fraction II. This has been used in the following experiment.

Experiment

To Determine the Effect of the Addition of Known Quantities of Bovine Gamma Globulin (Armour) to the Serum of Newborn Colostrum Deprived Calves

Gamma Globulin (Armour) to the Serum of Newborn Colostrum Deprived Calves

were made up in distilled water at concentrations of 4 and 5 mg. per ml. respectively. By means of serial dilutions from these concentrations, solutions of the following concentrations were made up: 5, 3.75, 2.5, 1.25, 0.62, 0.31, 0.15 mg. per ml. and 4, 3, 2, 1, 0.5, 0.25 mg. per ml. Due to the fact that 0.1 ml. of serum is used in the test, these concentrations are the equivalent of the range from 50 to 1.5 mg. BGG per ml. serum. Serum samples were obtained from 10 newly born colostrum deprived calves, split into 2 groups, each containing 5 samples and submitted to the zinc sulphate turbidity test using the standard technique.

The turbidity test on the samples containing BGG was performed as

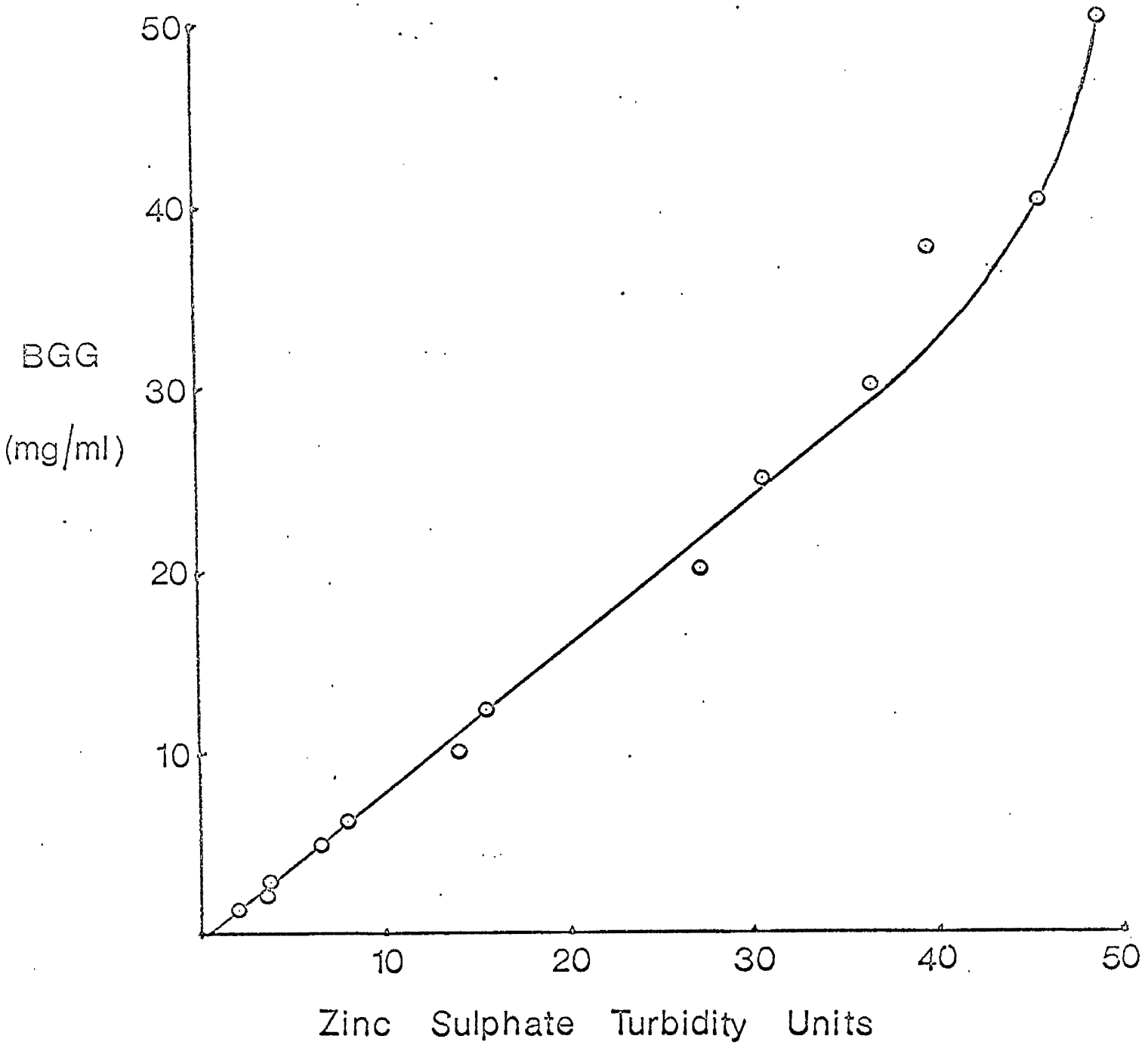
follows. Each concentration of BGG in a series of dilutions was tested against 5 of the serum samples; therefore, for each concentration 5 colorimeter tubes were set up and to each was added 5 ml of zinc sulphate solution. This solution was made up at a strength of 250 mg $ZnSO_4 7H_2O$ per litre, in order to allow for the dilution which occurs when the globulin solutions are added. To each of the 5 tubes, 1 ml of the appropriate solution was added. Finally, 0.1 ml of serum was taken from each serum sample and added to one tube. Thereafter, the performance of the test was as described in the standard procedure. The average turbidity obtained from each concentration of BGG used was calculated and the results are shown in Figure No. 1.6.

It would appear that the relationship existing between the turbidity developed by the zinc sulphate test and the concentration of BGG added to the serum is linear only up to levels of about 30 mg BGG per ml. Thereafter, the turbidity would appear to increase in an exponential manner.

Discussion

The regression equation for the correlation between zinc sulphate turbidity and BGG concentration below values of 30 mg per ml is $y = 0.8x - 0.2$. Comparing this equation with the equation calculated for the correlation between zinc sulphate turbidity and IgG concentration, namely $y = 0.97x - 1.95$, it would appear that the slope of the two lines is quite different. An explanation for this may be that during the process of fractionation of BGG certain changes may have occurred in the structure of the gamma globulins which are reflected in the turbidity reaction. In addition, it can be demonstrated by means of

Fig. 1.6. The effect on the zinc sulphate turbidity reaction of adding a bovine gamma globulin preparation to hypogammaglobulinaemic calf serum.



Immunoelectrophoresis that the BGG is not a preparation of pure IgG but contains traces of other globulin fractions (Penhale, personal communication).

Conclusion

The effect of adding known quantities of a preparation of bovine gamma globulin to hypogammaglobulinaemic calf serum has been investigated. A linear relationship appears to exist between the amount of BGG added and the turbidity developed by the zinc sulphate test only up to concentrations of about 30 mg BGG per ml. The reason why this relationship does not hold at higher concentrations of bovine gamma globulin preparation is not known.

SUB-SECTION G

The Comparative Turbidities Developed by Serum and Plasma

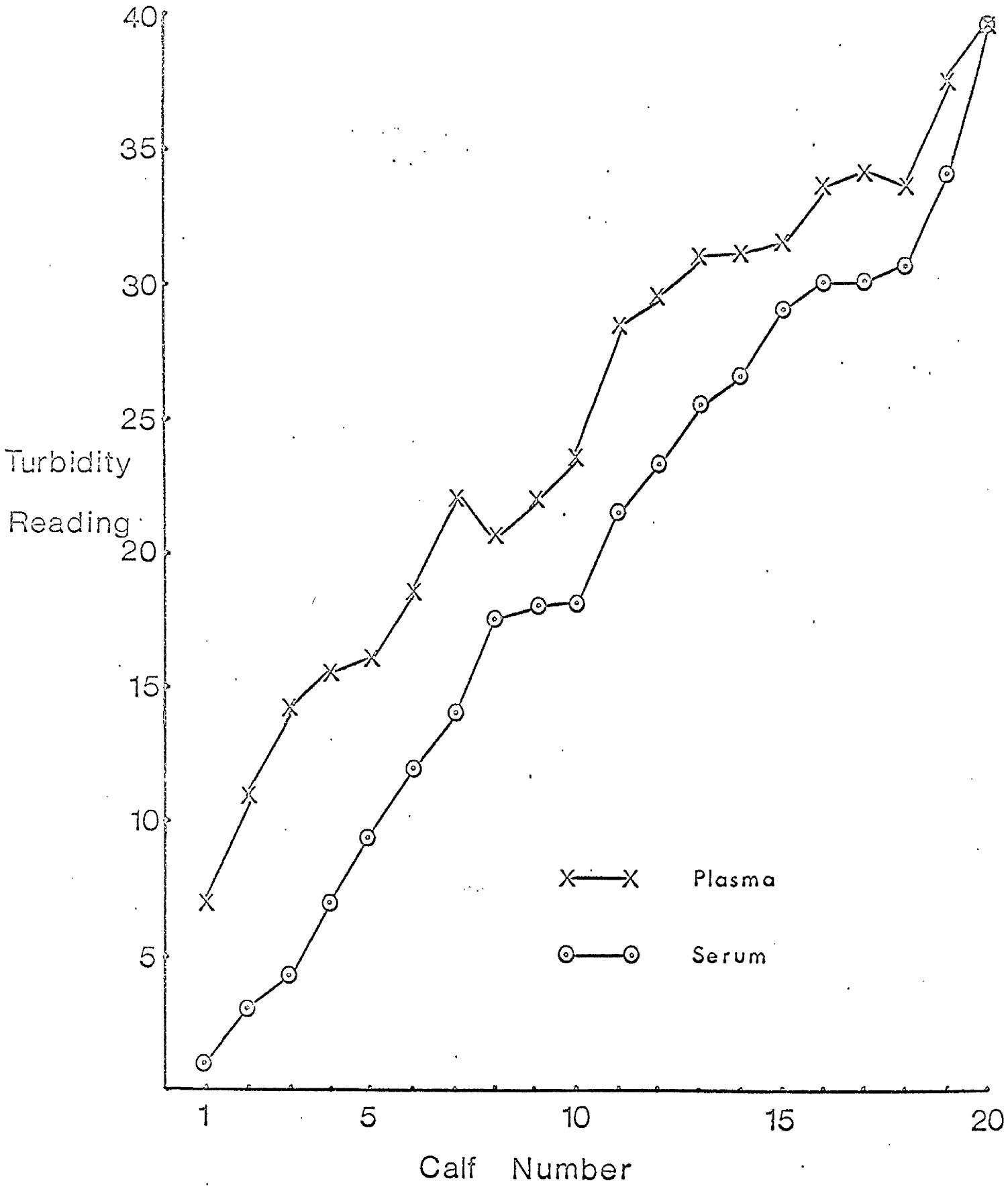
It has generally been accepted by all workers who have used the zinc sulphate turbidity test that it should be performed on serum rather than plasma. The reasons for this are several. The effect of whatever anti-coagulant is used on the turbidity reaction must be ascertained. Heparin is reported by Horn and Kovacs (1959) to cause a decrease in zinc sulphate turbidity. These agents which inhibit the clotting mechanism by selectively binding calcium ions may possibly influence the reaction either by their effect on the ionic strength of the reagents or indirectly by their effect on the calcium ions. The role of calcium in the zinc sulphate turbidity reaction is unknown. The action of some anti-coagulants is not long and this may allow the development of fibrin clots if samples are stored for some time. Finally, the effect of variations in the fibrinogen concentration may cause different turbidity readings from samples with the same gamma globulin concentration.

Experiment

To Compare the Zinc Sulphate Turbidity of Serum and Plasma Taken from the same Animal

Serum and plasma samples were taken from a group of neonatal calves. Heparin was used as the anti-coagulant for plasma samples. These samples were then submitted to the zinc sulphate turbidity test and the results obtained are shown in Figure No. 1.7. It would appear that plasma gives a greater turbidity reaction than serum.

Fig. 1.7. Comparison of the zinc sulphate turbidities developed by plasma and serum.



Discussion

The difference in turbidities between serum and plasma is most marked in the samples with the lowest turbidities, but becomes less so in samples in the higher ranges. The average plasma turbidities were higher by 7.8, 5.4, 5.1 and 2.8 zinc sulphate turbidity units than the corresponding serum turbidities in the ranges of 0 to 10, 10 to 20, 20 to 30 and 30 to 40 respectively.

The depressant action of heparin on the zinc sulphate turbidity reaction reported by Horn and Kovacs (1959) is not apparent in these results. Possibly this effect has been masked by the presence of fibrinogen.

Conclusion

The use of plasma in place of serum is not recommended since the effect of fibrinogen is not equal throughout the range of turbidities likely to be encountered.

SUB-SECTION H

Repeatability of the Zinc Sulphate Turbidity Test

To ascertain the repeatability of the zinc sulphate turbidity test, the following experiment was performed.

Materials and Methods

Five separate determinations were made on six different serum samples in the following manner.

A solution of zinc sulphate (208 mg. $ZnSO_4 7H_2O$ per litre) was prepared in a volumetric flask using distilled water which had been boiled in order to remove dissolved carbon dioxide. This solution was protected against the uptake of carbon dioxide by the insertion of a soda lime tube into the stopper of the container.

The test was performed in sets of matched 10 ml. colorimeter tubes. For each serum sample a set of 5 "control" tubes containing 6 ml. of distilled water and 5 "test" tubes containing 6 ml. of the zinc sulphate reagent were set up. To each of these tubes 0.1 ml. of serum was delivered from an autozero high precision pipette. The solution was then gently shaken in order to ensure proper mixing, and then the tubes were allowed to stand at room temperature for 60 minutes. After this period, the tubes were reshaken to ensure an even suspension of the precipitate and then inserted into an EEL colorimeter, using an Ilford 623 filter. The meter had been previously zeroed on a colorimeter tube containing 6 ml. of distilled water.

The results are shown in Table 1.5.

TABLE 1.5

The Repeatability of the Zinc Sulphate Turbidity Test

	Test	Control	Reading	Mean and Standard Deviation
Serum 1	11.5	2.0	9.5	
	11.5	2.5	9.0	
	10.0	2.5	7.5	9.0 ± 1.1
	11.25	0.75	10.5	
	11.5	2.75	8.75	
Serum 2	20.75	0.25	20.5	
	20.25	1.75	18.5	
	20.0	1.5	18.5	19.0 ± 0.8
	20.75	1.75	19.0	
	20.25	1.75	18.5	
Serum 3	27.5	1.0	26.5	
	27.0	2.75	24.25	
	27.5	2.5	25.0	25.1 ± 0.9
	28.25	2.75	25.5	
	27.0	2.75	24.25	
Serum 4	34.75	2.25	32.5	
	33.5	2.0	31.5	
	35.5	2.0	33.5	33.0 ± 1.4
	34.0	1.75	32.25	
	35.5	0.25	35.25	
Serum 5	38.75	1.25	37.5	
	39.5	1.25	38.25	
	39.75	1.0	38.75	38.1 ± 1.1
	39.5	0	39.5	
	37.5	1.0	36.5	
Serum 6	41.5	1.5	40.0	
	43.0	1.75	41.25	
	43.5	1.5	42.0	40.9 ± 0.8
	41.5	1.25	40.25	
	42.5	1.25	41.25	

Conclusion

The standard deviation calculated from all the determinations is $\pm$ 0.97. It would appear, therefore, that in this laboratory the zinc sulphate turbidity test is repeatable with an accuracy of about $\pm$ 1 unit.

GENERAL DISCUSSION

Before attempting to calibrate the zinc sulphate turbidity test, it was found necessary to ascertain what influence certain factors exerted on the zinc sulphate turbidity reaction. Moreover, the repeatability of the reaction remained to be established.

Greater sensitivity in measuring the amount of turbidity developed by the reaction was found by use of a Blue-green filter (Ilford No. 623). Use of this filter increased the sensitivity of the colorimeter to haemoglobin, but this source of error was minimised by the use of control samples.

The amount of turbidity was found to be influenced by the temperature of the reagents and the time allowed for the reaction to develop. Variations in the temperature, especially over 25°C produced large variations in the turbidity. This latter fact would suggest that in the performance of the test the temperature of the reagents should be controlled and be less than 25°C.

Buffers used by other workers had the effect of rendering the zinc sulphate solution alkaline and consequently depressed the development of turbidity. That this should be so may indicate that the prevention of carbon dioxide uptake by the zinc sulphate solution is an important precaution, in addition to the boiling of the distilled water prior to making up the solution.

An increase in the ionic strength of the zinc sulphate solution resulted in a depression of the turbidity. Similarly, albumin had the effect of depressing turbidity if added to the zinc sulphate solution in concentrations greater than 5 g. per 100 ml. It has been noted previously that concentrations of this magnitude are not normally

found and therefore no correction factor or estimation of albumin concentration are necessary.

A preparation of bovine gamma globulin (Armour) caused the turbidity to increase inalinear fashion until a concentration of 30 mg. per ml. was attained. The deviation from linearity at higher concentrations in addition to the difference between the regression equation calculated for this preparation and the specific immune globulin fractions (Section B) precludes the use of this preparation in the calibration of the zinc sulphate turbidity reaction.

It was found that plasma samples gave rise to higher zinc sulphate turbidity readings than the corresponding serum samples. The effect of fibrinogen was most marked in those samples with the lowest turbidities but became less so when higher turbidities were reached. Serum, therefore, would appear to be the more suitable substance on which to attempt a calibration.

Finally, under standard conditions, the test was repeatable with an accuracy of $\pm$ 1.0 unit.

In view of the findings of other workers and the results obtained from the preceding experiments, the following technique was adopted in the performance of the zinc sulphate turbidity test.

Zinc Sulphate Turbidity Test - Procedure

Considering the technical difficulties inherent in obtaining anaerobic blood samples together with the fact that the mineral oil seal may itself cause an elevated reading for unknown reasons, Yonan and Reinhold (1957), it was decided not to employ this technique. All sera were separated from the clots and no attempt was made to prevent the sera from equilibrating with the atmosphere.

A solution of zinc sulphate (208 mg. $ZnSO_4 7H_2O$ per litre) was prepared in a volumetric flask using distilled water which had been boiled for 10 - 15 minutes in order to remove dissolved carbon dioxide. This solution was then kept in an aspirator bottle and was further protected against the uptake of carbon dioxide by the insertion of a soda lime tube into the stopper. The aspirator bottle was connected by means of polythene tubing to an automatically dispensing pipette (W. G. Flage & Sons Ltd., Excelo Works, Margate Road, Broadstairs, Kent) set to deliver 6 ml. An identical arrangement was used to deliver the distilled water for the control tubes.

The test was performed in sets of matched 10 ml. colorimeter tubes. For each serum sample tested, a "control" tube containing 6 ml. of distilled water and a "test" tube containing 6 ml. of zinc sulphate solution were set up. To each of these, 0.1 ml. of serum was delivered from an autozero high precision pipette, (H. J. Elliot Ltd., E-Mil Works, Treforest, Glamorgan). The solution was then gently shaken in order to ensure mixing of the reagents and tubes were allowed to stand for 60 minutes at room temperature, which was 20°C. After this time had elapsed, the tubes were shaken again to ensure an even

redistribution of the precipitate and inserted into an EEL colorimeter. Using an Ilford No. 623 filter (blue-green) the colorimeter had been previously zeroed by means of a tube containing distilled water. The turbidity reading was found by subtracting the reading obtained from the "control" tube from that of the "test" tube.

In order to eliminate variations in the sensitivity of the colorimeter and to facilitate comparison of the results obtained in this laboratory with results possibly obtained in the future in other laboratories, the barium sulphate standard recommended by Shank and Hoagland (1946) for use in the thymol turbidity test was adopted. Three ml of a barium chloride solution containing 1.15 g $BaCl_2.2H_2O$ per 100 ml were made up to 100 ml in a volumetric flask with 0.2 N sulphuric acid. The turbidity produced by this standard when measured under the same conditions as used in the zinc sulphate turbidity test was such that it caused a deflection of 20 on the colorimeter scale.

SECTION B

Calibration of the Zinc Sulphate Turbidity Test

Introduction

Several workers have attempted to correlate the degree of turbidity produced by the zinc sulphate reagent with the concentration of the globulin fraction of serum. Kunkel (1947) found close correlation between the turbidity and the gamma globulin concentration as calculated from electrophoretic patterns of 9 pathological sera showing approximately normal albumin concentrations. A correlation was shown also to exist between the turbidity and the total globulin level as determined by the Howe Fractionation technique (1921a and b). A comparison was made by de la Huerga et al (1950) between the results of two turbidity tests and moving boundary electrophoretic determination of gamma globulin. The turbidity tests examined were the zinc sulphate reaction described by Kunkel (1947) and the gamma globulin test using a mixture of ammonium sulphate and sodium chloride as described by de la Huerga et al (1950). Statistically the zinc sulphate method correlated less well with the electrophoretically determined gamma globulin ($r = 0.67$) and the expected error was almost twice that obtained using the ammonium sulphate/sodium chloride reagent. This was attributed to several factors such as the concentration of serum lipid and albumin. A similar correlation was attempted by Ricketts et al (1951) using the same techniques as the above authors. They found the correlation between the tests to be within 20% if the sera were from normal subjects. Portal and biliary cirrhosis, however, caused marked differences between turbidimetric and electrophoretic values.

Discombe et al (1954) performed duplicate estimations on 45 sera using the Kunkel reagent and zone electrophoresis to determine the

gamma globulin concentration. A close correlation ($r = 0.91$) was found to exist but the authors did not state whether the sera were normal or not. Wilson, Brown and Hainline (1957) found a correlation coefficient of 0.59 in the relationship between gamma globulin as determined by moving boundary electrophoresis and zinc sulphate turbidity. Adner (1957), however, found a very close correlation ($r = 0.88$) between zinc sulphate turbidity and gamma globulin concentration as measured by zone electrophoresis. These estimations were made on samples taken from patients suffering from a wide range of diseases, but were selected in so far as they all had increased erythrocyte sedimentation rates.

Using the modified test, Aschaffenburg, (1949) demonstrated that changes in turbidity were accompanied by changes in the euglobulin nitrogen and total nitrogen of calf sera. No attempt was made to obtain a closer correlation between the results. Nelmann-Sorensen, Konggaard and Kruse (1966) found a close correlation ($r = 0.93$) between the zinc sulphate turbidity and the gamma globulin concentration as determined by zone electrophoresis of calf serum. Using this correlation turbidities were expressed as relative percentages of gamma globulin by use of the formula:

$$\text{Relative Percentage of Gamma Globulin} = \frac{(2 + \log \text{transmission})}{0.634} \times 100$$

The constant 0.634 was found by determining the levels of gamma globulin in the serum of 10 normal cows.

Patterson (1967) also found a correlation to exist between the degree of turbidity developed by the zinc sulphate test and the gamma globulin concentration as determined by zone electrophoresis, expressed either as a percentage of total protein ($r = 0.942$) or gms/100 ml of

serum ($r = 0.887$). The turbidity was found to be inversely related ($r = -0.508$) to the albumin globulin ratio for values up to 0.83.

No references have been found in which quantitation has been attempted by adding various amounts of a gamma globulin preparation to normal or hypogammaglobulinaemic calf serum. Adner (1957) noted the effect on the zinc sulphate turbidity test of adding gamma globulin to serum taken from a patient with agammaglobulinaemia. At levels greater than 1 gm/100 ml there was a linear response between the optical density of the turbidity produced and the gamma globulin concentration.

In the work described in Sub-section F of the preceding section it was found that a linear response existed between the concentration of bovine gamma globulin added to hypogammaglobulinaemic serum and the zinc sulphate turbidity reaction. At concentrations higher than 30 mg per ml, however, the turbidity reaction appeared to increase exponentially. Because the reason for this is not known and in view of divergencies found by various workers in the correlation between gamma globulin concentration and zinc sulphate turbidity in man, it was decided to investigate how close the relationship was in neonatal calf serum. Attempts have therefore been made to correlated the zinc sulphate turbidity with the total protein concentration, the total globulin concentration and the concentration of IgG.

Subsection a)

Correlation of the Zinc Sulphate Turbidity Test with Total Serum Protein Concentration

It has been found that newly born unfed calves have a protein concentration of about 4.2 gms/100 ml serum (Roberts, et al., 1954; Pierce, 1955a). Protein estimations made on the serum of 30 newly born colostrum deprived calves, born at the University Veterinary Hospital, revealed concentrations of 4.4 ± 0.25 g per 100 ml. Since the increase in total protein concentration subsequent to the ingestion of colostrum is due almost entirely to immune globulins, the zinc sulphate turbidity test may provide an indication of the serum protein concentration.

Experiment

In order to investigate this possibility, the zinc sulphate turbidity test was performed on the sera of 50 neonatal calves. The total serum protein concentration of these sera was then measured and compared with the turbidity readings. The results are shown in Figure 1.8.

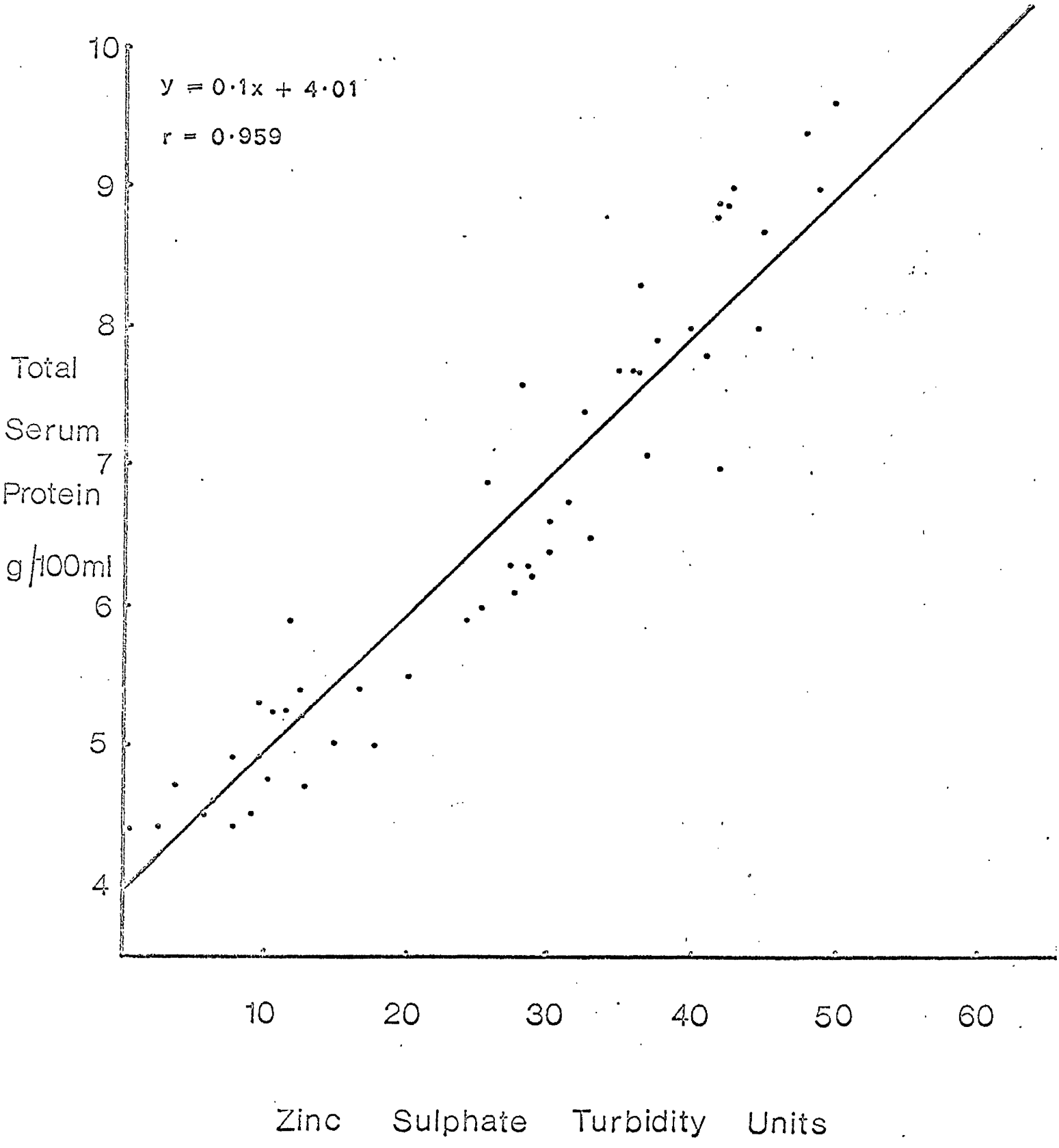
Discussion

There would appear to be a highly significant correlation ($r = 0.76$, $p < 0.001$), between the zinc sulphate turbidity test and the total serum protein concentration. The regression equation for this correlation has been calculated as $y = 0.1x + 4.01$. It will be noticed that the regression line intercepts the y axis at 4 g per 100 ml, a value which is not very different from the values found by other authors.

Conclusion

A significant correlation was found between the zinc sulphate turbidity test and the total serum protein concentration.

Fig. 1.8. Correlation between total serum protein concentration and the turbidity developed by the zinc sulphate turbidity test.



SUB-SECTION B

Correlation of the Zinc Sulphate Turbidity Test with Total Serum Globulin Concentration

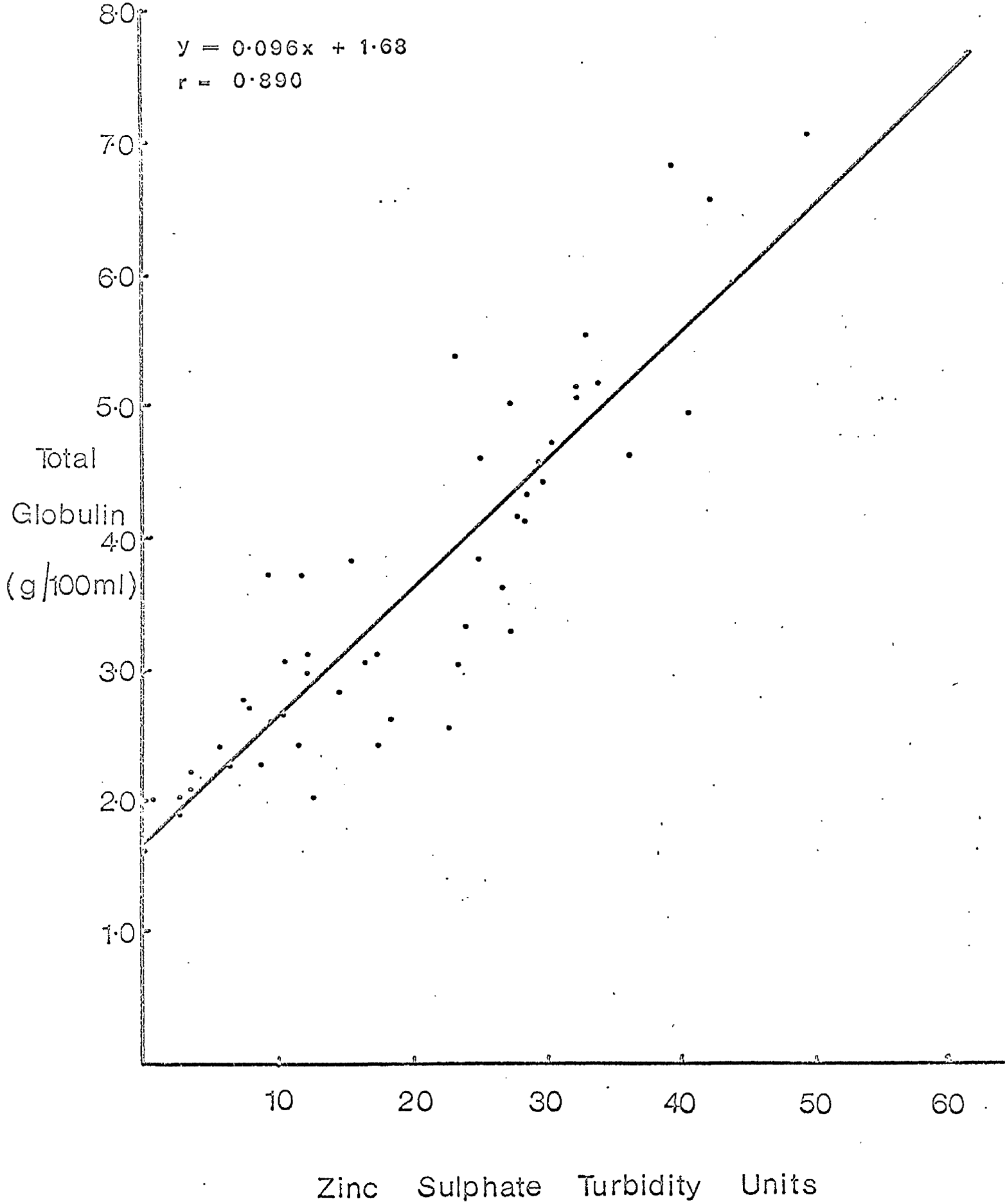
It has been shown by Pierce (1955a) that after the ingestion of colostrum and during the first few days of life, changes occur in the absolute and relative values of the serum globulin fractions. The most marked of these changes are found in the gamma globulin fraction and are probably associated with the equilibration of the immune globulins between the intravascular and extravascular spaces. To determine whether in spite of these changes a correlation could be found between the turbidity developed by the zinc sulphate test and the total globulin concentration, the following experiment was performed.

Experiment

The turbidity readings of 50 neonatal sera were determined by the zinc sulphate test as described previously.

Using 12 x 2.5 cm cellulose acetate strips (Oxoid Ltd., Southwark Bridge Road, London, S.E.1) electrophoresis of the serum samples was carried out. A barbitone n-octoate buffer of pH 8.6 was used and a current of 1mA per strip was applied for 2 hours. The strips were fixed in 5% trichloracetic acid and then placed for 10 minutes in a staining solution containing 0.2% Ponceau S in 3% aqueous trichloracetic acid. After staining the strip was washed in several changes of 5% aqueous acetic acid until the background was completely white. The strip was then scanned using an EEL Scanner (Evans Electroselenium Ltd., Halstead, Essex, England), and the relative proportions of albumin and globulin were determined by cutting the tracing into albumin and globulin sections and then weighing them.

Fig. 1.9. Correlation between the total serum globulin concentration and the turbidity developed by the zinc sulphate turbidity test.



The biuret method described by Varley (1963) was used to estimate the serum protein concentration. The test was standardised by use of a commercially prepared standard of known protein concentration (Versatol, W. R. Warner and Co. Ltd., Eastleigh, Hampshire, England).

Discussion

There appears to be a highly significant correlation ($r = 0.89$, $p < 0.001$), between the zinc sulphate turbidity test and the total globulin content of the sera. The regression line calculated for this relationship was $y = 0.96x + 1.68$. The regression line intercepts the y axis at a value of 1.68 g per 100 ml. This compares with the values of total globulin of newborn colostrum deprived calves obtained by Roberts, Worden and Rees-Evans (1954) and Pierce (1955a, 1961b).

Conclusion

A highly significant correlation was found to exist between the zinc sulphate turbidity test and the total serum globulin concentration in neonatal calves.

Subsection c)

Correlation of the Zinc Sulphate Turbidity Test with Serum Immune Globulin Concentration

The zinc sulphate turbidity test described by Kunkel (1947) was designed specifically to measure the relative increases in gamma globulin concentration which occurred in certain human diseases. In the original paper, Kunkel demonstrated that by increasing the concentration of the reagent used a corresponding increase occurred in the percentage of protein precipitated. Use was made of this fact by Aschaffenburg, (1949c) in his adaptation of the test for use in neonatal calf serum. This test has now become one which measures the absolute concentration of gamma globulin rather than increases above specific levels. In view of the close correlation obtained between the zinc sulphate test and the total globulin concentration, an attempt was made to determine what relationship existed between this test and the immune globulin concentration of serum.

Experiment

A series of 53 calf sera on which the zinc sulphate turbidity test had been performed were sent to Dr. W. J. Penhale, Department of Veterinary Pathology, Royal (Dick) School of Veterinary Studies, Edinburgh, where determinations of the IgG and IgM concentrations were made without knowledge of the zinc sulphate turbidity. The immune globulin concentrations were determined by a quantitative immuno-diffusion technique, Penhale and Christie (1968). The correlations existing between the zinc sulphate turbidity test and the concentration of IgG, IgM and IgG plus IgM are shown in figures nos. 1.10, 1.11, 1.12 respectively.

Fig. 1.10. Correlation between the serum IgG concentration and the turbidity developed by the zinc sulphate turbidity test.

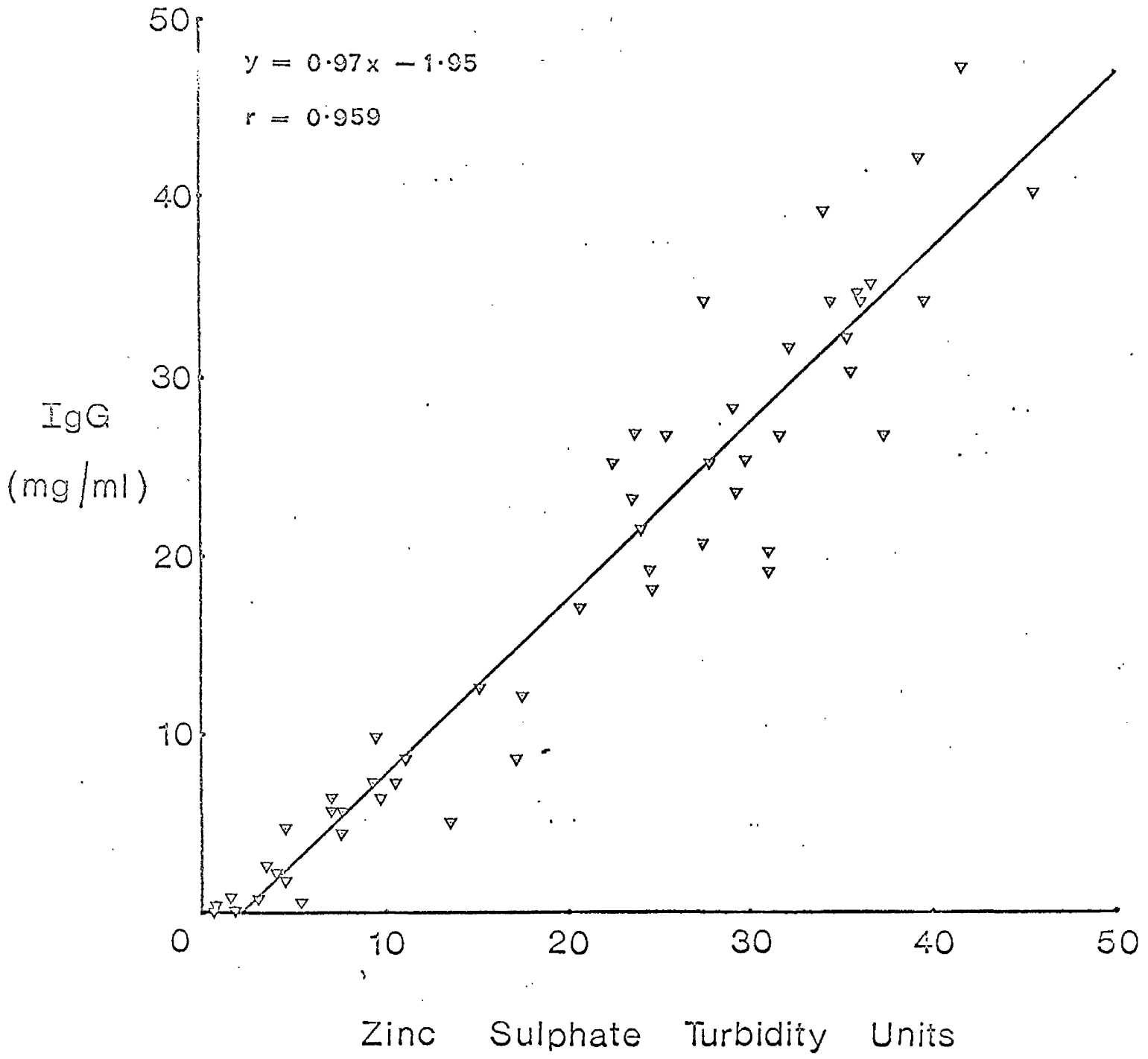


Fig. 1.11. Correlation between the serum IgM concentration and the turbidity developed by the zinc sulphate turbidity test.

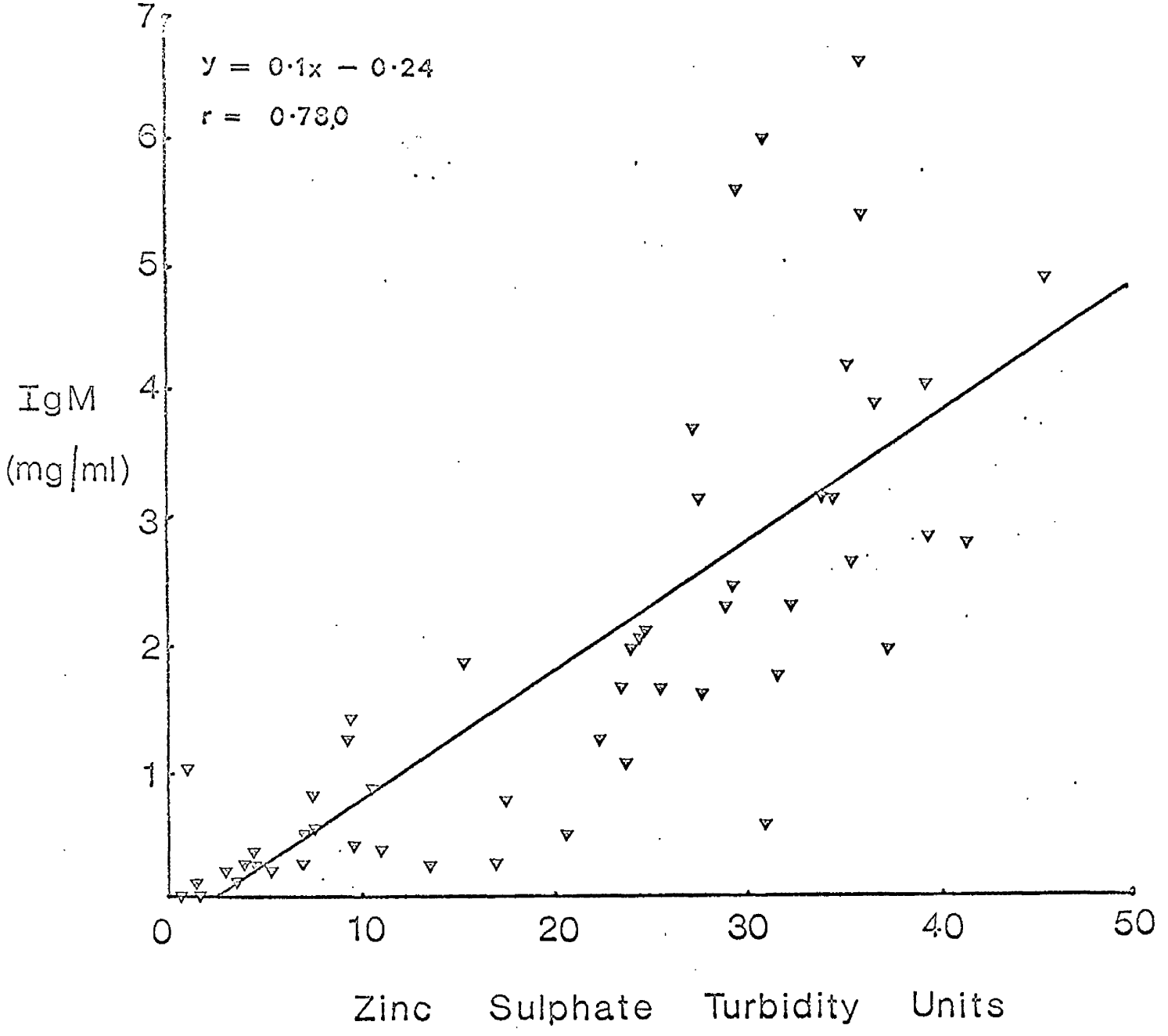
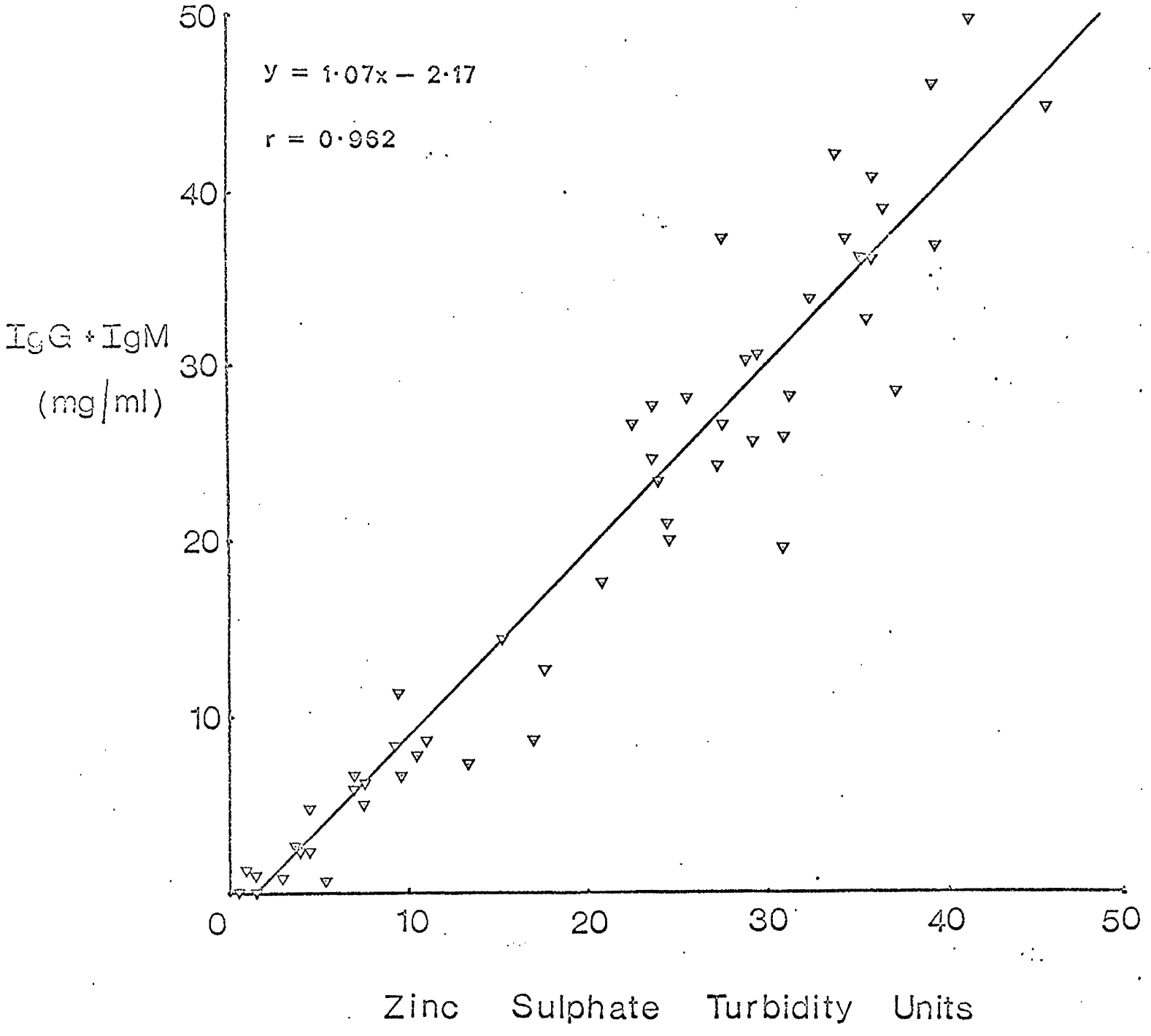


Fig. 1.12. Correlation between the serum Immune globulin (IgG + IgM) concentration and the turbidity developed by the zinc sulphate turbidity test.



Results

a) IgG

There is a highly significant correlation ($r = 0.959$, $p < 0.001$) between the IgG concentration and the turbidity developed by the zinc sulphate turbidity test. A regression equation was calculated as $y = 0.97x - 1.95$, when y = IgG concentration (mg per ml) and x = zinc sulphate turbidity (ZST units).

b) IgM

Again, there is a highly significant correlation ($r = 0.780$, $p < 0.001$) between the IgM concentration and the zinc sulphate turbidity test. The regression equation expressing this relationship is $y = 0.1x - 0.24$ when y = IgM concentration (mg per ml) and x = zinc sulphate turbidity (ZST units).

c) IgG and IgM

The correlation existing between the sum of the individual immune globulins and the zinc sulphate turbidity is the highest yet obtained ($r = 0.962$, $p < 0.001$) and the regression equation is $y + 1.07x - 2.17$ when y = (IgG and IgM) concentration (mg per ml) and x = zinc sulphate turbidity (ZST units).

Discussion

These results indicate that the turbidity developed by the zinc sulphate test is influenced mainly by the concentration of IgG and to a lesser extent by the IgM concentration.

Jones (1967) using urea starch gel electrophoresis and the zinc sulphate turbidity test as described by Patterson (1967) failed to find any relationship between the turbidity readings and the presence of IgG in 12 calves. Further work (Jones, 1968) using the same sera and the

Immunodiffusion method of estimating IgG, Penhale and Christie (1968) showed that a correlation did exist. A coefficient of correlation of 0.75 was found between the zinc sulphate turbidity reaction and the IgG concentration.

Because of the high correlation between this test and the immune globulin concentration of serum, there exists the possibility that the test may be used on serum samples from other ages of animals or on other biological fluids, e.g. colostrum or cerebrospinal fluid. Whereas a correlation may exist, it is probable that it may not be the same as that found for neonatal calf serum, the main reasons for this difference being the presence of other protein fractions and the influence of different ionic strengths. For example, in the preparation of colostrum whey, rennet is often used, which has a high salt content. This would tend to depress the turbidity reaction.

Conclusion

Highly significant correlations were found to exist between the zinc sulphate turbidity test and the concentration of two serum immune globulin fractions (IgG and IgM).

General Conclusions

There is a highly significant relationship between the zinc sulphate turbidity test and the total protein concentration, the total globulin concentration and the immune globulin (IgG and IgM) concentration of neonatal calf serum. The equations expressing these relationships are given below. x = the zinc sulphate turbidity expressed in units. r = coefficient of correlation.

Total protein (y) g. per 100 ml.

$y = 0.1x + 4.01$ $r = 0.959$

Total globulin (y) g. per 100 ml.

$y = 0.096x + 1.68$ $r = 0.890$

IgG (y) mg. per ml.

$y = 0.97x - 1.95$ $r = 0.959$

IgM (y) mg. per ml.

$y = 0.1x - 0.24$ $r = 0.780$

IgG and IgM (y) mg. per ml.

$y = 1.07x - 2.17$ $r = 0.962$

PART II

USE OF THE ZINC SULPHATE TURBIDITY TEST IN NEONATAL CALVES

Introduction

In the previous section it was established that the zinc sulphate turbidity test gave an accurate estimation of the immune globulin content of calf serum. It was decided, therefore, to apply this test to the serum of neonatal calves passing through the University Veterinary Hospital. These calves were predominantly Ayrshire bull calves which had been purchased in local markets and were of approximately one week of age. The principal objects of this investigation were to ascertain normal serum immune globulin levels, their relationship to neonatal disease, and the changes which occur in the immune globulin levels during early life. Before describing the results of these investigations it is relevant to review briefly the literature concerning the immunological status of the newborn calf with particular reference to neonatal disease.

a) The Immunological Status of the Newborn Calf

1) Presence of Immunoglobulin at Birth

The presence of immunoglobulin in newborn calf serum was not detected by earlier workers, Howe (1921, 1924), Jameson, Alvarez-Tostado and Sortor (1942), Smith and Holm (1948), Polson (1953). Small amounts, however, have been detected by other workers. Hansen and Phillips (1947) noted small but consistent amounts of gamma globulin by the electrophoresis of the serum of newborn unfed calves. Using salt fractionation, Aschaffenburg (1949) found measurable amounts of euglobulin in all but one of a series of calves. Roberts, Worden and Rees-Evans (1954) found that the euglobulin pseudoglobulin I fraction averaged 0.25 ± 0.16 g. per 100 ml., i.e. 5.9% of the total serum protein.

Pierce found levels of about 1.4% (1955) and 3% (1961), and Johnson and Pierce (1959) detected levels of about 2%. Knlazeff, Rimmer and Gaeta (1967) using an immunodiffusion technique detected the presence of gamma globulin in 7 out of 40 foetuses between four and six months of gestation.

2) Active Synthesis and Passive Decline of Immunoglobulin

Hansen and Phillips (1947) using colostrum deprived calves noted an increase in the relative amount of gamma globulin between one and 8 weeks of age. Smith and Holm (1948) using colostrum fed calves followed over four months the development of the gamma globulin fraction and the decline of the immune lactoglobulin component. These workers found an increasing concentration of gamma globulin after 50 days of age. A gradual increase in the euglobulin pseudoglobulin I fraction was found by Roberts et al. (1954). In colostrum deprived calves this fraction increased from birth until at 70 - 80 days of age there was a concentration of 1 g. per 100 ml. of serum. Approximately similar values were found by Pierce (1955) in a study which lasted over the same period. By 70 - 80 days colostrum deprived calves had serum concentrations of gamma globulin of about 1 g. per 100 ml. In colostrum fed animals by the 10th day of life active synthesis of immune globulin was suggested by the development of a second component in the lactoglobulin profile. In 3 out of 4 partially colostrum deprived calves the production of gamma globulin began within the first five days of life with an increase in the gamma 1 globulin fraction. Varnell, Erwin, Smith and Fleming (1960) detected a relative increase in the gamma globulin fraction between the 7th and 14th days of life in colostrum deprived calves.

Johnson and Pierce (1959) using electrophoretic and ultracentrifugal techniques also found a gradual increase in the autogenous gamma globulin after one week, and Pierce (1961b) showed by electrophoresis in colostrum deprived calves the early development, under one week, of serum gamma globulin.

The decline of euglobulin-pseudoglobulin 1 fraction during early life was first described in the calf by Howe (1924) using a salt fractionation technique. Smith and Holm (1948) performed electrophoretic analysis on the serum proteins of a calf and found that the colostrum globulin increased logarithmically and had a half life of 20 days. They suggested that the true half life would be greater than the calculated value since the animal was growing rapidly and dilution of the immune globulin would occur. Using a modification of Howe's technique, Roberts et al. (1954) found that the rate of decline of the serum euglobulin-pseudoglobulin 1 fraction in colostrum fed calves was initially fast, but slowed down gradually, i.e. apparently logarithmic. No attempt was made to calculate the half life of this fraction. Dixon, Talmage, Maurer and Delchmiller (1952) in a study of the half life of ^{131}I labelled homologous gamma globulin in 7 different species found an average half life of 21.2 $\pm$ 1.7 days in 2 steers and 2 non-lactating cows. In a later study a half life of 10 days was observed in adult Holstein cows (Dixon, Weigle and Vazquez, 1961). It was suggested from these findings that immature cattle have a lower rate of serum protein catabolism than adults. In normoimmunoglobulinaemic adult cattle, Nansen and Neilsen (1966) using ^{131}I labelled IgG found a mean plasma half life of 15.7 days.

Pierce (1955a,b) made detailed electrophoretic and immunological

studies of the changes taking place in the sera of colostrum fed and colostrum deprived calves during the first 80 days of life. The early autogenous production of immune globulin complicated the calculation of the rate of elimination of passively acquired lactoglobulin due to the formation by 10 days after birth of 2 components replacing the single lactoglobulin component. He also found that minimal concentrations of gamma globulin occurred between the 40th and 60th days. Similarly Varnell et al. (1960) found that the relative concentration of gamma globulin was lowest during approximately the same period. Unfortunately absolute values were not given.

3) Active Synthesis and Passive Decline of Specific Antibodies

Fennestad and Borg-Petersen (1962) found that at 132 days' gestation the calf foetus responded to Leptospira saxkoebing by producing antibodies. At birth Brown (1958) found the colostrum deprived calf or the calf from a non-immune dam to be capable of responding to Rinderpest vaccination. This finding was confirmed by Strickland (1963). Smith and Ingram (1965) found that the acquisition of immunological competence occurred at different ages and depended on the characteristics of the antigen. Using human serum albumin by intravenous and intramuscular inoculation they failed to produce a detectable response. When this antigen was combined with Freund's incomplete adjuvant and inoculated at one day of age, antibody production was detected within 7 - 8 days. On the other hand, no response to Klebsiella pneumoniae polysaccharide occurred in calves inoculated while under one month of age.

Carroll, Thellen and Leighton (1968) investigated the immunological competence of thymectomised neonatal calves. Three thymectomised and one control calf were used. Using as antigen egg albumin with Freund's

complete adjuvant and tetanus toxoid, these workers detected by the 4th week after the initial inoculation, the presence of precipitins to the tetanus toxoid. Precipitins to egg albumin were detected only in one of the thymectomised calves and not in the control calf.

Other authors have also shown that the ability of the very young calves to respond to antigenic stimulation is limited and varies with the quality of the antigen. Kerr (1956) compared the response to inoculation with antigens prepared from Brucella abortus and Salmonella dublin. Five of the 6 calves were inoculated at seven days of age and did not respond by producing detectable circulating antibodies. When reinoculation took place at 4 months antibodies were detectable in some cases within 3 days, suggesting a secondary response and hence a previous sensitisation. Nagy and Hignett (1967) exposed newborn calves to a very heavy dose of Brucella abortus in the milk for the first 15 days of life. Active production of agglutinins was not detectable in the sera of the 6 test calves until 20 - 38 days of age and the titres remained low. On subsequent challenge by parenteral exposure to Brucella abortus a secondary response was obtained.

McDiarmid (1946) measured the decline in the serum agglutination titre for Brucella abortus and found that there was a steady logarithmic rate of decline from week to week. Smith and Holm (1948) studied the rate of decline of passively acquired antibodies to 3 different antigens. These antigens were diphtheria toxoid, a killed culture of Haemophilus pertussis and Vaccinia virus. They estimated that the half life of diphtheria antitoxin was 16 days, whereas Haemophilus pertussis and Vaccinia antibodies had a half life of about 50 days.

The rates of decline of passively acquired antibodies to Brucella abortus and Trichomonas foetus were measured by Kerr and Robertson (1954).

They found that both were eliminated logarithmically and had approximate half lives of between 14 and 20 days, although in one calf with a very high initial titre to T. foetus the rate appeared to be 57 days. Pierce (1955b) also studied the rate of elimination of the agglutinating antibodies to T. foetus and found that it was logarithmic.

The rate of elimination of passively acquired neutralising antibody to Rinderpest was measured in calves by Brown (1958). He established the mean half life of this antibody as 36.7 days and the extinction point as 10.9 months. This work was supported by Strickland (1962) who studied the thermal reaction of calves to Rinderpest vaccine. Whereas no reaction occurred in calves under four months of age and born to immune dams the percentage of calves which did react increased with age until by 8 - 9 months 95% reacted. In another study of the decline of Rinderpest neutralising antibodies Singh, Osman, El Cicy and Baz (1967) found the half life of maternal antibodies in buffalo and Friesian calves to be 30 and 29 days respectively.

Graves (1963) studied the persistance of neutralising antibody to Foot and Mouth disease. He found that the neutralising antibody had a half life of between 15 and 19 days.

Collnet, Kaeckenbeeck and Schoenaers (1961) used the agglutinating antibodies to E. coli to measure the rate of elimination. They concluded that the half life of these antibodies was between 20 and 27.6 days, the mean value being 23.2 days.

4) The influence of passive immunity upon the development of active immunity

There is evidence that passively acquired immunity interferes to some extent with the active synthesis of specific antibody. This has been demonstrated by the failure of passively immune animals to respond to vaccination in a manner similar to animals of the same age either born of non immune dams or having been deprived of colostrum. There are many examples of this phenomenon in other species, e.g. dog, Gillespie, Baker, Burgher, Robson and Gilman (1958); sheep, Batty, Thomson and Hepple (1954) and man, Perkins, Yetts and Gaisford (1959). Because of this state vaccination of young animals is not usually carried out till they are over one to two months of age.

Hemming (1953) attempted to vaccinate the calves of cows previously immunised with a formolised Salmonella dublin vaccine. No appreciable agglutinogenic response was obtainable from any of these calves although the majority of calves born to unimmunised cows showed a definite rise in the H titre 7 days after the first inoculation. This blocking effect persisted until at least 30 days after birth and as the passively acquired agglutinins were demonstrable in the sera of these calves for two to three months, it was suggested that a response to vaccination might not be obtained until this time. Roberts et al. (1954) noted that the antibody response of colostrum deprived and colostrum fed calves to inoculation with a heat-killed Salmonella dublin vaccine was similar. These workers, however, did not describe whether Salmonella dublin antibodies were present or absent in the colostrum.

Brown (1958b), found that the serological response to caprinised Rinderpest vaccine depended on the level of passive immunity. There appeared to be upper and lower levels of immunity above and below which there was no or a good response respectively. The response of animals lying within these limits appeared to be inversely proportional to the inoculation titre. Strickland (1962) using the same vaccine, supported Brown's work in that he found calves up to four months of age and born of immune dams failed to respond to vaccination. Between four and nine months there was an increasing percentage of responses.

The effect of vaccinating with inactivated Foot and Mouth Disease virus two three-week old calves born from an immune and a susceptible dam was studied by Graves (1963) and the responses compared. The susceptible calf responded in a way similar to the susceptible adult animal, whereas there was no response from the passively immune calf. When revaccination was carried out 120 days later, the initially susceptible calf showed a definite anamnestic response and the other gave a primary response.

b) <u>The relationship between serum immune globulin levels and neonatal disease</u>

The relationship between colostrum deprivation and susceptibility to disease was noted in calves by Smith and Little (1922). In a series involving 22 newborn calves, 8 of the 12

colostrum deprived calves died and 1 was killed in a moribund state. Among the 10 calves which received colostrum there were no fatalities.

The importance of feeding colostrum has been re-emphasised by subsequent workers, McEwen (1950) noted the marked protective effect found by feeding calves from a common pool of colostrum. The results of the various trials described by McEwen give strong support to the importance of immune globulins to the calf. Of all the calves which were given immune globulins by various means, e.g. by feeding colostrum, colostrum whey or bovine serum or by injection of colostrum whey or bovine serum only 4 (5.7%) out of 70 died. The mortality rate amongst calves deprived of colostrum or other source of immune globulin was 20 (71.4%) out of 28.

Aschaffenburg and coworkers (1949a, b, 1951 a, b) in a series of papers observed that only the calves which received the aqueous fraction of colostrum made satisfactory progress. The others which received different fractions either died or failed to make satisfactory weight gains. These workers found that some degree of protection was given by 80 ml of the aqueous fraction or 1¼ gms of immune lactoglobulin in that these quantities prevented deaths but did not prevent diarrhoea or allow normal weight gains.

Ingram, Lovell, Wood, Aschaffenburg, Bartlett, Kon, Roy and Sears (1953) showed the value of colostrum to the newborn calf. Of 103 colostrum deprived animals, only 9 (8.7%) survived, whereas 118 (73.3%) of 161 colostrum fed animals survived. Unfortunately, the serum immune globulin concentrations of calves fed colostrum were

not ascertained by any of the workers mentioned previously, and so the proportion of colostrum fed calves which were in fact still hypogammaglobulinaemic is still unknown. In retrospect it is possible that a high proportion of the colostrum fed calves which died and which were described by these authors were in this state.

Fey and Margadant (1961) examined the serum of calves which had died as naturally occurring cases of coli septicaemia. Of the 22 cases examined 21 (95.5%) were hypogammaglobulinaemic or agamma-globulinaemic as measured by a semi-quantitative immunodiffusion test. In comparison 5 (10.9%) out of 46 calves of the same age had more or less pronounced hypoglobulinaemia in spite of having been fed colostrum. The possibility of this deficiency being due to a lack of gamma globulin in the colostrum was examined by Fey and Hunyady (1962). From 51 samples they found that the mean concentration of gamma globulin in the colostrum whey was 7.8 $\pm$ 2.2 gms per 100 ml. These figures suggested that it is unlikely that there is an absolute deficiency of gamma globulin in colostrum.

Smith (1962) using the zinc sulphate turbidity test described by Aschaffenburg (1949) noted a large variation in the turbidity level of Jersey bull calves which had been left with their dams for a period of 48 hours after birth. Six calves of the 52 tested had turbidity readings below 5 as measured on an absorptiometer. The others ranged from this level to 80. Three of these 6 calves eventually developed a bacteraemia. In addition, there appeared to be no correlation between the amount of immune lactoglobulin absorbed and the severity of diarrhoea developed by the calf.

Gay, McKay and Barnum (1964a), in a study of the antibody acquired by calves as a result of vaccination of the dam, found 11 (13.25%) out of 83 calves to have low or negative antibody titres to the O and K antigens of certain strains of E. coli. Six of these 11 calves were hypogammaglobulinaemic and 3 of these 6 died of colibacillosis.

Many workers have attempted experimentally to reproduce collisepticaemia using strains of E. coli isolated from naturally occurring cases (Schoenaers and Kaeckenbeec, 1958; Fey, 1962; Smith, 1962; Gay, McKay and Barnum, 1964). In general, attempts have failed if use is made of calves which have been allowed access to colostrum. The association of K antigens with pathogenic strains of E. coli isolated from septicaemic calves (Briggs, 1951a, b) has suggested that the protective factor in colostrum might be the presence of antibody against this antigen. This view was promoted by the work of Ingram and his colleagues (1953, 1956), but since then it has become apparent that K agglutinins are not the immune factors which protect calves. A critical review of the nature of the protection offered by colostrum has been made by Gay (1965).

In the United Kingdom several surveys have recorded that incidence of deaths in calves is seasonal, late winter and early spring being the worst periods (Jordan, 1933; Lovell and Hill, 1940; Withers, 1952, 1953; Veterinary Investigation Service, 1964). In southwest Scotland many farmers have difficulty in rearing newborn calves, and during the months of January to April a very heavy death

rate is experienced (Inglis, 1960). Many reasons have been forwarded as to why this should be so, most of them falling into the categories of infection, nutrition, housing, stockmanship and transport. These aspects have been described in several review articles (Inglis, 1960; Manton, 1963; Loosemore, 1964; Edgson, 1964; Roy, 1964; Ministry of Agriculture, 1966).

Although the relationship between colisepticaemia and agamma-globulinaemia has been recognised for many years, there does not seem to have been any investigation into the levels of immune globulin present in the serum of calves suffering from neonatal disease until Fay (1962) re-emphasised the relationship in naturally occurring cases. A possible reason for this delay is that it has been assumed generally and has appeared in most textbooks that the calf intestine retains for 24-36 hours the ability to absorb intact the immune lactoglobulin molecules present in colostrum. During this period a calf would receive three or four feeds of colostrum and hence have access to more than enough immune globulin to give it a high level of passive immunity. Very little is known about the speed and efficiency of the absorption mechanism in calves and this again has led to the assumption that the time of feeding and the quantity fed are relatively unimportant.

Aims of the present investigation

In view of our knowledge of the immunological status of the newborn calf and its relationship to disease the following aspects of this subject have been studied utilising the zinc sulphate turbidity test as a measure of serum immune globulin concentration.

A) Changes in the serum immune globulin concentration of newborn calves over a three week period.

B) The relationship between serum immune globulin concentration and susceptibility to neonatal disease.

C) Survey of serum immune globulin concentration of neonatal calves in relation to season.

SECTION A

Changes in the serum immune globulin concentration of newborn calves over a three week period

The method used to study the changes in serum immune globulin concentration was as follows. Three calves under one week of age were each allotted to a specific group depending on the initial zinc sulphate turbidity of their serum. The groups were established as follows.

Group	Immune Globulin Concentration (ZST Units)
I	0 - 5
II	5 - 15
III	15 - 25
IV	25 - 35
V	35 - 45

The calves were retested at 7 day intervals until 21 days had elapsed. Under local conditions a certain amount of disease, e.g. septicaemia, diarrhoea, pneumonia, is present. Therefore, any calf which clinically appeared unwell was removed from the series, and observations were started on another calf under one week of age. The average turbidity for each group was found from the three calves, and the results are shown in Figure No. 21 and in Appendix No. 2.1.

Results

The statistical significance of these changes are shown in Table No. 2.1. The greatest rates of decline of passively acquired circulating immune globulin occurred in the calves which had the

Fig. 2.1. Changes in the serum immune globulin concentration of newborn calves over a 3 week period.

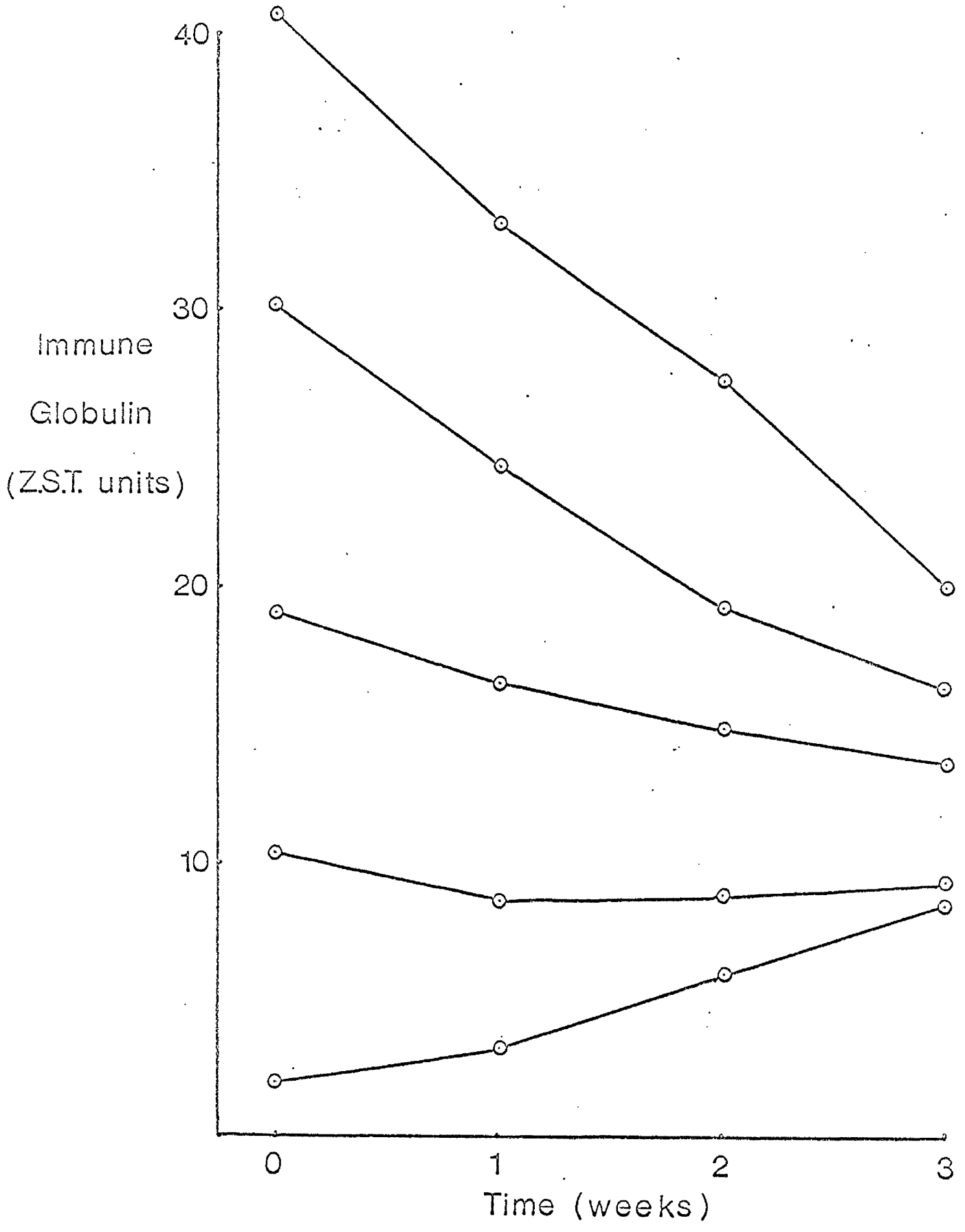


TABLE 2.1

Significance of the Changes in the Zinc Sulphate Turbidity during a 21 Day Period

	Time (days)		
Class	0 - 7	0 - 14	0 - 21
I	n.s.	0.05	0.01
II	0.01	n.s.	n.s.
III	n.s.	0.02	0.01
IV	n.s.	0.1	0.02
V	0.02	0.001	0.001

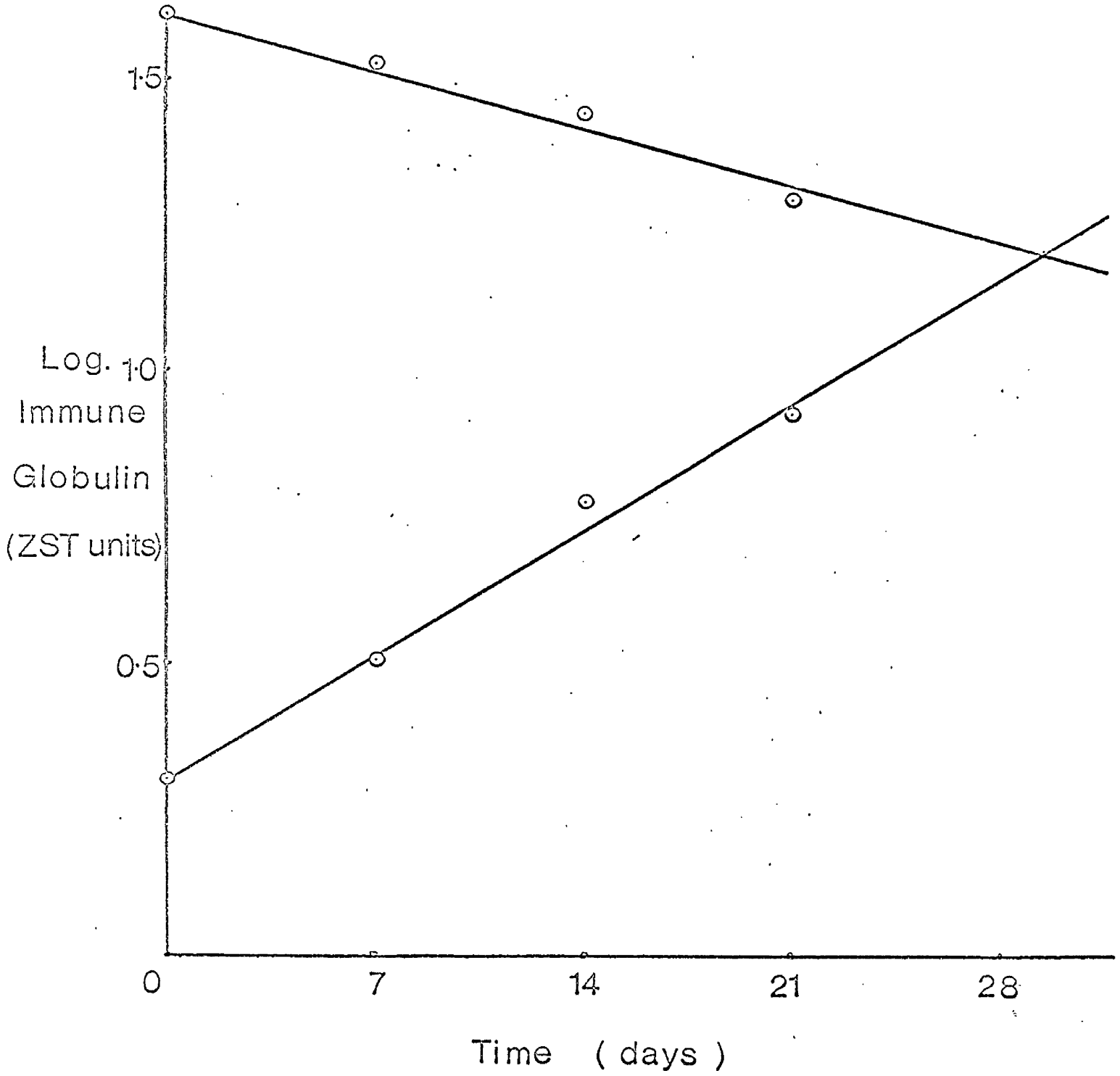
The figures in the Table refer to the probability (p).

n.s. - not significant

highest initial turbidity readings. This suggests that elimination is occurring in a logarithmic manner. The fact that within each class the rate of elimination would appear to be almost linear may be explained by the relative shortness of the period studied in relation to the half life of the passively acquired immune globulin.

In the group with the lowest initial turbidity it will be noted that a significant increase in turbidity occurs within fourteen days. This is probably due to the autogenous production of immune globulin. This active production has been shown by Pierce (1955) to cause an apparent falling off in the rate of elimination of passively acquired immune globulin when measured by electrophoretic methods. This phenomenon will be reflected in the zinc sulphate turbidity test, since it does not distinguish between the source of immune globulin. The effect may be minimised, however, by measuring the rate of decline of the group with the highest turbidity readings. Reference to Figure 2.2 in which the turbidity changes of Groups I and V have been plotted against a logarithmic scale shows that these changes are occurring in a logarithmic manner. The regression equation for Group V may be calculated as $y = 1.61 - 0.014x$ and for Group I as $y = 0.0303x + 0.3058$. From the equation pertaining to Group V, it may be calculated that a halving of the concentration of passively acquired immune globulin occurs every 21.5 days. Similarly, in colostrum deprived calves (Group I) during early life the serum immune globulins double their concentration every 9.9 days.

Fig. 2.2. Changes in the serum immune globulin concentration of calves in Groups I and V.



Discussion

As far as can be seen, there exist in the literature few references to the use of turbidity test to measure the decline of passive immunity and the development of active immunity in calves. Niemann-Sorensen, Konggaard and Kruse (1966) using the zinc sulphate test followed the changes which occurred in the turbidity of the serum of 6 colostrum deprived calves over a period of 100 days. A rise in turbidity was detected between the 8th and 16th days. Using four colostrum fed calves the same authors noted the changes which occurred during the first 70 days of life. From the initial high turbidity levels seen 48 hours after the birth, the levels fell to minimal values by the 32nd day. Patterson (1967) followed the changes in zinc sulphate turbidity of one colostrum deprived calf during the first 80 days of life and found the reaction to be negative for the first four weeks. In human infants it has been shown that the zinc sulphate turbidity undergoes marked changes during the first weeks of life (Harris, Anderson and Day, 1954). Levels similar to these seen in adults were found at birth, but in 2-4 weeks they had dropped to minimal values.

The major objection to the use of such a test as zinc sulphate turbidity is that the amount of turbidity developed is to some extent influenced by the concentration of other components of the serum (Kunkel, 1947; de la Huerga et al., 1950; Adner, 1957; Reinhold, 1960).

In calves during the first 30 days of life the serum albumin concentration rises from approximately 2 gms to 3 gms per 100 ml

(Pierce, 1955a). According to results of Kunkel (1947) and dela Huerga et al. (1950), this increase in concentration may depress slightly the turbidity readings and give rise to apparent rates of decline of immune globulin. Similarly, the detection of the production of immune globulins by the colostrum deprived calf may be delayed. Reference to the results obtained in the previous section studying the influence of increasing albumin concentration on zinc sulphate turbidity would suggest that this effect is negligible. The influence of alpha and beta globulins on the zinc sulphate turbidity test in calves is unknown, and it has been shown by Pierce (1955), Varnell et al. (1960) that these components do undergo significant fluctuations during the first eighty days of life as do the lipids (Shannon and Lascelles, 1966) and glycoproteins and lipoproteins (Varnell et al., 1960).

The influence of fetuin, a glycoprotein which migrates with the alpha globulins (Pedersen, 1944, 1947) is also unknown. It has been noted that sera of newborn calves have contained relatively large amounts of alpha globulin (Jameson et al., 1942; Hansen and Phillips, 1947; Deutch, 1954; Sprio, 1960). Bergman et al. (1962) found by means of quantitative complement fixation assay that the level of fetuin in the calf foetus at 9 months' gestation was 21.7 mgms per ml of serum. In adult cattle sera approximately 0.4 mgms per ml of fetuin was detected, but unfortunately since the sera of calves of various ages were not tested, no rate of decline was described. From the work of Varnell et al. (1960) it can be seen that F action II of the glycoproteins equivalent to the alpha globulin fraction, decreased from birth until 42 days and then remained constant.

SECTION B

The relationship between serum immune globulin concentration and susceptibility to neonatal disease

During the course of a year a large number of calves are brought into the Glasgow University Veterinary Hospital for teaching and experimental purposes. These are mostly Ayrshire bull calves under one week of age and are bought in the local markets. During the first weeks a certain amount of disease in the form of colisepticaemia and neonatal diarrhoea is usual. In an attempt to confirm the relationship between colisepticaemia and hypogammaglobulinaemia and possibly to predict which calves would be susceptible to this disease, the zinc sulphate turbidity test was performed on the sera of these calves as soon as they arrived at the Hospital. The results of this test were then correlated with the fate of those calves during the first three weeks after arrival.

Post-mortem examination

A necropsy examination of all calves which died was carried out. This consisted of a macroscopic inspection of the thoracic and abdominal organs and of the major joints, i.e. carpal, tarsal, coxo-femoral and atlanto-occipital. Routinely attempts were made to recover *E. coli* from the spleen and kidney by culture on blood agar and MacConkey agar of material taken from these organs. A diagnosis of septicaemia was made only if *E. coli* was recovered from both organs. Additional supportive evidence was supplied by the clinical history of the animal and the changes observed in certain organs. Enlargement

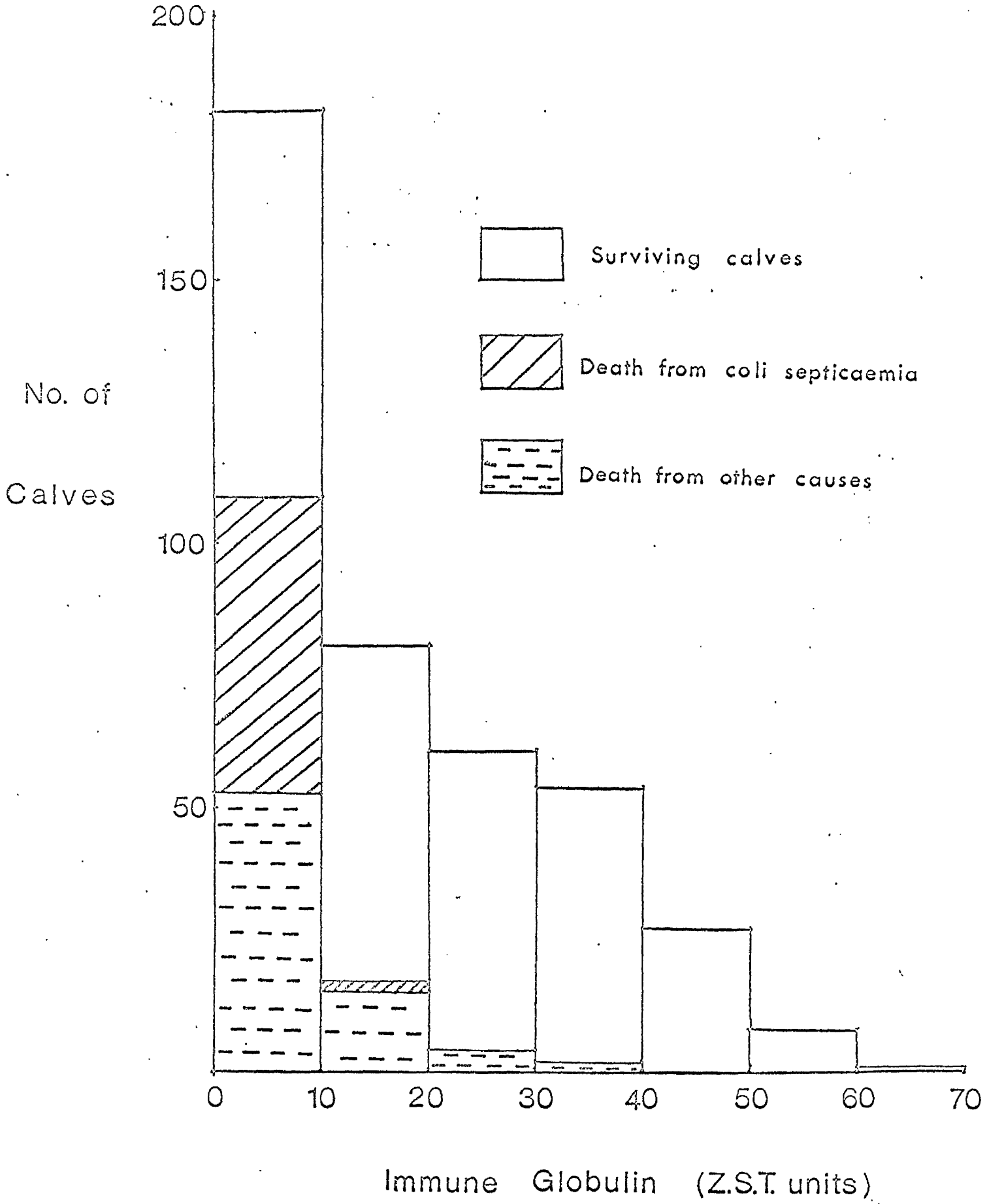
of the spleen was frequently observed in collisepticaemia, but cases were encountered in which this was not the notable feature. Petechiae were often found on the surface of the spleen and on the endocardium and epicardium especially in the region of the coronary arteries. It was noted that petechiae are present on the epicardium of normal animals, but they are not so marked as in collisepticaemia. Diagnosis of death from the effects of diarrhoea was made on the clinical history, the failure to isolate E. coli from spleen or kidney and from the absence of any specific lesion at post-mortem examination.

Results

The initial results of this trial have been described elsewhere, (Gay, Anderson, Fisher, McEwan, 1964). Since the publication of these results, this test has been used constantly in the selection of neonatal market calves. The relationship between neonatal disease and hypogammaglobulinaemia has been further confirmed. Figure 2.3 shows the results of this investigation which involved 415 calves.

It can be seen that with two exceptions all the septicaemic deaths have occurred in the group with the lowest immune globulin levels. Diarrhoea did occur in most of the calves, but deaths arising from this syndrome were seen again mainly in the calves with the lowest levels of immune globulin. It is important to emphasise here that the calves included in this investigation have been kept as control animals during trials of various methods of treatment and prophylaxis.

Fig. 2.3. The relationship between serum Immune globulin concentration and neonatal mortality.



The overall mortality rate in this group of calves was 31.8%. Deaths arising from colisepticaemia accounted for 14% of the losses, the remaining 17.8% being due to death from the effects of diarrhoea. This mortality rate is much higher than the national or regional average. The reasons for this probably lie in the source, selection and management of these animals. That these calves had all passed through a market, that many of them had been selected because of their low immune globulin concentrations, and that no attempt was made either to treat or to prevent neonatal disease must be considered as contributory towards the high mortality rate.

The impression that calves which died of colisepticaemia did so mainly during the first week after arrival was verified (Table 2.2). It can be seen from this table that the mean survival period of 50 septicaemic calves was 5.0 $\pm$ 3.2 days. The mean serum immune globulin concentration of these calves was 2.1 $\pm$ 2.4 ZST units.

Discussion

The mean survival period after arrival of the 50 calves which died of colisepticaemia was 5.0 $\pm$ 3.2 days. In view of the fact that some of the calves included in this group may have been 6 or 7 days of age at the time of purchase, it is possible that 2 or 3 days should be added to the mean survival period so that the actual mean age of the calves might be found. It is interesting to note, however, that in order to reproduce this condition experimentally and with a degree of regularity, most workers have had to use colostrum deprived calves of 2 or 3 days of age only (Dunne, Glantz, Hokanson and Dortree, 1956;

TABLE 2.2

Immune Globulin Concentration (ZST units) and the Survival of 50 Septicaemic Calves

Calf No.	Zinc Sulphate Turbidity Units	Survival (days)	Calf No.	Zinc Sulphate Turbidity Units	Survival (days)
1	0.25	4	26	2.5	1
2	0	3	27	0.5	3
3	6.0	14	28	0.6	5
4	1.75	11	29	0.2	6
5	2.5	4	30	0.6	6
6	2.5	8	31	0.1	3
7	0.25	14	32	1.9	8
8	3.0	11	33	1.7	3
9	2.0	3	34	1.3	2
10	1.5	4	35	2.3	2
11	6.0	4	36	0.9	5
12	12.0	4	37	2.6	1
13	8.5	12	38	1.8	5
14	0.5	4	39	1.2	8
15	2.0	9	40	0	2
16	2.25	4	41	0.1	2
17	0	3	42	1.5	7
18	0	3	43	1.7	5
19	8.5	5	44	0	3
20	2.5	3	45	4.4	5
21	2.5	2	46	1.8	5
22	5.25	9	47	0.4	2
23	1.75	3	48	1.1	2
24	0.5	2	49	0.9	3
25	2.0	7	50	0.25	2

Zinc sulphate turbidity mean $\pm$ S.D. = 2.1 $\pm$ 2.4.

Survival (days) mean $\pm$ S.D. = 5.0 $\pm$ 3.2.

Glantz, 1959; Penhale, 1965). This would suggest that under natural conditions other factors are involved in the pathogenesis of this disease.

The very low immune globulin concentrations found in these calves confirms the findings of Foy and Margadant (1961). These very low levels suggest that either these calves have not been allowed colostrum at all or that they have been given it after the absorptive ability of the intestine has ceased. An investigation to ascertain the relative importance of these two possibilities would certainly be worthwhile.

Death is often a convenient criterion by which to judge the severity or incidence of certain diseases. In relation to neonatal calf diarrhoea and septicaemia, however, it is well recognised that although many animals die from the effects of these syndromes, a much larger proportion develop the clinical signs from which after a variable period they make a recovery. The effect of this on the calf is to temporarily check the growth rate and as a result, there may be no gain in weight over this period (Aschaffenburg, et al., 1949, 1950; Dalton, Fisher and McIntyre, 1960, 1965). Moreover, in a certain proportion of calves there is no full clinical recovery. These animals may have continually soft or fluid faeces or may develop secondarily a chronic pneumonia or arthritis. The economic importance of the check in liveweight gain and the subsequent development of poorly thriving animals is difficult to determine since with the exception of intensive veal producing units, calves are kept under semi-intensive or extensive management.

In relation to this problem an interesting point arises from the negative correlation in serum immune globulin levels and death in neonatal calves. If the effects of neonatal diarrhoea are seen most in those calves with low immune globulin levels, there may exist a positive correlation between immune globulin levels and liveweight gains. Although no direct evidence supporting this has been found, various authors have noted the difference in liveweight gains which exist between colostrum deprived and colostrum fed animals. Aschaffenburg et al. (1949a, b) showed that the mean liveweight gains of calves when measured at 21 days of age was greatest in those which had been fed the largest quantities of the non-fatty fraction of colostrum, Table 2.3.

TABLE 2.3

Quantity of Non-Fatty Fraction (ml)	No. of Calves	Mean Birth Wt. (lb)	Mean Liveweight Gain During First 21 Days (lb)
7.200	8	83	14 ± 2.2*
3.000	2	80	12
900	2	84	8
400	6	86	2 ± 0.7
200	6	83	4 ± 1.9
80	6	77	3 ± 2.3

* Values with their standard errors.

In the work of Roberts et al. (1954) on some effects of colostrum deprivation in the calf, observations were made on liveweight gains of colostrum fed and deprived calves. The mean weight gain of 13 colostrum deprived calves which survived to three weeks of age was 13.4 ± 8.4 lbs. Eight colostrum fed calves over the same period had

mean weight gains of 17.9 $\pm$ 3.74 lbs. Whereas the difference between these two groups is not significant, it will be observed that the standard deviation of the colostrum deprived group is much larger. This could be interpreted as an indication of the various levels of health of these calves.

Perry and Watson (1967) compared the growth rates of piglets which were classified according to their levels of passive immunity. Up to 7 days post-partum there was little difference between the classes, but between 7 and 28 days an increasing difference was observed between those piglets which had absorbed most antibody and those which had absorbed the least amount.

From the correlation between immunoglobulin levels and the fate of calves the question arises as to what is the exact protective function of the proteins. The protection from septicaemia offered by relatively small amounts of immune globulins taken together with the obvious association between the syndrome and an infectious agent leaves little doubt that an immunological function is being performed. The precise nature of the specific protecting antibody is not yet known (Gay, 1965). The aetiology of neonatal diarrhoea is not apparent and the multiplicity of strains of E. coli isolated from such cases has caused some doubt as to whether the syndrome had an infectious aetiology (Smith, 1962). The possibility exists, therefore, that these proteins may have a function other than an immunological one.

The infusion of high molecular weight substances intravenously is a well-recognised technique for the expansion of plasma volume. Such an effect may occur after the absorption of immune globulin molecules intact by the intestine. The osmotic effect of these molecules may expand the plasma volume and concurrently give the animal greater reserves of fluid and electrolytes from which to draw during a diarrhoeic episode. In addition proteins have long been recognised as a part of the buffering mechanisms existing within the body to preserve 'le milieu interieur.' The effect of the loss of fluid and electrolytes through diarrhoea is to produce a metabolic acidosis, an augmented ability to maintain the pH of the body fluids within the range compatible with life may aid the survival of acidotic animals. Investigations into these possibilities have been undertaken and are reported later.

SECTION C

Survey of serum immune globulin concentration of neonatal calves in relation to season

During the investigation into the relationship between immune globulin levels and mortality, it was soon noted that during the winter months the average zinc sulphate turbidity of the market calves bought in was very much lower than those seen during the summer and autumn months. It was decided therefore to conduct a survey of the variations in immune globulin seen during the course of a year. The results of this survey for 1964/1965 have been published (Gay, Fisher, McEwan, 1965). The survey has been continued for another year and the combined results are shown below, Figure 2.4. The calves studied in this survey include animals which were brought into the Veterinary Hospital for treating and experimental purposes and calves passing through the Paisley market which were sold for immediate slaughter as veal. Since the calves bought by the Hospital were drawn from this source, no attempt has been made to differentiate between the two groups in the survey.

Results

It would appear that in these market calves there is a very marked seasonal variation in immune globulin level. The high levels seen during the summer and early autumn start to decline by November, reach their lowest values in February and March and then increase to maximum values between June and September. The relative distribution of immune globulin concentrations found monthly is given in Table 2.4.

Fig. 2.4. Seasonal variations in the average serum immune globulin concentration of neonatal calves.

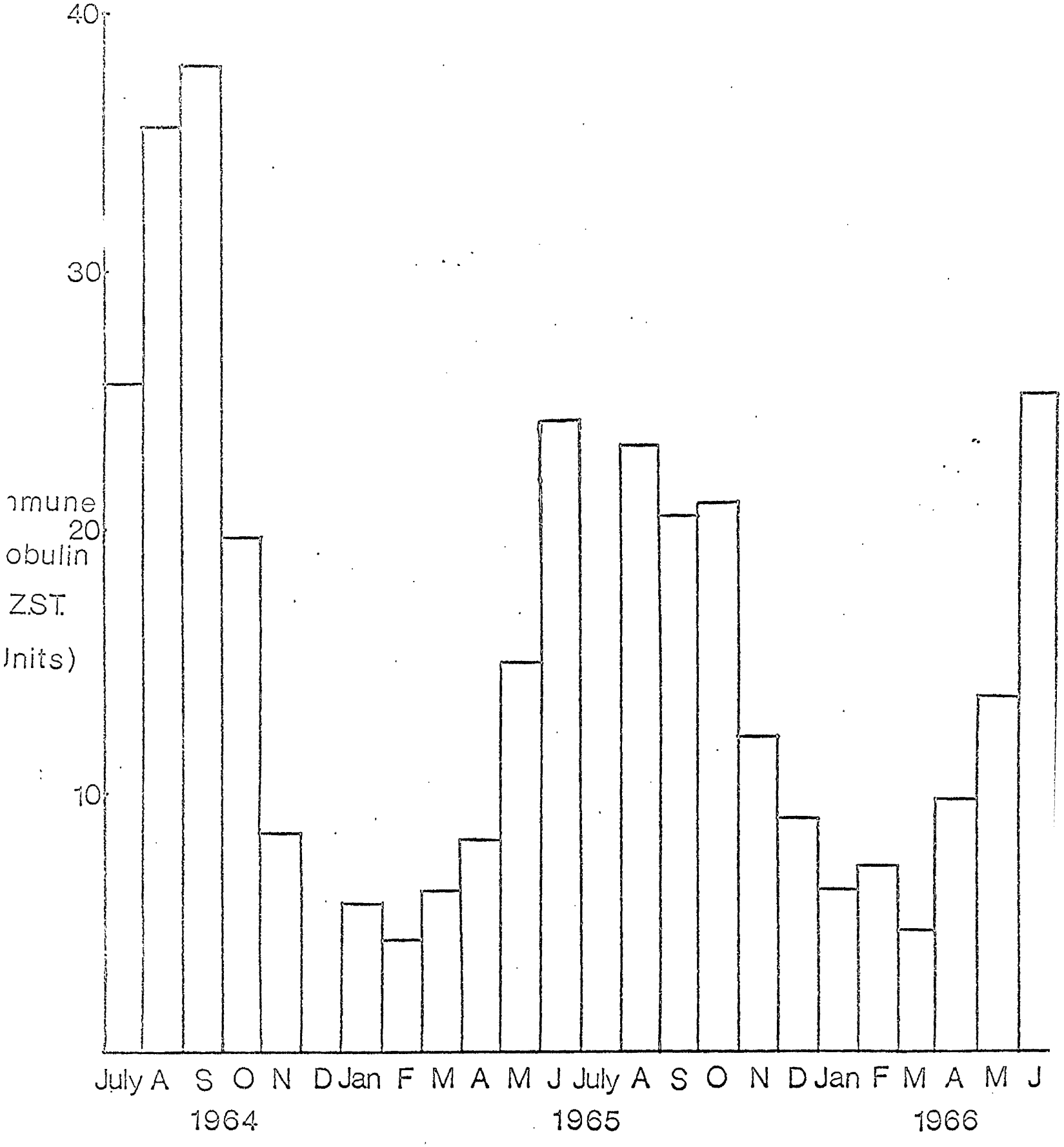


TABLE 2.4

The Relative Distribution (%) of the Serum Immune Globulin Levels of Neonatal Market Calves During a Two Year Period

Month	Zinc Sulphate Turbidity Units							No. of Calves
	0-9	10-19	20-29	30-39	40-49	50-59	60-69	
July 1964	33.3	8.3	16.7	25.0	16.7			12
Aug.	10.0	10.0	5.0	25.0	30.0	15.0	5.0	20
Sept.	16.6	0	0	16.6	50.0	16.6		6
Oct.	32.0	19.5	19.5	21.0	7.0	0	1.0	97
Nov.	65.0	30.0	5.0					20
Dec.	-	-	-	-	-	-	-	0
Jan. 1965	87.5	12.5						40
Feb.	90.0	10.0						20
Mar.	75.0	20.0	5.0					20
April	61.0	30.5	5.0	3.5				59
May	47.0	19.7	17.0	12.0	3.5	0.8		117
June	37.5	10.0	20.0	17.5	10.0	5.0		72
July	-	-	-	-	-	-	-	0
Aug.	13.3	33.3	26.7	13.3	10.0	3.3		30
Sept.	34.7	11.1	26.4	15.3	11.1	1.4		72
Oct.	36.7	15.0	11.6	21.7	13.3	1.7		60
Nov.	49.3	32.5	10.4	5.2	1.3	1.3		77
Dec.	66.6	20.5	10.3	2.6				39
Jan. 1966	77.5	17.5	2.5	2.5				40
Feb.	75.7	19.0	2.7	1.3	1.3			74
Mar.	76.0	20.0	0	4.0				24
April	65.0	20.0	10.0	5.0				40
May	50.7	23.3	10.9	8.2	6.8			73
June	9.0	18.2	41.0	31.8				22

Discussion

Following upon the publication of the results of this survey after one year (Gay, Fisher and McEwan, 1965), a smaller survey in England was conducted by Smith, O'Neill and Simmons (1967). This involved the determination of the immune globulin concentration of the sera of 230 calves, 190 of which were home-bred, the remaining 40 being obtained from markets. Direct comparison of the results of both surveys is difficult since the techniques of using the zinc sulphate turbidity test were not identical. Although the appearance of low levels of immune globulin was not apparent in the English survey during the winter months, an increase in the proportion of calves with high levels was found during the summer.

The reason for this apparent difference may be due to regional differences in the management of the cow and her newborn calf. The majority of calves studied by Smith et al. (1967) obtained colostrum by suckling. During the winter months in southwest Scotland the majority of dairy cattle are housed in byres. It is standard practice to allow the cows to calve there and then to remove the calf to a pen where it may receive some colostrum. In general, colostrum is not fed until after the next milking time which may be 12-15 hours after the birth of the calf. On farms where calving takes place in a loose box kept specially for this purpose the procedure is generally similar in that the calf is not allowed to be suckled, but is removed and fed colostrum from a bucket later on. The reasons given by farmers for this procedure are various. The advantages claimed are that it is known that the calf has fed, that training the calf to drink out of a

bucket is easier if it has never been suckled and that cattle do not resent machine milking if they have not been allowed to suckle a calf. This procedure also removes the factor of poor mothering ability as a result of which a cow may take little or no interest in the calf and may refuse to allow it access to her udder.

Information on the influence of feeding methods on immune globulin levels of the serum of calves is scant. Smith et al. (1967) noted that differences in the levels occurred between calves fed by bucket, suckling or by a mixture of both methods. No obvious difference in the levels was found between those fed by suckling and those fed by suckling and bucket. The calves fed by bucket alone, however, had much lower levels. Other indirect evidence that the amount of immune globulin absorbed is less if colostrum is fed by bucket comes from the survey on mortality and disease in calves conducted by Withers (1952). A comparison of the mortality rates of calves up to one month of age which had been fed colostrum by suckling or by bucket revealed that bucket feeding was constantly associated with a high mortality rate. In view of the evidence cited earlier on the relationship between neonatal disease and colliseptic-aemia, in particular, and hypogammaglobulinaemia, it is very probable that the bucket fed calves described by Withers had lower serum concentrations of immune globulin than the suckled calves.

Although suckling is the most natural method of feeding, it would appear that even this cannot be relied upon to give high levels of immune globulin. Smith (1962) noted that in 52 male Jersey calves which were allowed to remain with their mothers for 2 days, 6,

i.e. 11.5%, had turbidity readings between 0 and 5. Smith et al. (1967) reported that of 80 calves which were observed to have suckled within a few hours of birth, 8, i.e. 10%, had serum levels which yielded turbidity readings of less than 10.

During some preliminary work investigating the influence of the method of feeding, results were obtained from 10 Ayrshire cows which were allowed to calve in loose boxes and to remain with the calves for a period of 24 hours. Serum from the calves was taken at 48 hours post-partum and tested by the zinc sulphate turbidity test. The results are shown below.

TABLE 2.5

Calf No.	1	2	3	4	5	6	7	8	9	10
Immune Globulin ZST Units	5	5	8.5	13	18	18	19	34	37	45

These results would appear to agree with those of Smith in that access to the cow during the first day of life does not guarantee a high level of immune globulin. This small trial was conducted during the months of February and March when the seasonal average was lowest, and it is interesting to see that relatively high levels can be obtained during this period of the year. These results taken in conjunction with those of Smith (1962, 1967) raise questions about the frequency and time of feeding and the quantities obtained by the newborn calf in relation to the amount of immune globulin which they ultimately absorb. These questions will be discussed in Part IV.

PART III

INVESTIGATIONS INTO NON-IMMUNOLOGICAL FUNCTIONS OF THE PASSIVELY ACQUIRED GLOBULINS

GENERAL INTRODUCTION

It has been shown in an earlier section that deaths from the effects of diarrhoea occurred only in those calves with low or moderate levels of circulating immune globulin. The reason why calves with high globulin levels do not die although they may develop diarrhoea is not clear. It is a well established fact that the principal function of the globulins acquired passively from the dam is an immunological one. However, the absorption of intact protein molecules must have important physiological as well as immunological effects. One possible function common to all large molecules within the circulation is that of causing an expansion of the plasma volume. If the expansion of the plasma volume is proportional to the amount of globulin absorbed by the animal, those with the highest concentrations of circulating globulin and possibly the largest plasma volumes will have larger reserves of fluid and electrolyte on which to draw during a diarrhoeic episode. In addition, proteins have been recognised to be involved in the buffering mechanisms of the body. In severe diarrhoea a metabolic acidosis develops due to the loss of bicarbonate and sodium in the faeces (Bland, 1963). An increased concentration of protein buffer may add significantly to the ability of the animal to withstand such an acidosis.

One further possibility is that during the process of natural elimination of passively acquired immune globulin a source of amino acids is available from which the animal may draw. The significance of this function in either the anabolic or catabolic state is not known, and metabolic studies on gnotobiotic animals would have to be made to determine its validity.

a) Plasma Volume Expansion

Many workers have studied the quantitative and qualitative changes in the protein components of serum and plasma of the newborn calf before and after the ingestion of colostrum (Howe, 1921; Jameson, Alvarez-Tostado and Sortor, 1942; San Clemente and Huddleston, 1943; Pedersen, 1945; Hansen and Phillips, 1947; Polson, 1952; Pierce, 1955a). This passive transference of globulin to the young calf may result in a doubling of the serum protein concentration within 24-36 hours of birth. In other species increases of this magnitude have been observed (Earle, 1935; Nordbring and Olsson, 1957; Ramirez et al., 1963).

It is known that the osmotic pressure developed by a dissolved substance is proportional to the number of non-diffusible molecules and not to the mass of the substance dissolved. By comparing the average molecular weights of the major protein fractions of plasma, i.e. Albumin, 69,000; Globulin, 170,000; Fibrinogen, 330,000, one will see that for equal weights of these proteins the number of molecules present will be in the ratio of 5:2, 5:1 respectively. It has been calculated by Scatchard et al. (1944) that albumin is responsible for 75% of the colloid osmotic pressure of human plasma proteins. The reason for this, in addition to the relatively low molecular weight, is that the albumin molecule has a relatively high average net charge due to its low isoelectric point (pI = 4.7) and its powerful binding activity for anions.

The colloid osmotic pressure of 5 fractions of plasma proteins at different concentrations are shown by Guyton (1961) in a figure modified from Ott (1956). From this figure it can be seen that the

relationship between protein concentration and osmotic pressure is not linear due to the Donnan equilibrium effect. Low concentrations of protein, i.e. below 4 g per 100 ml, produce a disproportionately small osmotic pressure. The concentration of gamma globulins found in the serum of colostrum fed animals rarely exceeds 4 g per 100 ml serum, and so the expected increase in plasma osmotic pressure will be very slight.

The contribution made by the plasma proteins towards the total plasma osmotic pressure is not large, a pressure of 28 mm Hg being accepted as normal; whereas plasma osmotic pressure is in the region of 5000 mm Hg. However, the importance of this contribution was recognised by Starling (1896) and subsequent workers who have studied the mechanical factors influencing lymph production. Due to the intravascular location of the proteins, the osmotic pressure is greater than the hydrostatic pressure at the venous end of capillary vessels. This is a major factor governing the reabsorption of fluid and the maintenance of an osmotic equilibrium between the intravascular and extravascular spaces. An increase in the concentration of protein may lead to an increase in the amount of fluid entering the circulation and hence an expansion of the plasma volume.

The ability of large molecules within the circulation to cause such an expansion has been utilised clinically in the treatment of cases in which hypovolaemia occurs. Albumin has been used for this purpose (Cournand et al., 1944). The results of infusing albumin into the circulation of an analbuminaemic patient were described by Ott (1957).

The concentration of albumin in the serum rose from 0.1 g to 2.7 g per 100 ml, although the serum proteins only rose from 5.4 to 5.6 g per 100 ml, a fact suggesting that a large expansion of plasma volume had occurred.

An expansion of the plasma volume of rabbits has been detected by Rothschild, Oratz, Franklin and Schreiber (1962) and Bjorneboe (1967) as a result of immunisation. It was pointed out by the latter author that in order to achieve this expansion, the rise in gamma globulin concentration should be large and rapid. Nansen and Nellsen (1966) noted that adult cattle with hyperimmunoglobulinaemia tended to have higher plasma volumes than normal animals.

It has been suggested that in piglets following the ingestion of colostrum, one of the physiological results of the assimilation of a large quantity of protein within the vascular space is an expansion of the plasma volume (McCance and Widdowson, 1959). This view was supported by Ramirez et al. (1963). It was postulated that the absorption of colostral proteins into the blood stream caused an increase in the osmotic pressure of the plasma. This increase in osmolarity would result in the attraction of fluid into the vascular system and cause an expansion of plasma volume.

From the available evidence it would appear that an expansion of plasma volume does occur in early life. McCance and Widdowson (1959) demonstrated that colostrum fed piglets had at 24 hours of age plasma volumes which were greater than at birth, and this finding was supported by Ramirez et al. (1963). In calves no comparable measurements appear to have been made although there exists a certain

amount of circumstantial evidence. It was noted by McCance and Widdowson (1959) that piglets which were allowed to ingest colostrum as well as having higher concentrations of protein in the serum, had consistently lower haematocrits. Observations made by several workers on the changes in the haematocrit of calves during the first days of life show a similar tendency (Wise, et al., 1947; Roberts, et al., 1954; Greatorex, 1954; Holman, 1956; Penhale, 1965; Shannon and Lascelles, 1966). Dalton (1967), however, did not notice any change in the haematocrit of calves during the first 3 weeks of life. Of comparative interest, a marked fall in the haematocrit of newborn goat kids has been observed during the first 32 hours of life by Holman and Dew (1965), but no measurements of plasma volume were made.

In order to confirm that an expansion of the plasma volume of calves occurs during early life and to determine the role of the immune globulins in this occurrence a series of measurements have been made on colostrum fed and milk fed calves.

b) Buffering Capacity

Due to the fact that the physiological pH of the body is more alkaline than the isoelectric point of the plasma proteins, these proteins exist in the plasma as anions. As such, they play a role along with the carbonic acid - bicarbonate system and the dihydrogen phosphate - mono hydrogen phosphate system in the maintenance of a stable pH through their buffering characteristics. The relative buffering powers of the different buffers are demonstrated by Guyton (1961). Quantitatively the intracellular buffers, protein and

phosphate, are responsible for most of the buffering activity of the body fluids. In blood, assuming that the buffering power of the bicarbonate system is unity, the relative powers of the bicarbonate, phosphate, plasma protein and haemoglobin buffers are 1.0:0.3:1.4:5.3 respectively. However, if blood and interstitial fluid are considered together, the relative powers of the plasma protein and haemoglobin are decreased due to the dilution of these substances by the interstitial fluids.

Since the effect of increasing proportionately the concentration of the components of a buffering system is to increase the buffering capacity, it may be assumed that the increase which occurs after immune globulins have absorbed from colostrum, will increase the buffering capacity of the plasma proteins. The relative importance of this increase in terms of the total buffering capacity of the body is not known. By the adoption of *in vitro* techniques, the buffering characteristics of a protein may be found by the addition of known quantities of acid or alkali to a solution of the protein. *In vivo*, however, the tendency to displacement of the pH to one or the other side of the physiological norm is countered not only by the buffering systems but also by compensatory renal and respiratory mechanisms. In an attempt to determine the influence of the protein buffer system on the ability of the whole body to withstand the effects of a metabolic acidosis, a series of experiments were undertaken, using calves with high and low immune globulin levels.

SECTION A

PLASMA VOLUME EXPANSION

Experimental Design

In order to determine the role of immune globulins in the probable expansion of plasma volume which occurs after birth, the following experiment was devised. Newborn calves were removed from their dams immediately after birth and blood samples were taken for estimation of the concentration of serum protein, electrolytes, packed cell volume and osmotic pressure. The plasma volume was then measured and the calves were then allowed to drink either colostrum or milk. Five calves were put on a milk diet. Both groups of calves were then fed milk until 3 days of age when repeat estimations were made of the parameters measured at birth.

Plasma Volume Estimation

For the estimation of plasma volume a stock solution of 1% Evan's Blue dye (T1824, The British Drug Houses Ltd., Poole) in water was used. Approximately 2 ml of this solution was taken up into a 2 ml plastic disposable syringe (Johnson's Ethical Plastics Ltd., Slough, England) and a 1½" 18G needle was attached to the syringe. The syringe containing the dye and the needle were then weighed together. After the dye had been injected intravenously (see Management of Calves) the syringe and needle were then reweighed so that an accurate estimate of how much dye had been injected was obtained. The blood samples taken before and 10 minutes after injection of the dye, were collected in heparinised polythene centrifuge tubes. Plasma from these samples was obtained by centrifugation. The time interval of 10 minutes was chosen since it has been shown by Dalton and Fisher (1961) that the concentration of the dye in the plasma of cows was very similar to that at zero time obtained by forward extrapolation of the excretion curve of the dye.

A standard concentration of dye in plasma was made by adding 10 microlitres of the dye solution by means of a disposable micro-pipette (Microcaps' Drummond Scientific Co., Broomhall, Pa., U.S.A.) to 5 ml of plasma. The concentration of dye in the 10 minute sample was estimated by comparing the intensity of the colour of this sample with that of the 'standard' by means of an EEL portable colorimeter (Evans Electroselenium Ltd., Harlow, Essex) using matched 1 ml colorimeter tubes and an Ilford 607 (orange) filter. The colorimeter is previously zeroed on a tube containing 1 ml of pre-injection plasma. Having determined the concentration of dye in the plasma 10 minutes after injection, the volume of plasma was determined by dividing the amount of dye injected by this figure. The plasma volume was expressed on a body weight basis, i.e. ml per kilogram body weight.

Haematocrit

This was determined using a micro haematocrit centrifuge (Hawksley & Sons Ltd., London). The centrifugation time was 10 minutes. No correction factor was used in estimating the error due to the effect of trapped plasma, mainly because this factor is not accurately known for calf serum and also because the factor may be influenced by the change in globulin concentrations occuring as a result of colostrum feeding.

Serum Protein Concentration

The biuret method described by Varley (1963) was used to estimate the serum protein concentration. The test was standardised by use of a commercially prepared standard of known protein concentration (Versatol, W.R. Warner and Co. Ltd., Eastleigh, Hampshire, England).

Zinc Sulphate Turbidity Test

This test was performed by the standard method described previously.

Osmotic Pressure

The osmotic pressure of plasma was determined usually within 1 hour of the sample being obtained from a calf. The instrument used was a Knauer semi micro osmometer (Shandon Scientific Products Ltd., London), which utilised the cryoscopic method of determining osmotic pressure. Before each sample was tested, the instrument was calibrated against distilled water and a salt solution of known osmolarity.

Sodium and Potassium

The serum sodium and potassium concentrations were determined on an EEL Flame photometer using the method described by Varley (1963).

Management of Calves

As soon as a calf was born, it was removed from the cow, dried and then weighed. On both sides of the neck overlying the jugular veins, the hair was removed by means of mechanical clippers so that the underlying vessels might be more easily located through the skin. The calf was then placed in lateral recumbency on a table with its head and part of its neck overhanging the edge. This position was found to be best both in respect of restraint of the animal and access to the jugular vein. A sterile 1½" 18 Gauge needle, which had been weighed previously with the syringe was inserted into the jugular vein, in the direction of the blood flow. A 25 ml sample of blood was taken and distributed into heparinised polythene test tubes

(15 ml) for those estimations requiring plasma and a Universal bottle (10 ml) for estimations on serum.

Having ensured that the tip of the needle was in the lumen of the vein by noting the rate of flow of blood through the needle when the syringe was detached, the 2 ml syringe containing the dye solution was connected to it and the solution injected. The syringe and needle were then withdrawn together, wiped clean of any blood which may have adhered and put aside for re-weighing. A stopwatch was started immediately the dye was injected so that another sample could be taken 10 minutes later. When this time had elapsed the calf was placed on the table in the same position but on its other side so that the other jugular vein was exposed. This procedure was adopted so that there would be no chance contamination of the second sample by any Evan's blue which may have leaked into the tissues during the withdrawal of the needle after the dye had been injected. A 10 ml sample of blood was taken and put into a heparinised test tube.

After this sample had been taken the calf was given by bucket some of its dam's colostrum or some milk depending on the type of feeding to which it had been allocated. The volume ingested by the calf was found by measuring the volume of the colostrum before and after the calf had fed. All the calves were allowed to drink to satiation. During the ensuing 15 hours colostrum or milk was offered again to the calf and the amount taken was noted. Thereafter, only milk was offered. A sample of colostrum (60 ml) was taken so that an estimate of the total quantity of immune globulins presented to the calf could be calculated. The calves were all kept in individual pens and were

bedded in straw. The ambient temperature was never below 20°C. Milk was fed at the rate of 3-4 pints, twice daily. Three days after birth a second estimation of the plasma volume was made using the same technique. This time interval was allowed to pass for several reasons; namely, to ensure that absorption of the immune globulins from the intestine had been completed, to allow time during which the immune globulins would equilibrate themselves between the extravascular and intravascular spaces and to allow time for the excretion of beta lactoglobulins by the kidney (Pierce, 1959) and so minimise any physiological effect which they may have.

Results

The individual results are shown in Tables 3.1 and 3.2.

Zinc Sulphate Turbidity

Before feeding both groups of calves have negligible turbidity levels; however, after feeding, only in the colostrum fed calves was there a significant increase ($p < 0.001$).

Serum Proteins

The concentrations of the serum proteins in both groups before feeding are not significantly different from each other. After feeding, the levels in the colostrum fed group rise significantly ($p < 0.001$), whereas in the milk fed group the levels are unchanged.

Albumin

In both groups before and after feeding there is no significant difference between the serum albumin concentrations.

TABLE 3.1

Changes in the Serum Proteins, Plasma Electrolytes, Plasma Osmotic Pressure, Haematocrit and Plasma Volume of Calves Fed Colostrum

		Calf Number					
		1	2	3	4	5	Mean ± S.D.
Zinc Sulphate Turbidity	Pre	1.0	0.5	0.75	1.25	1.0	0.9 ± 0.3
	Post	21.25	32.0	21.25	27.0	30.0	26.3 ± 4
Serum Protein (g/100 ml)	Pre	4.2	4.4	4.7	4.7	4.0	4.4 ± 0.3
	Post	6.6	7.5	6.6	7.2	7.1	7.0 ± 0.4
Albumin (g/100 ml)	Pre	2.1	2.25	2.1	2.05	1.6	2.0 ± 0.25
	Post	1.95	2.05	1.8	2.05	1.9	1.95 ± 0.1
Globulin (g/100 ml)	Pre	2.1	2.15	2.25	2.65	2.4	2.3 ± 0.2
	Post	4.65	5.45	4.8	5.15	5.2	5.0 ± 0.4
Sodium (m.eq/l)	Pre	155.8	150	152.6	155.8	155.6	153.9 ± 2.4
	Post	146.8	136.4	146.8	146.8	146.8	144.5 ± 5.1
Potassium (m.eq/l)	Pre	5.76	5.94	5.19	5.76	5.38	5.6 ± 0.3
	Post	6.12	5.2	5.58	6.86	5.38	5.8 ± 0.4
Osmotic Pressure (m.osmol/l)	Pre	289	293	291	299	296	293.6 ± 3.0
	Post	286	286	286	285	287	286 ± 0.7
Haematocrit (%)	Pre	39	38	44	42.5	39	40.5 ± 2.5
	Post	40	36.5	38	40	33.5	37.6 ± 2.7
Plasma Volume (ml/Kg Body Wt.)	Pre	72	75.5	61.4	56.6	62.3	65.6 ± 7.8
	Post	107.8	100.4	91.1	73.2	94.8	93.4 ± 12.9

TABLE 3.2

Changes in the Serum Proteins, Plasma Electrolytes, Plasma Osmotic Pressure, Haematocrit and Plasma Volume of Calves Fed Milk

		Calf Number					
		1	2	3	4	5	Mean ± S.D.
Zinc Sulphate Turbidity	Pre	1.5	1.0	1.5	0.5	1.0	1.1 ± 0.4
	Post	1.0	1.75	1.5	0.5	0	0.95 ± 0.6
Serum Protein (g/100 ml)	Pre	4.3	4.2	4.1	4.0	4.2	4.15 ± 0.1
	Post	4.5	3.7	4.2	3.8	3.9	4.0 ± 0.3
Albumin (g/100 ml.)	Pre	1.9	1.55	1.8	1.9	1.65	1.75 ± 0.15
	Post	2.05	1.5	1.75	1.75	1.55	1.7 ± 0.2
Globulin (g/100 ml)	Pre	2.4	2.65	2.3	2.1	2.55	2.3 ± 0.2
	Post	2.45	2.2	2.45	2.05	2.35	2.3 ± 0.17
Sodium (m.eq/l)	Pre	158.6	150	158.6	150	155.8	154.6 ± 4.3
	Post	144	144	141.2	138.6	147.0	142.9 ± 3.18
Potassium (m.eq/l)	Pre	6.1	6.28	5.34	5.0	5.16	5.57 ± 0.57
	Post	4.78	5.72	4.78	4.68	4.38	4.8 ± 0.5
Osmotic Pressure (m.osmol/l)	Pre	293	292	296	293	296	294 ± 2.0
	Post	279	284	281	286	280	282 ± 2.8
Haematocrit (%)	Pre	32	39	37.5	39	40	37.5 ± 3.6
	Post	27	35	33.5	39	31.5	33.1 ± 5.1
Plasma Volume (ml/Kg Body wt.)	Pre	54.8	65.9	69.7	60.5	63.5	62.9 ± 5.6
	Post	93.1	86.4	90.9	86.8	78.1	87.0 ± 5.7

Globulin

The changes in the serum globulin concentrations mirror those seen in the zinc sulphate turbidity and serum protein concentrations, i.e. a significant different ($p < 0.001$) is observed only in the colostrum fed group after feeding.

Sodium

The pre-feeding and post-feeding levels do not differ significantly between the groups, but within each group the post-feeding level is significantly lower ($p < 0.01$).

Potassium

There was no significant alteration in either group between the pre- and post-feeding concentrations of serum potassium.

Osmotic Pressure

The mean osmotic pressure of the plasma of the colostrum fed animals differed significantly ($p < 0.01$) as did that of the milk fed animals ($p < 0.001$) from the pre-feeding pressures. The post-feeding pressures in either group did not vary significantly from each other.

Haematocrit

The haematocrits of both groups of calves would appear to fall after feeding; however, there is not a significant difference between the pre- and post-feeding levels.

Plasma Volume

In both groups of calves there is a significant difference between the pre- and post-feeding volumes. The levels of significance for the colostrum fed group is $p < 0.01$, and for the milk fed group, $p < 0.001$.

Discussion

The main aims of this experiment were threefold. Firstly, it had to be shown that absorption of globulins from the intestines of the colostrum fed calves had occurred to a significant extent. Having established this, the changes in the plasma volumes of the two groups of calves have been determined and compared. Finally, in an attempt to explain the mechanism involved in the alteration of plasma volume, measurements have been made of other physiological parameters.

Evidence that globulins have been absorbed by the colostrum fed calves is hown by the increase in zinc sulphate turbidity, serum protein and total globulin concentrations. Compared with the milk fed calves, these measurements have all increased significantly.

The plasma volumes of both groups of calves, before and after feeding, were not significantly different from each other, although the volumes measured 3 days after birth were approximately 40% greater. This order of increase has been observed to occur in other species. McCance and Widdowson (1959) working with colostrum fed piglets noted that within 24 hours of birth the plasma volume increased from initial levels of 55 $\pm$ 7.7 ml/Kg to 81 $\pm$ 8.3 ml/Kg, an increase of 47%. Ramirez et al. (1963) have studied plasma volume changes in piglets during the first 5 weeks of life. From birth to 24 hours of age the plasma volume increased from 55 ml/Kg to 71.5 ml/Kg, an increase of 30%. This relative volume was maintained for 8 days after birth, whereupon it began to fall.

The reason for this decrease may be connected with the very fast growth rate of piglets. By consulting the mean bodyweights of the piglets described by Ramirez et al. (1963), it may be seen that during the first and second weeks of life, bodyweight increased by approximately 100 per cent per week. Although the growth rate of calves is not nearly so precocious, this may explain why the volumes measured at 3 days of age in the present experiment are significantly higher than those found by Dalton and Fisher (1961). These workers, using a similar technique to estimate plasma volume, determined values for 65 bull calves aged between 1 and 3 weeks. They found a mean of 66.0 ± 8.3 ml/Kg. Hansard et al. (1953) measured the blood volume of 2 calves, 2-6 days of age, and found a mean volume of 120 ml per Kg bodyweight, whereas a 3 week old calf had a volume of 85 ml per Kg bodyweight. Payne, Ryley and Gartner (1967) studied the changes in plasma volume of cattle from 2 months to 3 years of age, and have noted a progressive decline with age in this determination when expressed on a bodyweight basis.

As a result of certain experiments performed on newborn piglets (Widdowson and McCance, 1956; McCance and Widdowson, 1956, 1957), these authors noted that animals which had been fed either colostrum or early milk soon after birth had higher globulin concentrations and consistently lower haematocrits than those which were given water but no food. An attempt was made subsequently to study the effects of colostrum on the volume and composition of the plasma of newborn piglets (McCance and Widdowson, 1959). In this study blood was taken

from a litter immediately after birth for estimation of the plasma protein concentration and the haematocrit. The litter was then divided into two groups, one of which was allowed to obtain colostrum from the sow and the other was kept, unfed, in metabolic cages at 31° centigrade. Twenty-four hours later the plasma volumes were estimated by injecting Evan's Blue directly into the heart. It was found that colostrum fed piglets had larger plasma volumes, greater plasma protein concentrations and lower haematocrits than the control group. There was no significant change in the plasma protein concentration or haematocrit of the control piglets. The plasma volumes of the piglets at birth were not actually measured but estimated from the change in haematocrit reading, a method which assumes that the total number of erythrocytes in the circulation or the mean corpuscular volume does not change during the 24 hour period. It was concluded from this work that the absorption of globulins caused an increase in the plasma volume.

The chief criticism of this work is of the method of control. By starvation, not only are these animals being deprived of immune globulins but also of fluid, electrolytes and organic materials which are present in colostrum. Earlier work by these authors (McCance and Widdowson, 1956) has shown that starved piglets are in a catabolic state compared with the very marked anabolic state which exists in animals on normal diets. Also in this work a comparison can be made between the haematocrit and the serum protein levels of piglets fed naturally on colostrum or sows' milk by means of a stomach tube.

The haematocrit of the colostrum fed animals was lower than that of the milk fed ones, a possible interpretation of which is that a plasma volume expansion had occurred. However, the serum protein levels of the milk fed piglets, although lower than the levels seen in the colostrum fed group, were much higher than those seen in groups which had not been fed milk, a probable indication that protein absorption had occurred. The interpretation of these results is rendered even more difficult by the fact that the two studies were not concluded at the same ambient temperature. It has been shown by Morrill (1952) that the metabolism of the newborn piglet is profoundly influenced by the ambient temperature. Further evidence suggesting that the expansion of plasma volumes is not due to the absorption of immune globulins comes from the work of Sisson and Whalen (1960). These authors noted that in human infants, which are born with significant levels of circulating immune globulin, a 20% increase occurred in the plasma volume within the first 6 hours of life.

The osmotic pressures found in the plasma of the newly born calves are in close agreement with that of other workers. Dalton (1966), using calves of up to 19 days of age, found the mean osmolarity of 12 plasma samples to be 291 $\pm$ S.E. 3-5 m. osmol per litre. In later work he noted that the mean plasma osmolarity of 15 newborn calves was 290 m. osmol per litre and ranged from 276 to 306 m. osmol per litre. In adult cattle Blance (1966) found an average osmotic pressure of 4,943 mm Hg which is the equivalent of 290 m. osmol per litre. The fall in osmotic pressure observed in

both groups of calves at 3 days of age suggests that there may have occurred either a slight dilution of the plasma as a consequence of the absorption of a greater quantity of water than osmotically active material, or that there has been an absolute decrease in one or several of the osmotically active constituents of plasma. In view of the fact that an expansion of plasma volume has occurred simultaneously the former possibility is more attractive.

Reference to the changes which have occurred in some of the other parameters would also tend to support a dilution effect. The sodium concentration in both groups has fallen by a significant amount. In addition, although the difference between the prefeeding and post feeding haematocrits is not significant, there is a tendency for the post feeding levels to be lower. The concentration of serum albumin in both groups, however, remains virtually unchanged. Pierce (1955a) noted in colostrum fed calves that the concentration of serum albumin decreased during the first day of life, but by the second day was returning to the original level. He suggested that a mechanism involving the serum albumin concentration may be present for purposes of controlling protein osmotic pressure. It has been shown by Gitlin (1957) that a fall in the protein concentration in plasma is followed by a disproportionate fall in interstitial fluid protein, due to the flow of water from the capillaries. The restoration of the serum albumin concentration to the original level may have been effected via this mechanism acting upon the extravascular albumin pool.

The possibility that the transient albuminuria described in neonatal calves by Smith and Little (1924) and Howe (1924) was involved in this phenomenon was considered but later disproved by Pierce (1959, 1960, 1961), who demonstrated that the protein in the urine was beta lactoglobulin and not albumin.

The biochemical composition of the plasma or serum of calves has been studied by McSherry and Grinyer (1954), Roy et al. (1959), Fisher (1960), Dalton, Fisher and McIntyre (1965), and Dalton (1967). The ages of the calves studied ranged from birth to 3 or 4 weeks and apart from Roy et al. (1959) and Dalton (1967), no account was taken of possible changes arising as a function of age. Roy et al. (1959) noted that at birth the mean sodium values were 139-140 m. eq. per litre, but had fallen to 133.5 m. eq. per litre by the 5th day of life. The mean potassium levels at birth were 5.8-6.0 m. eq. per litre and rose on the first day to 6.1-6.5 m. eq. per litre. A rise in the potassium concentration was also noted by Dalton (1967), when comparing the levels found in newborn calves with those of 1 or 2 days of age or 3 to 5 days of age. No significant changes in the sodium concentration were observed by this author during the first 5 days of life, although there appears to be a slight increase in the levels found in 1 or 2 day old calves.

SECTION B

BUFFERING CAPACITY

Experimental Design

To ascertain the significance of the buffering capacity of the immune globulins present in the serum of colostrum fed calves, the following acute experiment was performed. Two groups each containing 5 calves were used. One group had high levels of circulating immunoglobulins and the other group had negligible levels. Each calf was anaesthetised and then prepared for continuous monitoring of electrocardiogram, systemic arterial blood pressure and blood pH. By means of slow intravenous drip, isotonic hydrochloric acid was infused into the calf until the animal died from the resulting acidosis. The amounts of acid required to kill each calf was taken as a measure of buffering capacity and the mean amounts required by each group was compared.

Materials and Methods

Preparation of Calves

For the purpose of this experiment, Ayrshire bull calves, 1-2 weeks of age, were obtained from the local market. These calves were chosen on the basis of the zinc sulphate turbidity test as having either high or negligible levels of circulating immunoglobulin. Before being used in the experiment, each calf was examined clinically to determine its fitness and to ensure that it was not diarrhoeic. The hair overlying the ventral aspect of the neck was removed by mechanical clippers so that the jugular groove on either side of the neck could be easily seen. The calf was then weighed and a sample of venous blood was taken from the jugular vein under anaerobic conditions and the pH estimated.

Anaesthesia was induced by slow intravenous injection of sodium pentobarbitone (Nembutal, Abbot Laboratories, Queensborough, Kent). After the calf had been placed in right lateral recumbency, a 4 inch incision was made through the skin overlying the jugular vein. By means of blunt dissection this vein and the carotid artery were identified and isolated. Into the jugular vein two catheters (Nylon Intravenous Cannula - 12" length - Portland Plastics Ltd., Hythe, Kent), one in a cranial direction for pH measurement and the other caudally for acid infusion. These were secured in position by means of nylon ligatures and were flushed through with heparinised saline to prevent clotting. This arrangement of catheters was used so that the acid would be well mixed and buffered before reaching the returning venous circulation from which pH was measured. A similar type of catheter was inserted in a caudal direction into the carotid artery. After being filled by heparinised saline, it was connected to the manometer so that systemic arterial blood pressure could be measured.

The trachea was then dissected from the surrounding tissue and when suitably exposed an incision was made between two of the cartilaginous rings in order that a metal endotracheal tube could be inserted. This was secured in position by means of nylon ligatures tied around the outside of the trachea. This tube was attached to a respiratory pump (Cyclator, British Oxygen Company Ltd., London), which was powered by a compressed air cylinder. The respiratory pump was adjusted so that the minute volume was about 7 litres. This volume was chosen after several measurements had been made of this

parameter in conscious calves using a close fitting face mask attached to a Wright respirometer. After an initial pH measurement had been made, the minute volume of the anaesthetised calf was adjusted so that the pH returned to the pre-anaesthetic level.

Prior to the start of infusion, blood was withdrawn for the estimation of the zinc sulphate turbidity. The plasma volume was also estimated using Evans Blue dye (T1824) and the same basic technique as described in the earlier part of this section. The only modifications were that a needle was dispensed with, the dye being injected through the venous catheter leading towards the heart. After injection, the catheter was flushed clean with heparinised saline. The 10 minute sample was taken from the carotid catheter.

Isotonic hydrochloric acid (155 m. eq. per litre) was prepared and accurately dispensed in 500 ml quantities into MRC transfusion bottles. A disposable recipient set (without filter) (Capon Heaton and Co. Ltd., Birmingham, England) was inserted into the bottle which was then inverted and hung on a transfusion stand. When acid infusion was due to start, the recipient set was connected to the venous catheter after air had been expelled from the tubing by allowing the acid solution to enter. The rate of flow was controlled by a regulating clamp and on average 500 ml of acid were infused in 25 minutes. At the end of each experiment the total volume infused was calculated from the number of empty bottles and the volume remaining in the bottle in use at the time of death.

pH

The pH was measured using Astrup Microequipment AME 1c (Radiometer Ltd., Copenhagen, Denmark). Blood was obtained anaerobically from the cranial section of the jugular vein by means of a catheter.

ECG

Electrocardiograms were recorded on a Mingograph 81 (Elema Schonander, Stockholm, Swedan). The animal was placed in right lateral recumbency and the standard limb leads were employed using needle electrodes. Leads I, II, III and AVR were recorded simultaneously.

Blood Pressure

The systemic arterial blood pressure was determined via a catheter inserted into the carotid artery. This catheter was attached to an impedence manometer, which was, in turn, connected to the Mingograph 81, so that a permanent record was made. The manometer was standardised electronically and against a mercury manometer previous to use.

For the purposes of presentation the results and discussion have been divided into two parts: a) attempted estimation of increased buffering capacity, and b) ECG and blood pressure changes induced by acid infusion. A summary of the results obtained from each group of calves is shown in Tables 3.3 and 3.4.

TABLE 3.3

Immune Globulin Concentrations and Plasma Volumes of Calves Infused with Acid

Calf No.	Zinc Sulphate Turbidity	Immune Globulin (mg/ml)	Plasma Vol. (ml/Kg)	Serum Immune Globulin (g/Kg)
1	37.0	37.4	65.1	2.43
2	34.0	34.2	53.4	1.82
3	30.5	30.4	61.5	1.86
4	45.0	45.9	69.0	3.16
5	29.5	29.4	71.0	2.08
Mean	35.2	35.4	64.0	2.27
S.E.	2.7	2.9	3.1	0.24
6	2.0	0	89.0	0
7	0.5	0	66.2	0
8	0	0	54.7	0
9	3.0	1.0	63.7	0.06
10	0.5	0	59.8	0
Mean	1.2	0.2	66.8	0.01
S.E.	0.5	0.2	5.9	0.01

TABLE 3.4

Infusion Rates and Amounts of Acid Required to Kill Calves With High and Low Serum Immune Globulin Concentrations

Calf No.	Wt. (Kg)	Time to Death (mins)	Infusion Rate (m.eq./Kg/min)	Amount Infused (m.eq./Kg)	Immune Globulin (g/Kg)
1	28.18	88	0.14	12.45	2.43
2	32.65	220	0.08	17.50	1.82
3	29.03	135	0.17	22.61	1.86
4	22.9	143	0.15	21.96	3.16
5	31.29	168	0.12	17.78	2.08
Mean	28.79	150.8	0.13	18.46	2.27
S.E.	1.67	21.6	0.02	1.8	0.24
6	23.13	160	0.11	18.09	0
7	30.84	100	0.11	10.75	0
8	31.52	83	0.10	8.53	0
9	32.65	185	0.08	15.62	0.06
10	30.84	142	0.10	13.64	0
Mean	29.79	134	0.10	13.32	0.01
S.E.	1.69	18.8	0.005	1.69	0.01

Results

a) Attempted Estimation of Increased Buffering Capacity

Weight

There is no significant difference between the weights of the two groups of calves.

Time to death

No significant difference between the length of time taken by either group to die was found.

Infusion rate

The infusion rates in both groups of calves are not significantly different.

Amount of acid infused

The difference between the amounts of acid infused into both groups of calves is not significant.

Plasma volume

The plasma volume found in these calves are not significantly Plasma volume different from each other, and are in close agreement with the values noted by Dalton and Fisher (1961).

Discussion

The overall contribution made by the plasma proteins relative to the other buffers was demonstrated by Pitts (1954). For this work nephrectomised dogs were used which were infused with 10 m. eq. hydrochloric acid per kilogram bodyweight. The resultant changes in the concentrations and quantities of the principle electrolytes were noted. Only 1.5% of the acid was neutralised by the plasma proteins in consequence of the change in pH of the blood. The protein and

bicarbonate buffer system together neutralised less than one third of the acid, the major buffering occurring within the cells and in extracellular structures (bone, cartilage, tendon).

The buffering capacity of the serum proteins of the horse and of man have been examined in vitro by Van Slyke, Wu and McLean (1923) and Van Slyke, Hastings, Hiller and Sendroy (1928), and a difference was noted. Horse serum at pH 7.4 was found to have per gram of protein nitrogen, only 93% of the buffering ability of normal human serum. This difference might be explained by the difference which existed between the relative proportions of albumin and globulin in the sera. The albumin/globulin ratio of the horse serum was 0.8, whereas that of human serum was 1.6. Differences in the buffering capacity of purified albumin and globulin were shown to exist in both species. For horse serum proteins over the range of pH 6.8 to 7.4 and at 38°C, the relationship between pH and the amount of alkali bound by the proteins was expressed by an empirical linear equation, M-eq. base bound per gram globulin N = 0.48 (pH - 4.89).

In the absence of a comparable equation for bovine immune globulins, this equation has been used to calculate the buffering capacity of the globulins acquired by the calf. In the pH range 6.8 to 7.4, 0.288 m. eq. of base are bound per gram of globulin nitrogen. Since the average nitrogen content of proteins is about 16%, this would mean that 6.5 grams of globulin, through this range of pH, would bind or liberate 0.228 m. eq. of base.

By consulting Table 3.3, the mean circulating immune globulin concentration of the group of calves with high zinc sulphate turbidities was about 2.27 g per Kg bodyweight. This would increase the buffering capacity of the calf by 0.07 m. eq. per Kg bodyweight.

This calculated increase in buffering capacity is probably an underestimate for two reasons. The range of pH studied by Van Slyke was smaller than the range observed in these experiments in which pH values of less than 6.6 were seen. The other factor which would tend to cause an underestimate is that the globulin concentration per Kg bodyweight has been calculated from the intravascular globulin; although exact figures for calves are not available at the time of writing, it is known that there exists for proteins a state of equilibrium between the intravascular and extravascular spaces. An identical quantity of globulin may exist in the extracellular fluid which would cause a doubling of the concentration of globulin when expressed on a bodyweight basis. Allowing for these errors, the difference in buffering capacity which might be expected is small.

In this experiment the failure to demonstrate an increased buffering capacity associated with high levels of circulating protein is perhaps understandable in view of the findings of the above authors. In order to detect an increase buffering capacity of this nature a more sensitive technique than the one used here would have to be utilised.

In an experiment of approximately the same design, hydrochloric acid was infused into neonatal calves in order to produce cardiac arrest (Stewart et al., 1965). Although the apparent rates of infusion of acid in this experiment and the one described here are not significantly different, cardiac arrest was produced in approximately half the time and using about half as much acid per kilogram of body-weight as was required in the present series. The reason for this may be associated with the concentration of acid used. These workers used 0.3 m hydrochloric acid, whereas in this experiment a concentration of 0.15 m was used which is isotonic. The longer duration of the present experiments would allow the renal and respiratory compensatory mechanisms to increase the amount of acid required. Finally, the method of controlling respiration may have influenced the results. In the experiments of Stewart et al. (1965) respiration was controlled by intermittent positive pressure inflation with pure oxygen. However, the figures demonstrated for calf No. 3 in these experiments show a raised pCO_2 in the arterial blood suggesting that there is simultaneously a marked respiratory acidosis. In the present series a mechanical respirator was used only at the beginning of the experiment to adjust the pH of the blood prior to infusion. Thereafter, respirations were allowed to occur spontaneously. Hyperpnoea and tachypnoea were both present during infusion.

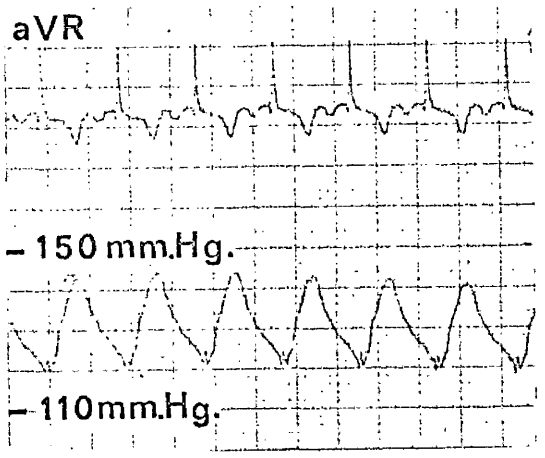
Electrocardiographic and Blood Pressure Changes

A summary of the experimental data obtained from each calf is given in Appendix No. 3.1 and the principal electrocardiographic changes have been summarised in Table No. 3.5. The following figures show the sequence of changes observed in the electrocardiogram and the blood pressure.

Initially there was tachycardia and slight hypertension - Figure 1.

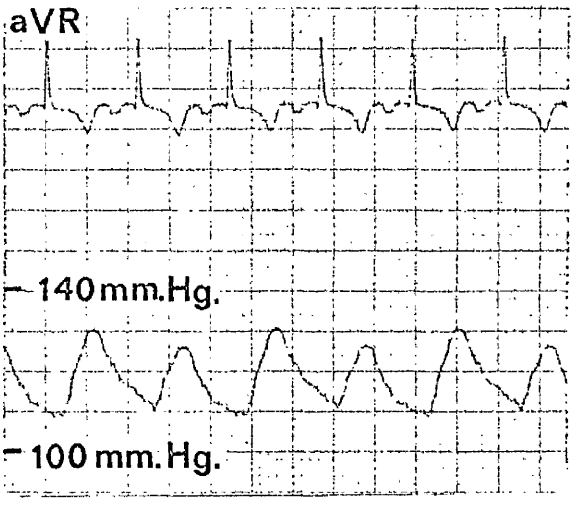
This was followed by a progressive slowing of the heart rate and decline in the blood pressure - Figure 2.

Fig.1



Heart Rate	159/min.	Blood Pressure	142/120mm. Hg.
PR Interval	0·1sec	pH	7·38
T Wave Amplitude	3mm	Paper Speed	25 mm./sec.

Fig. 2



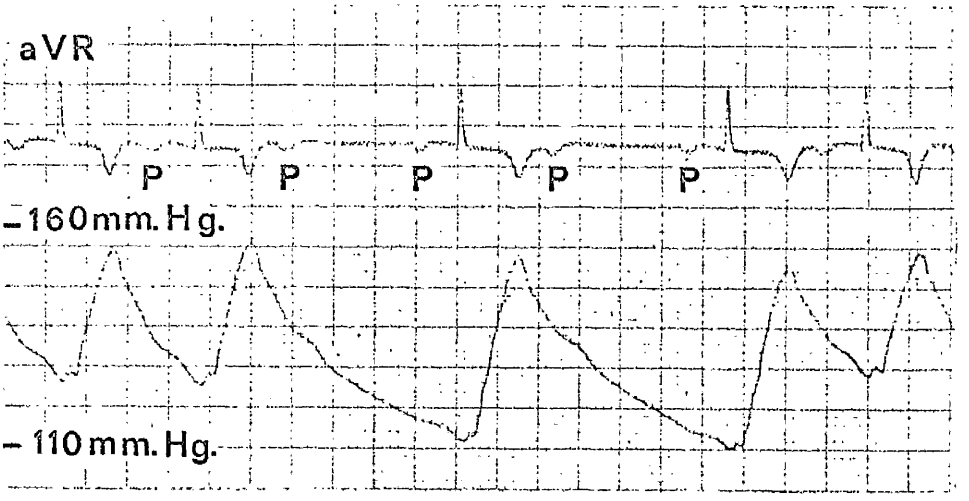
Heart Rate	135/min.	Blood Pressure	128/110 mm.Hg.
PR Interval	0·12 sec.	pH	7·06
T Wave Amplitude	3mm.	Paper Speed	25mm/sec.

The slowing of the heart rate continued until normal rates were observed, but this was accompanied by the development of an incomplete A.V. block. Lengthening of the PR interval became apparent. The blood pressure rose markedly at this stage - Figure 3.

As a result of continued infusion, a pronounced bradycardia developed. The blood pressure remained elevated - Figure 4.

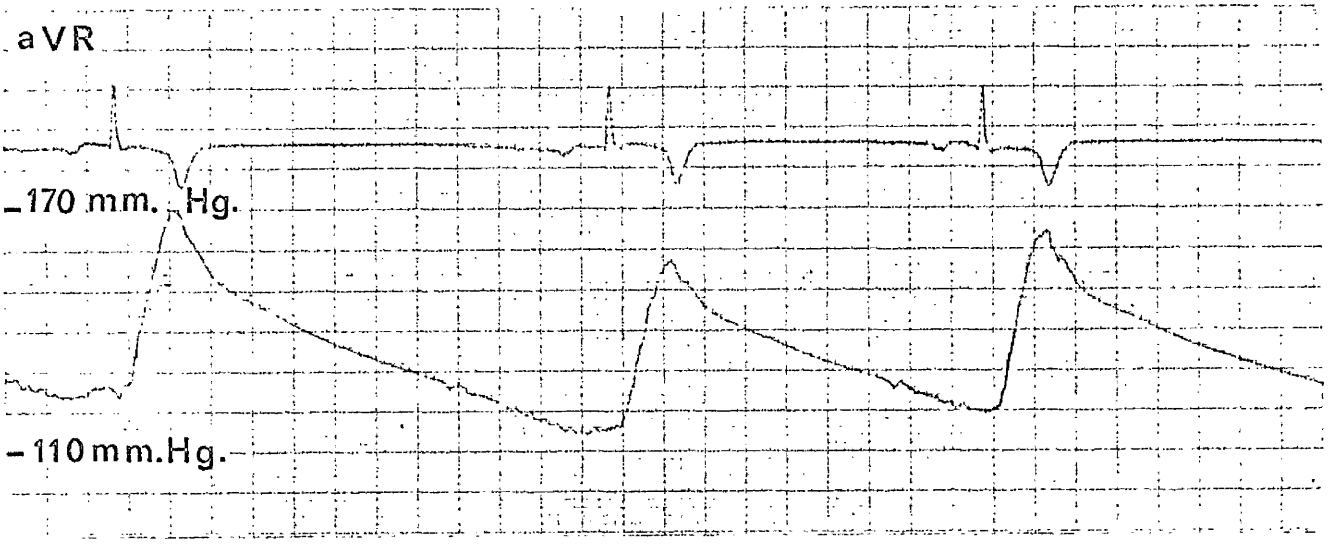
Incomplete A-V Block

Fig. 3



Heart Rate	55/min.	Blood Pressure	160/125 mm. Hg.
PR Interval	0·22 sec.	pH	6·80
T Wave Amplitude	3·5 mm.	Paper Speed	25 mm/sec.

Fig. 4

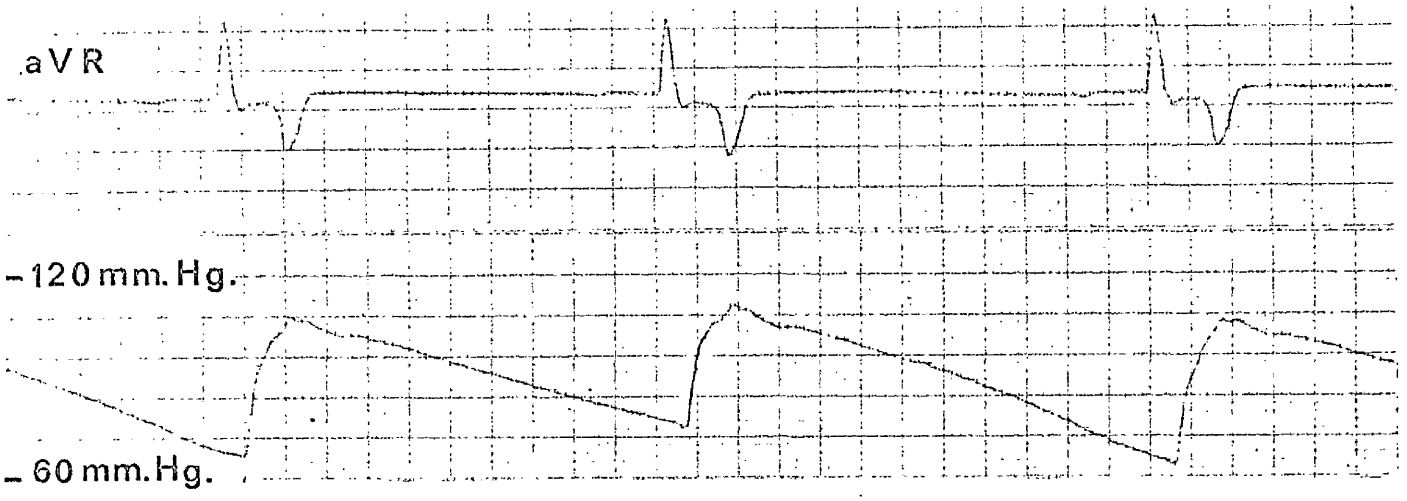


Heart Rate	28/min.	Blood Pressure	170/125 mm. Hg.
PR Interval	0·22 sec.	pH	6·70
T Wave Amplitude	5 mm.	Paper Speed	25 mm/sec.

Later, although the degree of bradycardia remained approximately the same, the blood pressure started to decline - Figure 5.

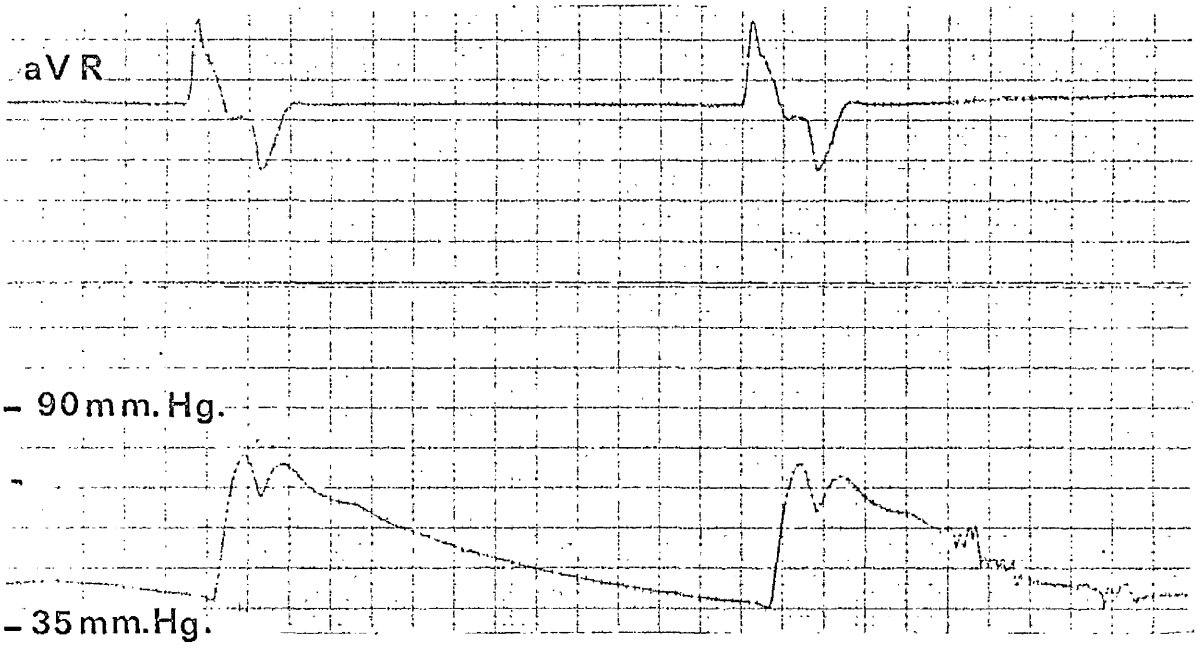
With the development of hypotension, a gross bradycardia developed. The increase in the amplitude of the T wave, shown in this and the preceding figures, was present only in a proportion of the calves. Similarly the broad RS complexes shown here were noted terminally in two calves only (Nos. 9 and 10) - Figure 6.

Fig. 5



Heart Rate	26/min.	Blood Pressure	110/75 mm. Hg.
PR Interval	0·34sec.	pH	6·60
T Wave Amplitude	8 mm.	Paper Speed	25 mm/sec.

Fig. 6



Heart Rate	22/min.	Blood Pressure	78/40 mm. Hg.
PR Interval	?	pH	< 6·60
T Wave Amplitude	8·5 mm.	Paper Speed	25 mm/sec.

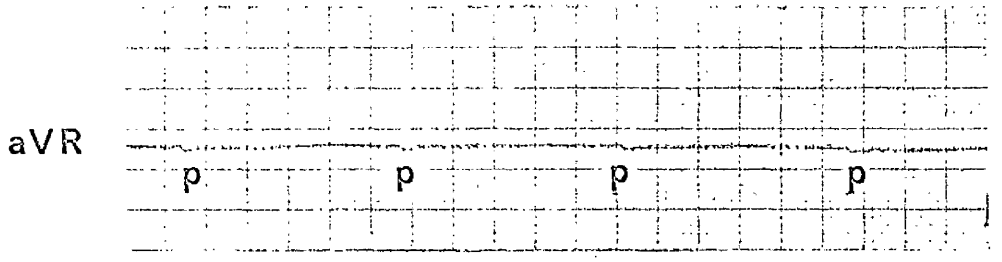
Terminally death was due to ventricular asystole in 6 calves - Figure 7a.

One calf (No. 7) developed ventricular fibrillation - Figure 7b.

In the remaining 3 calves (Nos. 6, 8, 9) complete cessation of electrical activity occurred after a period of gross bradycardia.

Electrical alternans was observed in one calf only (No. 8) at the beginning of the experiment and was associated with tachycardia - Figure 7c.

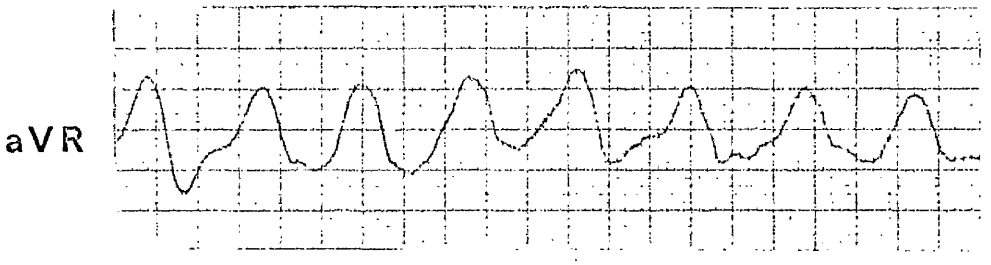
Fig. 7a.



Ventricular Asystole

Paper Speed 25 mm./sec.

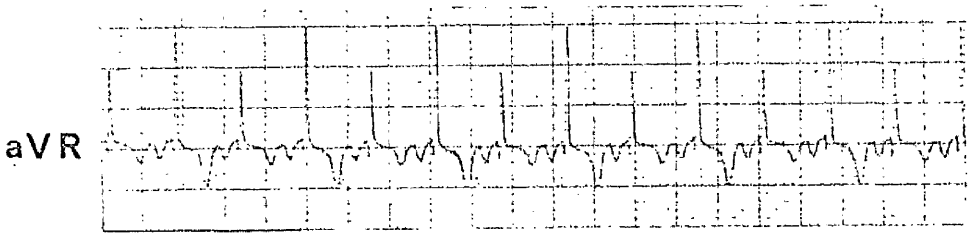
Fig. 7b.



Ventricular Fibrillation

Paper Speed 25mm./sec.

Fig. 7c.



Electrical Alternans

Paper Speed 25 mm./sec.

TABLE 3.5

Summary of Electrocardiographic Data

Calf No.	Sinus Tachycardia	Incomplete Heart Block	Terminal Bradycardia	Ventricular Asystole
1	+	-	+	+
2	+	+	+	+
3	+	-	-	+
4	+	+	+	+
5	+	+	+	+
6	+	-	+	-
7	+	-	-	-
8	+	+	+	-
9	+	+	+	-
10	+	-	+	+

\+ Present - Absent

Discussion

Stewart et al. (1965) in an investigation into the possibility that cardiac arrest in the human may be caused by a metabolic acidosis, designed an experiment using neonatal calves and a technique approximately similar to the one described here for the production of a severe acidosis. A sequence of electrocardiographic changes were recorded with the development of severe acidosis. These changes were sinus tachycardia, electrical alternans, 2 to 1 heart block, complete heart block and ultimately ventricular asystole. Of 10 calves used in this experiment, 4 were classed as atypical in that the expected sequence of electrocardiographic changes did not occur. In two of these calves hypotension, bradycardia and broad QRS complexes developed, another calf developed ventricular fibrillation and the fourth one developed idioventricular rhythm.

In the present experiment, cardiac arrest due to ventricular asystole was seen in a greater proportion of calves than those described by the above authors. A similar sequence of electrocardiographic abnormalities were not observed in that electrical alternans was seen only in one calf at the beginning of the experiment, incomplete heart block was seen but was not consistently of the 2 to 1 type, and complete heart block was not observed. The different and variable electrocardiographic results obtained by those workers in 4 calves were confirmed in this series. In both sets of experiments one calf developed ventricular fibrillation and two calves developed

broad RS complexes. The discrepancies which exist between the findings in this series and that reported by Stewart et al. may be due to differences in technique. For example, the influence of artificial ventilation and a superimposed respiratory acidosis are unknown, but possible sources of variation.

The electrocardiographic changes observed in neonatal calves with a metabolic acidosis arising from diarrhoea have been described by Fisher (1965). In this paper bradycardia was observed to be more marked in calves which were dying from the effects of diarrhoea. Of 25 calves which died, arrhythmias of various types were recorded in 8 (32%). These arrhythmias sometimes were associated with slow heart rates and no abnormality of the PQRST complex and sometimes with various degrees of A-V block. In one case only was a complete heart block detected.

The relationship between electrocardiographic abnormalities and the biochemical composition of calf serum have been studied by several workers. Bergman and Sellers (1954) found by elevating the serum potassium concentration to over 8 m. eq. per litre by means of injection of potassium chloride, the disappearance of the P wave, marked elevation of the T wave, widespread intraventricular block with occasional nodal rhythm and gradual cardiac arrest. Death occurred in one animal at a plasma potassium level of 12.7 m. eq./litre. Stewart et al. (1965) noted that during acid infusion broad QRS complexes were associated with hyperkalaemia, but that normal potassium levels were present in the calves which demonstrated the 'typical' sequence

of changes. This latter finding is also supported by Fisher (1965) who showed that in metabolic acidosis arising from neonatal calf diarrhoea, arrhythmias may occur with normal serum potassium levels. He also noted that hyperkalaemia may occur without arrhythmias and that in healthy calves elevation of the T wave may occur without hyperkalaemia.

Summary

Two possible physiological functions of immune globulins have been investigated. It has been confirmed that an expansion of plasma volume occurs in the immediate post natal life of the calf. This expansion is not due solely to an osmotic effect exerted by the globulins since an expansion of similar magnitude occurs in animals fed solely upon milk. This expansion is probably a temporary effect since using a similar measurement technique, other workers (Dalton and Fisher, 1967) have found lower values. The reason why such an expansion should take place at this time is not immediately apparent. One can only conjecture that the demands placed upon the cardiovascular system during the adaptation of the newborn animal to extra uterine life necessitate a large plasma volume.

The buffering capacity of the passively acquired globulins has proved not to be of any significant value in terms of the ability of the intact animal to withstand experimentally induced acidosis. The reactions of the calf to acid infusion have proved to be variable.

In view of these facts one must conclude that the major function of these globulins is their immunological one.

PART IV

INVESTIGATION INTO CERTAIN ASPECTS OF THE MECHANISM OF ABSORPTION OF GLOBULINS FROM COLOSTRUM

PART 4

Investigation into Certain aspects of the Absorption Mechanism in Calves

Introduction

The mechanism of the transfer of passive immunity from dam to offspring has been studied in many species and has been reviewed most recently by Brambell (1958). Species differences have been noted in the duration of the absorptive capacity and in the selectivity of the absorptive process. Possibly because of practical considerations the absorptive mechanism has been studied most intensely in laboratory rodents and relatively few investigations have been performed in domestic ruminants. In view of this a review has been made of the literature relating to the absorption mechanism in domestic animals. The aspects of the absorption mechanism in calves which have been investigated are the influence of the time at which colostrum is fed on the amount of immune globulin which is subsequently absorbed and the efficiency of the absorption mechanism.

Literature Review

The function of colostrum in transmitting to the offspring the antibodies necessary to ensure survival during early life was first recognised in mice by Ehrlich (1892). In 1912, Famulener published the results of observations made in goats immunised against sheep erythrocytes. Haemolysins were demonstrated in both the serum and the colostrum of immunised goats, the concentration being higher in colostrum. At birth no haemolysins were detected in the serum of the newborn kids until they had ingested colostrum, after which they were

detectable. Howe (1921) observed that the serum of newborn calves contained little or no euglobulin or pseudoglobulin I until after colostrum had been ingested. Following these observations, other workers have described in other species as well as cattle the changes which occur in the serum protein profile and antibody activity following the ingestion of colostrum (Mason, Dalling and Gordon, 1930; Earle, 1935; Schneider and Szathmary, 1938, 1939).

At one time it was thought that newborn calves were absolutely deficient of immune globulins, but with the development of more sensitive techniques of detection, small quantities have been found to be present (Pierce, 1955a, 1961; Kniazeff et al., 1967). These globulins probably represent antibodies synthesised by the foetus since it is known that the ability to produce immune globulin is present in the 132 day-old calf foetus (Fennstad and Borg Petersen, 1962).

In ruminants, horses and pigs, and to a lesser extent dogs and cats, there appears to be no or very little prenatal transference of antibodies. This situation is unlike that pertaining in man, the rabbit and guinea pig, the young of which species are born with substantial levels of circulating immune globulin. The reason for this difference has been suggested to lie in the structure of the placentae of these species. As the number of tissues interposed between the maternal and foetal blood streams decreases, there is an increase in the amount of immune globulin transferred to the foetus in utero. Using the classification of Grosser (1908, 1927), the chorio-allantoic placentae of mammals may be placed in one of four classes. These

classes are, in order of decreasing separation between maternal and foetal blood, epitheliochorial (horse, pig), syndesmochorial (cow, sheep, goat), endotheliochorial (dog, cat) and haemochorial (man, monkey, mice). A further class, haemoendothelial was suggested by Mossman (1926, 1937) to describe the placentation of the rat, rabbit and guinea pig. Under Grosser's original classification the placentae of cattle were described as syndesmochorial. Subsequent work by Bjorkman and Bloom (1957) and by Hamilton, Harrison and Young (1960) has shown that it should be described as epitheliochorial and as such placed in the same class as horses and pigs.

The work of Brambell and his colleagues (1958, 1966), however, suggests that this is not theexplanation of the necessity to transfer passive immunity by means of colostrum. These workers have noted that in the rabbit, immune globulins were transmitted by way of the uterine lumen and the yolk sac and not through the placenta. In those species in which the yolk sac is exposed to the uterine lumen during the major part of gestation, there is a high prenatal level of circulating immune globulin. In the domestic animals the yolk sac retracts into the umbilical cord in early gestation.

The histological changes which accompany the absorption of protein molecules have been described in most detail in rats and mice by Clark (1959) using light and electron microscopy. Similar changes have been described in calves by Comline et al. (1951b, 1953) and El-Nageh (1967c), in lambs and kids by Hill and Hardy (1956), and in piglets by Payne and Marsh (1962a and b), Mattisson and Karlsson (1964), and Lecce (1966). Protein molecules or other colloidal substances are taken up by the cell through the process of pinocytosis.

By means of this process a number of small vesicles are formed under the apical surface of the columnar epithelial cells. These small vesicles move away from the top of the cell and in the process fuse with one another, thus forming a much larger vesicle which finally discharges its contents into the interstitial fluid. From this location most of the protein would appear to enter the lacteals of the villi, pass into the main lymphatic vessels, and ultimately enter the general circulation by means of the thoracic duct (Comline et al., 1951a). The interval between the time of feeding and the detection of immune globulins in the thoracic duct lymph or in serum is about 2 hours (Table 4.1).

The regions of the small intestine at which absorption occurs has been determined by Pierce and Smith (1967b). These authors measured in vitro the transfer of bovine IgG across the wall of everted sacs of pig intestine, taken from different levels of the gut. It was noted that at the higher concentrations of IgG used, the greatest transfer occurred in the sacs taken from the middle third of the small intestine. By means of fluorescent microscopy El-Nageh (1967b) demonstrated the absence of pinocytotic vesicles in both the anterior duodenum and in the terminal ileum.

In studying the route of transmission of immunity it was observed by Batty et al. (1954b) that there were differences in the uptake of antitoxins by the yolk sac of the rabbit depending on the species from which they had been obtained. Assuming that the uptake of rabbit antitoxin was 100%, the relative entry of other antitoxins

TABLE 4.1

The Interval Between the Time of Feeding Colostrum and the Detection of the Absorption of Immune Globulins

Author	Time after Feeding	Method of Detection
A. Calf		
Little and Orcutt (1922)	1 hr.	*Br. abortus* agglutinins in serum
Schneider and Szathmary (1938)	90 mins.	Serum agglutinins and antitoxins
Kerr and Robertson (1943)	1 hr.	*Br. abortus* agglutinins in serum
McDiarmid (1946)	1-3 hrs.	*Br. abortus* agglutinins in serum
Comline, Roberts and Titchen (1951)	1-2 hrs.	*Br. abortus* agglutinins in thoracic duct lymph
Bangham *et al.* (1958)	3 hrs.	^{131}I labelled serum and colostrum protein in serum
Balfour and Comline (1962)	1-2 hrs.	^{131}I labelled gamma globulin in thoracic duct lymph
Schoenaers and Kaeckenbeeck (1963)	1-2 hrs.	Serum agglutinins to *E. coli* 0.137
Graves (1963)	1 hr.	Serum neutralising antibodies against Foot and Mouth disease
Schoenaers and Kaeckenbeeck (1964)	1½ hr.	Serum agglutinins to *E. coli* 0.137
El-Nageh (1967a)	1-2 hrs.	Rhodamine B200 labelled gamma globulin in serum
Singh *et al.* (1967)	2 hrs.	Rinderpest neutralising antibodies in serum
Hardy (1968)	1 hr. 40-2 hrs.	^{131}I labelled gamma globulin and PVP in lymph

Table 4.1 (cont'd)

Author	Time after Feeding	Method of Detection
B. Pig		
Young and Underdahl (1949)	½ hr.	Swine influenza haemagglutination - inhibition antibody in serum
Nordbring and Olsson (1957)	4 hrs.	Electrophoresis of serum
Kaeberle and Segre (1964)	2½ hrs.	Fluorescence microscopy and autoradiography
Pierce and Smith (1967)	1-3½ hrs.	Quantitative Immunodiffusion test

were: human, 81%; guinea pig, 53%; dog, 25%; horse, 4%; and cow, 1%. A similar type of selectivity has been shown to exist in the gut of the rat (Halliday, 1955, 1957). Further studies on this mechanism have demonstrated that certain heterologous antisera may interfere with the transmission of homologous antibodies (Halliday, 1957). It would appear from the work of Brambell et al. (1958) that the serum proteins which are responsible for this effect are located in the gamma globulin fraction. In view of this finding, it was suggested that within the gamma globulin molecule, there may be specific receptor sites which may be of importance in determining the selectivity of intestinal absorption. Utilising the method of fractionation of the gamma globulin molecule described by Porter (1959), it was found that fragment III (Fc piece) was absorbed almost as readily as the entire molecule and more readily than the other two fragments (Fab) (Brambell et al., 1960).

Hemmings (1960) observed during the course of work with serum protein preparations labelled with iodine 131 that the uptake of similar fractions by the intestine of 8 day old mice was influenced by the iodination levels of these molecules. Significant differences were noted between preparations in which the mean iodination level was 0.5 and 3 atoms of iodine per molecule of globulin. As a result of this finding, it was suggested that since iodine would only influence the number and distribution of tyrosine residues in the globulin molecule, these may be involved in the process of absorption.

In comparison with the absorption mechanism of the rabbit and rat, that of ruminants and pigs is remarkable by its relative non-selectivity. In the calf it has been shown that the gut is permeable to serum globulins, serum albumin, and dextran (Balfour and Comline, 1959). Pierce (1959, 1960, 1961a) has shown that the transient proteinuria which accompanies the feeding of colostrum is due to the absorption of beta lactoglobulins and its subsequent excretion via the kidneys. Gelatin was found to follow an identical route if given by mouth during the period of permeability. Later work has shown that insulin may be absorbed intact through the intestinal epithelium (Pierce et al., 1964) and also polyvinyl pyrrolidone polymers of molecular weights 50,000 and 220,000 (Bagliori et al., 1964). The close quantitative similarity between the absorption of isotopically labelled PVP and gamma globulin observed by Hardy (1968) suggests that solute specific carrier mechanisms are not involved in the process of absorption in calves. A similar lack of selectivity has been demonstrated in newborn piglets (Olsson, 1959 a and b; Lecce, Matrone and Morgan, 1961; Leece and Moran, 1962; Payne and Marsh, 1962; Kaeberle and Segre, 1964; Lecce, 1966 and Pierce and Smith, 1967 a and b).

A hypothesis concerning the mechanism of the transmission process was forwarded by Brambell et al. (1958, 1966). It was suggested that the process of pinocytosis was not selective, but that gamma globulin molecules became attached to receptor sites located in the walls of the pinocytotoic vesicle. This attachment would explain the specificity of the mechanism in that the receptors would be adapted to

homologous gamma globulin and heterologous globulin would interfere only to the extent to which it fitted the receptors. Attachment to a receptor would also protect the molecule from subsequent digestion, since it was noted that almost all of a dose of protein is absorbed by the cells, but only a proportion of it passes into the circulation.

An alternative hypothesis has been suggested by Pierce and Smith (1967 a and b). It was shown by these workers, using everted sacs of pig intestine *in vitro*, that human serum albumin was transferred preferentially to bovine immune globulin and that interference with the transfer of bovine immune globulin occurred when albumin is present in an equal concentration at the mucosal surface. There was no evidence of enzymic degradation. *In vivo* experiments, however, showed no evidence of selectivity or interference. It was pointed out, however, that digestion was occurring and the concentration of proteins presented at the mucosal surface was not known. In view of these findings, it was suggested that attachment to receptors may take place at the brush border membrane before pinocytosis occurs. Provided that a small vesicle is formed, the amount of non-selectively absorbed protein would be low in relation to the bound antibody and transfer of bound antibody would be favoured irrespective of intravesicular digestion.

It is possible that the cessation of absorption of immune globulins may be due to factors other than a change in the permeability of the intestinal mucosa. Such a factor may be that the maturation of the digestive processes may destroy or alter the immune globulin molecules. In a study of the gastric development of the

newborn lamb, it was found that the acidity of the abomasum gradually increased during the first 36 hours of life until levels were reached at which pepsin would be activated (Hill, 1956). Comparative studies made in rats showed that in the young rat, the gastric glands were not fully developed until 3 weeks after birth (Kammeraad,1942; Hill, 1956). The intestine of the young hedgehog has been shown to retain its ability to absorb antibodies for at least 41 days (Morris and Steel, 1964). Studies made in this species have shown that gastric pH declines from near neutrality at birth to between pH 3 to 4 during the fourth and fifth weeks of life. Assays of pepsin in the fundic mucosa revealed that pepsin was present in significant amounts at 9 days of age, and it was suggested, therefore, that the delay in the development of proteolytic digestion was because the hydrogen ion concentration was not adequate (Morris and Steel, 1967).

It was demonstrated by Moog (1953) that adrenocortical hormones will produce an increase in the alkaline phosphatase activity of suckling mice. Halliday (1959) noted that in rats the normal decline in absorption coincided with the increase in alkaline phosphatase activity and that both processes could be induced prematurely by the administration of large doses of deoxycorticosterone acetate or cortisone acetate. Clark (1959) observed that the administration of cortisone acetate to 8 to 10 day old rats altered the columnar absorptive cells of the intestinal mucosa so that they resembled those of the adult animal. In contradistinction to these findings, the work of Morris and Steel (1964) has shown that cortisone acetate has little

effect upon the amount of duodenal alkaline phosphatase in the hedgehog. It was found also that alkaline phosphatase activity fell during the first 30 days of life. It is possible that the amount of this enzyme present may not be a suitable index of intestinal differentiation in this species.

The effects of administering cortisone (200-350 mg) or ACTH (120 I.V.) to calves was examined by Deutch and Smith (1957) and no change in intestinal permeability was noted during the first 40 hours of life. Similar results in puppies were found by Gillette and Filkins (1966) in that hydrocortisone injected before being fed hyperimmune serum did not significantly alter antibody absorption. The possibility that in these latter experiments enough time was not allowed for the adrenocortical hormones to act is supported by the fact that the administration of ACTH to bitches 24 hours before parturition caused a significant reduction in the antibody absorption of the pups (Gillette and Filkins, 1966). In the experiments of Moog (1951), Clark (1959) and Halliday (1959), the effects of a single injection of hormone required 2 to 3 days before becoming apparent. Similarly the injection of cortisone acetate into starved newborn pigs appeared to affect the mechanism so that no absorption of gamma globulin was detected 48 and 72 hours after the injection (Payne and Marsh, 1962).

Following upon the findings of Laskowski and Laskowski (1951) that the colostrum of sows contained a trypsin inhibitor, several investigations have been carried out to ascertain the relationship

between this substance and the cessation of antibody absorption in piglets (Barrick, 1954; Nordbring and Olsson, 1958 b and c; and Chamberlain, Perry and Jones, 1965) and calves (Deutch and Smith, 1957). In all these investigations the common finding has been that the absorption of immune globulins is not influenced to a significant degree by the presence of this inhibitor substance. Negative results were also obtained by Deutch and Smith (1957) in attempting to prolong the duration of absorption in calves by use of an inhibitor of the enzyme ribonuclease.

Steel (1965) observed that no absorption of antibody occurred after the injection of immune serum into the intact, but isolated, duodenum of 28 day old rats. When previous to the injection of the immune serum, the lumen of the gut was washed with warm isotonic saline and the pancreatic and bile ducts were severed, absorption did occur. From this work, it was suggested that in the rat and hedgehog the cessation of antibody absorption is brought about at least initially as a result of a change in the hydrogen ion concentration which, in turn, allows the proteolytic enzymes to become active. In view of the lack of similar information on the absorption mechanism of ungulates, it is not unreasonable to assume the presence of a similar mechanism. The failure to observe the uptake of fluorescent gamma globulin molecules by the intestine of older animals of other species in vitro suggests that other factors are involved (Lecce, 1966).

A study of some of the factors present in bovine colostrum which were found to influence the absorption of globulin from the small intestine has been made by Balfour and Comline (1962). A maximum rate of absorption of ^{131}I labelled globulin was obtained when this protein was administered in solutions containing a low molecular weight protein fraction (probably beta lactoglobulin), glucose-6-phosphate and inorganic phosphate, all present in concentrations normally found in colostrum. Using a similar experimental design as the above authors, a study has been made of the effect on the rate of absorption of metabolites of known metabolic activity (Hardy, 1968). These substances were the sodium and potassium salts of lactate, pyruvate and citrate, and of certain lower volatile fatty acids. Absorption was facilitated by solutions containing lactate and pyruvate and especially by potassium butyrate, which appeared to be even more effective than colostrum or whey. Qualitative differences were observed in the concentration and volume of the thoracic duct lymph obtained after the administration of butyrate solutions and colostral whey.

Using everted sacs of neonatal pig intestine, *in vitro*, Lecce (1966) observed that the uptake of immune globulin required oxygen and sodium. By use of metabolic antagonists such as iodoacetate, arsenate, fluoride, 4,6-dinitro cresol, phlorrhizin, anaerobiosis and cold, the uptake could be reversibly inhibited. Surface active agents did not produce non-specific absorption artefacts. An increase in the oxygen consumption of everted ileal sacs of 18 day old rats was noted during the absorption of immune globulins (Bamford, 1967).

The effects of various hormones on the absorption of antibodies has been studied by several authors. Halliday (1959) observed that aldosterone, progesterone, testosterone and stilboestrol did not have any appreciable effect on antibody absorption in rats. Diethylstilboestrol and progesterone, singly and in combination, were administered to calves by Deutch and Smith (1957), again without any detectable change in permeability occurring. Smith, Reed and Erwin (1964) could detect no difference between the absorption of 2 newborn calves which had received 30 mg of somatotrophin at birth and 2 control calves. In puppies, Gillette and Filkins (1966) found no difference in absorption between animals dosed with progesterone and control animals.

The possibility that the duration of absorption is a function of gestational age was investigated in piglets by Payne and Marsh (1962). Premature piglets of about 100 days and others of 118 days gestation did not exhibit any difference from animals farrowed at the normal time, i.e. 114 days. Similarly Smith et al. (1964) could not detect any difference in the absorption mechanism of calves removed by Caesarian section 14 to 19 days before the expected end of gestation.

In an attempt to observe whether gamma globulin would be absorbed by the bovine foetus, Smith et al. (1964) infused 30 to 50 g of gamma globulin into the amniotic fluid of 3 foetuses of 6, 7 and 8 months gestation. Serum samples taken at birth failed to show a globulin content higher than that expected in untreated calves. The possibility that amniotic fluid swallowed by the foetus may contain a permeability factor was investigated by Deutch and Smith (1957) with negative results.

The influence of a high level of circulating immune globulin on the absorptive ability of the intestine has been studied in piglets by Payne and Marsh (1962) and in calves by Kaeckenbeeck, El-Nageh and Schoenaers (1967). In the experiments reported by these authors no inhibition of the uptake of immune globulins by the intestine was noted.

A relationship has been suggested between the cessation of intestinal absorption of antibody and the renewal of intestinal epithelium (El-Nageh, 1967d). This hypothesis is supported by the histological differences observed in the pattern of absorption of newly born calves and those over 24 hours of age. In very young animals absorption may be observed along the whole length of the intestinal villus except within the crypts of Hiebarkuhn, whereas in slightly older animals absorption is confined to the apical regions of the villi.

Finally the removal and maintenance of young rats from their mothers was shown to result in the cessation of antibody absorption 3 days earlier than in non-deprived litter mates (Halliday, 1959). The possibility exists that this procedure may have a "stress" effect which would result in the stimulation of the adrenal cortex.

SECTION A

THE INFLUENCE OF TIME OF FEEDING ON THE ABSORPTION OF IMMUNE GLOBULINS

In Part II a relationship between neonatal calf disease and low serum immune globulin levels was demonstrated. In neonatal bull calves a very pronounced seasonal variation was observed in the immune globulin levels of serum. Possible reasons for this variation were forwarded in the discussion, among which was the possibility that calves were not being fed as soon as if calving had occurred under more natural conditions and the dams were allowed to suckle them. Hence, the immune globulins might be presented to the intestinal epithelium during the period when its absorptive capacity was on the wane.

The technique used by other workers to determine the duration of the absorptive ability in domestic animals have been numerous and varied. Because of the large size of an average litter, the piglet has been studied more intensively than other species. In general, it has been assumed that the duration of absorption in this species was no longer than that of the other domestic animals, namely 24-36 hours. Nordbring and Olsson (1958a), however, noted that absorption in piglets could continue for 72 hours after birth if the piglets were maintained on oral and parenteral fluids. Payne and Marsh (1962a) found that absorption had ceased completely in piglets 12 hours after birth if they were allowed to be suckled immediately after birth or were fed modified cows' milk. In marked contrast, piglets which had been starved or fed on water only, retained their absorptive capacity for over 106 hours. Asplund et al. (1962) did not mention any difficulty in the absorption of antibodies by piglets which before

receiving colostrum were fed on skimmed milk. Under these conditions it was suggested that the cessation of absorption of gamma globulins is 21 to 27 hours after birth. The findings of Payne and Marsh (1962) are to a large extent supported by the work of Lecce and Morgan (1962), who, using starved piglets and lambs, detected the absorption of polyvinylpyrrolidone 86 and 48 hours respectively after birth. No attempt was made to estimate what proportion of the test dose had been absorbed.

This subject had been further pursued by Lecce (1966) who observed in piglets that the capacity of the intestine to absorb was diminished by feeding pure aqueous solutions of certain substances, e.g. glucose, galactose, xylose, sucrose and lactose. If more than 300 m. eq. of glucose were taken within 24 hours of birth, the absorption of egg proteins appeared to be suppressed. This state could only be induced after 12 hours had elapsed since birth and did not seem to be influenced by either the volume or concentration of the solution inducing the cessation of absorption but solely on the amount. The relevance of these findings to the absorption mechanism in calves is not yet known, but should they be valid, special regard must be paid to the methods described in the literature and used to maintain calves before testing the absorption mechanism.

There is confusion in the literature about the influence of starvation or the ingestion of another food previous to colostrum feeding, on the absorption of immune globulins by calves. Deutch and Smith (1957) failed to prolong the duration of permeability by intravenous feeding. Of 3 calves fed milk for 21-22 hours after birth,

only one failed to absorb Br. abortus agglutinins from the colostrum (Little and Orcutt, 1922). Steck (1961) before giving colostrum to calves first gave them cows' milk, without inhibiting the absorption of antibody. Ulbrich (1962) noted that the previous ingestion of colostrum deficient in Brucella agglutinins did not interfere with the absorption of these antibodies when given later in another colostrum. The ingestion of glucose water and the exclusion of all other food or drink did not appear to prolong the period of permeability of the calf intestine according to the work of Schoenaers and Kaeckenbeeck (1964) and Pierce (1961a). These former authors also noted that proteins (cows' milk and egg white) or glucose water did not reduce the ability of the intestine to absorb antibodies contained in a later feed of colostrum, and this fact was confirmed in later work (Kaeckenbeeck, El-Nageh and Schoenaers, 1967). Baglioni (1966) noted that PVP given by mouth appears to have the ability to suppress the absorption of colostrum proteins in calves. This ability seems to be related to the molecular weight of the polymer used in that 25% solutions of molecular weight 50,000 were not effective, whereas absorption was decreased by a 25% solution of molecular weight 220,000 and was virtually suppressed by a 10% solution of molecular weight 700,000.

In contrast with the findings of the above-mentioned authors, Henning (1953) noted that when one litre of serum with a high titre of S. dublin agglutinins was administered by mouth shortly after birth, the feeding of colostrum from immune cows 12 hours afterwards had no effect on the titre of the calves' serum. However, when calves were fed non-immune colostrum or milk 12 hours before being

given immune serum by mouth, their capacity to absorb these agglutinins was noticeably reduced. Similarly, Graves (1963) found that by feeding 1.4 litres of powdered skim milk to one calf, 3 hours before being allowed to receive colostrum from its dam, no specific antibody could be detected in its serum, although high levels were present in the colostrum. Another calf was given a meal consisting of 80 ml of bovine serum diluted in 500 ml of physiological saline. A slight rise in the specific antibody titre of the calf occurred following this meal, but none was observed following the ingestion of colostrum 3 hours later.

The discrepancies between the findings of Schoenaer and Kaeckenbeeck (1964) and Graves (1963) on the influence of the previous ingestion of milk may be explicable in terms of the amounts given to the calves and the time allowed to elapse before colostrum was fed. The former workers administered 300 ml of milk and tested 5-7 hours later, whereas Graves fed 1.4 litres of milk and then allowed the calf to be suckled by its dam. Due to the large variations seen in the titres of the calves tested by Schoenaers and Kaeckenbeeck, there is some difficulty in interpreting the results. That this variation may be due to the use of a constant volume of colostrum rather than dosing on a bodyweight basis, must be considered. A criticism of the experiment reported by Graves is that it was performed on one calf only which was then placed with its dam and assumed to have obtained colostrum.

Few conclusions may be drawn from these conflicting reports. Although no test of absorptive ability similar to that performed by Payne and Marsh (1962a) has been reported, it would appear that the capacity of the small intestine of the calf is very much reduced after the first day of life and possibly after milk or colostrum feeding.

A summary of the literature relating to the duration of absorption in calves and other domestic animals is given in Table 4.2.

TABLE 4.2

Duration of Absorption Ability

Author	Duration	Method of Detection	Management
		A. CALF	
Howe (1921)	21 hrs.	Salt fractionation of serum proteins	Milk fed until tested with colostrum
Little & Orcutt (1922)	21 hrs.	Br. abortus agglutinins in serum	As above
Smith & Little (1924)	22 hrs.	Development of proteinuria	As above
Howe (1924)	22 hrs.	Salt fractionation of serum proteins	As above
Mason, Dalling & Gordon (1930)	12 hrs.	Serum antitoxin levels	Allowed suckling then fed heterologous antiserum
McDiarmid (1946)	15½ hrs.	Br. abortus agglutinins in serum	Starved until allowed to suck from cow
Hansen & Phillips (1947)	24 hrs.	Serum electrophoresis	Milk fed until tested with colostrum
Comline, Roberts & Titchen (1951)	>27 but <63 hrs.	Salt fractionation of lymph and serum and Br. abortus agglutinins	Direct introduction of whey into duodenum
Deutch and Smith (1957)	24-30 hrs.	Ultracentrifugation and electrophoresis	Milk fed until tested with colostrum
Bangham et al. (1958)	16 hrs.	^{131}I labelled colostrum protein	Fed labelled protein in milk

Table 4.2 - A. Calf (cont'd)

Author	Duration	Method of Detection	Management
Pierce (1959)	17-26 hrs.	Change in rate of renal clearance of protein	Fed colostrum
Smith & Erwin (1959)	>18 but<48 hrs.	Electrophoresis of serum	Fed milk but colostrum introduced directly into duodenum
Kaeckenbeeck et al. (1961)	36 hrs.	Serum agglutination titre to E. coli (0.137)	Fed colostrum and milk if not tested within 20 hours
Pierce (1961)	14-27 hrs.	Change in rate of urine protein excretion	Fed colostrum
Steck (1961)	36 hrs.	Immunoelectrophoresis and semi-quantitative immunodiffusion	Fed milk until tested with colostrum
Balfour & Comline (1962)	About 24 hrs.	^{131}I labelled globulin in thoracic duct lymph	Infusion of whey directly into duodenum
El Nageh (1967a)	60 hrs.	Fluorescent microscopy	Fed colostrum, milk or glucose water
		B. PIG	
Earle (1935)	24 hrs.	Salt fractionation of serum	Natural suckling
Young & Underdahl (1949)	8-24 hrs.	Serum HI titre to swine influenza	Natural suckling
Nordbring and Olsson (1957)	24 hrs.	Electrophoresis of serum	Natural suckling
Nordbring and Olsson (1958a)	72 hrs.	Serum agglutination titre to paratyphoid H	Maintained on oral and parenteral fluids before feeding
Nordbring and Olsson (1958b)	40 hrs.	Serum electrophoresis and agglutination titre to paratyphoid H	Maintained on parenteral 5% glucose saline

Table 4.2 - B. Pig (cont'd)

Author	Duration	Method of Detection	Management
Rutquist (1958)	48 hrs.	Electrophoresis of serum	Natural suckling
Vesselinovitch (1959)	24 hrs.	Electrophoresis of serum	Natural suckling
Speer *et al.* (1959)	24 hrs.	Serum haemagglutination titre to *E. coli*	Natural suckling followed by test dose of colostrum
Miller *et al.* (1961)	24 hrs.	Electrophoresis of serum	Natural suckling
Miller *et al.* (1962)	36 hrs.	Serum agglutination titre to *Salm pullorum*	Management until test dose of serum not given
Asplund *et al.* (1962)	27 hrs.	Electrophoresis of serum	Fed reconstituted skim milk before test dose of colostrum
Lecce & Morgan (1962)	36 hrs.	Absorption of PVP	Natural suckling
Payne & Marsh (1962)	12 hrs. (a) 106 hrs. (b)	Fluorescent microscopy	a) Fed milk before test dose b) Starved or allowed water only before test dose
Chamberlain *et al.* (1965)	60 hrs.	^{131}I labelled gamma globulin	Maintenance regime not given
Lecce (1966)	12 hrs.	Microimmunoelectrophoresis of serum	Maintained on diets of varying amount and concentration of glucose
Sharpe (1966)	26 hrs.	Serum agglutination titre against *E. coli*	Maintained on lactose and water then allowed natural suckling
Perry & Watson (1967)	24 hrs.	Serum agglutination titre against *Salm pullorum*	Natural suckling until given test dose of serum

Table 4.2 (cont'd)

Author	Duration	Method of Detection	Management
		C. LAMB	
Mason, Dalling & Gordon (1930)	18 hrs.	Diphtheria and tetanus antitoxin levels in serum	Natural suckling until dosed with heterologous antiserum
McCarthy & McDougall (1953)	>29 but<48 hrs	Salt fractionation, electrophoresis and agglutination titre of serum	Fed milk before colostrum given
Lecce & Morgan (1962)	24 hrs. (a) 48 hrs. (b)	Absorption of PVP	a) Fed colostrum b) Starved until tested
Payne & Marsh (1962)	48 hrs.	Absorption of PVP	Starved until dosed with PVP
		D. HORSE	
Bardelli (1930)	10 days	Tetanus antitoxin levels in serum	Natural suckling
Bruner et al. (1948)	5 days	Haemagglutination titre of serum (Salm. abortus equi)	Natural suckling
Roberts & Archer (1966)	36 hrs.	Direct Coombs test	Natural suckling
		E. DOG	
Gilette & Filkins (1966)	24 hrs.	Serum agglutination titre	Fed hyperimmune serum
Filkins and Gilette (1966)	24 hrs. (a) 36 hrs. (b)	Serum agglutination titre (Salm. pullorum)	a) Natural suckling b) Maintained on synthetic diet

Table 4.2 (cont'd)

Author	Duration	Method of Detection	Management
		F. CAT	
Harding et al. (1961)	24 hrs.	Serum haemagglutination titre (Salm. montevideo)	Natural suckling
Miller-Ben Shaul (1965)	20 days	Precipitin reaction	Feeding antigens to naturally suckled animals

In view of the lack of exact information on the cessation of intestinal permeability the possibility exists that calves may be being fed colostrum too late for them to absorb the maximum amount of globulins. A trial was carried out with the aim of observing the influence of time of feeding on the amount of globulins absorbed.

Materials and Methods

Management of cows and calves. The purpose of this experiment was to stimulate the conditions under which calves are born and reared on local farms. Accordingly 50 cows were allowed to calve either standing in a byre or in a loose box. As soon as calving had taken place, the calf was removed from the cow and placed in a small cubicle which was bedded with straw. The calf was dried with sacking or straw. Depending on the group to which the calf had been allocated, 2-3 pints of colostrum were offered between 0-6 hours, 6-12 hours, 12-18 hours after birth. The calves in all groups were fed a similar volume of colostrum approximately 12 hours later and thereafter they were given milk.

Immune globulin levels of the calf sera were measured 48 hours after birth using the zinc sulphate turbidity test as described in Part I.

Results

The means and standard errors of each group of calves are shown in Table 4.3.

TABLE 4.3

Time of Feeding (hrs.)	No. of Calves	Mean ± S.E. (ZST Units)
0 - 6	24	20 ± 1.8
6 - 12	11	11.4 ± 2.2
12 - 18	15	8 ± 1.8

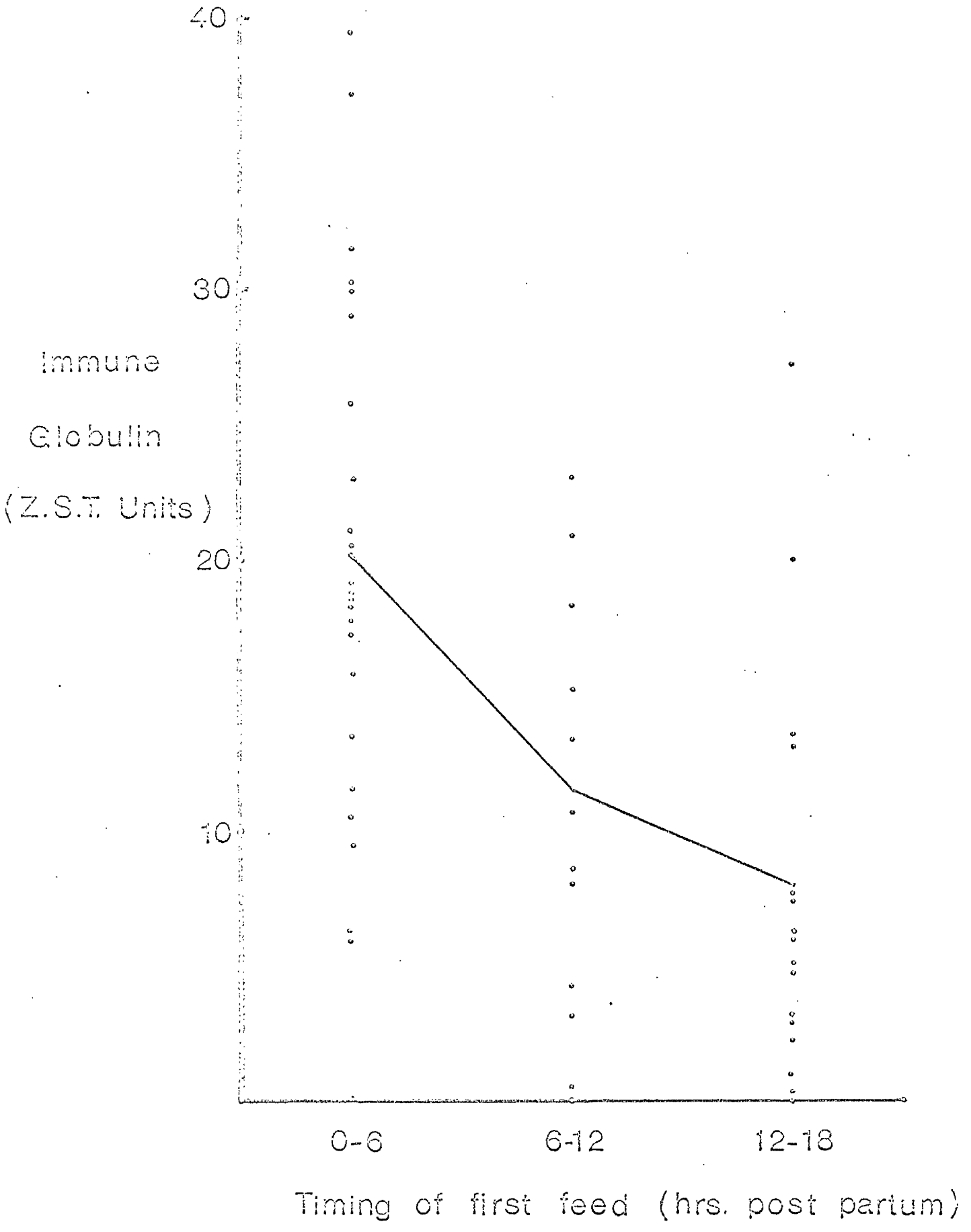
There exists a statistical difference of $p < 0.001$ between the serum immune globulin concentration of those calves fed within 6 hours of birth and those fed after this time. No significant difference exists between those calves fed between 6 and 12 hours and those fed between 12 and 18 hours after birth.

The individual results which make up each group are shown in Figure 4.1. The means of each group are connected by a line.

Discussion

The primary object of this investigation was to establish in quantitative terms what immune globulin levels may be obtained by calves fed colostrum after certain times had elapsed since birth and under a system of management similar to that used on local farms. In such an experiment there are several sources of variation, the main one being the amount of globulin taken by the calf. This factor is probably contributory to the large scatter which exists in each group in that sub-maximal doses have been taken. Large variations

Fig. 4.1. The influence of time of feeding on the absorption of immune globulins from colostrum.



have been noted in the uptake of colostrum proteins in other species under one system of management (Speer et al., 1959; Nordbring and Olsson, 1957; Miller et al., 1962; Filkins and Gillette, 1966; Perry and Watson, 1967 a and b). In spite of these variables a significant difference in the mean levels of immune globulin can be observed between the 0-6 hour and the 6-12 hour groups.

Several points of importance have been highlighted by the results of this trial. In the group of calves fed within 6 hours of birth, 50% have immune globulin levels of less than 20 zinc sulphate turbidity units. By referring to the correlation, demonstrated in a previous section, between immune globulin levels and fate, it can be seen that these calves would be very susceptible to neonatal diarrhoea and colisepticaemia. One possible reason why these calves should have low levels of passive immunity might be a low immune globulin content of the colostrum. Although actual measurements were not made on each sample, a small survey of the immune globulin content of colostrum taken from 18 newly calved cows revealed mean levels of 8.0 ± 2.5 g/100 ml. Fey and Hunyady (1962) have also looked at the immune globulin levels of colostrum in an attempt to find a correlation between hypogammaglobulinaemia and colisepticaemia in calves. These authors found in 51 samples mean levels of 7.8 ± 2.2 g per 100 ml whey. These findings are also supported by the work of Meyer and Steinbach (1965) who found mean levels of 9.9 ± 3.3 g per 100 ml whey.

The other factor more likely to cause these low levels is the volume of colostrum ingested. A proportion of calves do not take readily to drinking milk from a bucket. Some may refuse to drink at all or may drink a little slowly while others attempt to teat seek by extending their head and neck forwards and then attempting to butt by raising their heads forcefully upwards. Faced with such a calf, a stockman may be inclined either to be satisfied after a relatively small volume of colostrum has been taken or to abandon the attempt for several hours in the belief that the calf will be hungrier and possibly more tractable. Unfortunately, in this trial the volume taken by each calf initially was not recorded, neither was the readiness to drink from a bucket.

A trial of a similar nature to this one has been reported by Smith et al. (1967). The immune globulin content of the serum of 30 calves fed colostrum from a bucket up to 12 hours after birth was measured. The results are very similar to the ones obtained here, but again no measure was taken of the volume of colostrum taken by each calf.

A point of practical importance is illustrated by the amount of absorption which has occurred in the calves fed 12-18 hours after birth. The majority of these calves have absorbed immune globulins to some small degree. It has been assumed generally that the intestine of the newborn calf is permeable to immune globulins for 24-36 hours after birth (see Table 4.2). This statement may be factually correct in that small quantities may enter the circulation, but from the point of view of disease prevention, it appears that the amounts absorbed would be insufficient.

It is interesting to compare the results of both this trial and that reported by Smith et al. (1967) with the investigation carried out by Kaeckenbeeck et al. (1961). These workers noted that under their system of testing the absorptive ability of the intestine, a decrease in the absorptive capacity started between 12 and 16 hours after birth and complete cessation occurred after 36 hours. In this trial and that of Smith a decrease in absorptive ability would appear to occur between 6 and 14 hours. The reason for this difference may lie in the methods of testing absorption. The system employed by Kaeckenbeeck et al. (1961) involved the feeding of a test dose of 200 ml of colostrum with a high titre to E. coli 0.137, whereas in the present series a much larger volume, and hence a higher dose of globulin, was given. If the amount of globulin presented to the intestine is less than the amount which can be absorbed from the time of dosing until cessation of absorption occurs, then the process of shutdown will not be detected. Only when the capacity to absorb is less than the amount of globulin presented will detection be possible. This may explain the differences encountered in the apparent onset of shutdown of absorptive ability. Similarly the use of a small dose of colostrum has suggested to Kaeckenbeeck et al. (1961) that the rate of absorption is described by a sigmoid curve, being at maximum for several hours, then declining rapidly, and finally persisting at a much reduced level. In view of the criticism mentioned previously, it may be possible that the state of maximum absorption rate does not persist for several hours, but starts to decrease in a linear fashion from birth.

As a result of trials conducted in a dairy herd, Meyer and Steinbach (1965) concluded that absorption begins to be suppressed about 8 hours after birth. However, it was pointed out by these authors that the calculated regression between the time of drinking and the gamma globulin content of the calf serum, was unreliable for the period 0-5 hours after birth due to the lack of observations during this period. In addition, the volume of colostrum offered to the calves was less than would have been ingested if fed to satiations, and so the timing of the shutdown may have been overestimated.

SECTION B

ESTIMATION OF THE EFFICIENCY OF THE ABSORPTION MECHANISM

In Section II of this thesis it was shown that calves with high levels of circulating immune globulins were not susceptible to die from either colisepticaemia or neonatal diarrhoea. The calves with the highest levels of passive immunity may have a serum globulin content which is 2-4 g per 100 ml higher than colostrum deprived animals. Assuming a plasma volume of 7% of the total bodyweight, a calf weighing 35 Kg would have circulating within its vascular system approximately 50-100 g of immune globulin. The question which arises from this fact is how much colostrum must be fed in order that this amount may accumulate within the circulation?

Several attempts have been made to estimate the efficiency of the absorption mechanism in calves and piglets. Bangham et al. (1958) fed ^{131}I labelled adult bovine serum and colostrum proteins to newborn calves. Within 3 hours of feeding, 8-16% of the labelled proteins were found in the circulation and by 20 hours, this percentage had decreased to 5-10%. The inaccuracies inherent in the technique used to estimate absorption efficiency were pointed out by these authors. Among the unknown factors are the percentage of the dose which reaches the cell surface and hence is available for absorption, and the rate of equilibrium of the proteins between the intravascular and extravascular pools.

In a study of some factors which influence the rate of absorption in calves, Balfour and Comline (1962) noted that within 300 minutes of infusing ^{131}I labelled bovine gamma globulin, between 12 and 25% of the labelled protein could be recovered from the thoracic duct.

Pierce (1961) examined the serum of a calf after being fed a mixture of hyperimmune equine serum containing diphtheria antitoxin and maternal colostrum. It was calculated that 30% of the antitoxin which was fed was circulating in the plasma. As in the work of Bangham et al. (1958) the plasma volume was not actually measured, and no allowance was made for extravascular losses.

In piglets, absorption efficiencies of approximately the same magnitude as have been found by the above authors have been reported (Nordbring and Olsson, 1958 a and b; Olsson, 1959 a and b; Perry and Watson, 1967 and Pierce and Smith, 1967 a).

There exists in the literature some disagreement about the efficiency of the absorption mechanism at different dosage rates. Olsson (1959 a and b) and Payne and Marsh (1962) both state that the amount of globulin absorbed is proportional to the amount fed. In a series of carefully conducted trials in which different amounts of globulin were fed to piglets in a volume of 2.1 ml per 100 g bodyweight, Pierce and Smith (1967 b) found that below certain doses, the absorption was not proportional. When 9.5 g bovine IgG was fed, only 1% was absorbed; but when 2 g were fed, the proportion increased to 10%. With doses of 2-4 g, this percentage remained constant.

In contrast with these findings, Perry and Watson (1967 a) observed that the efficiency of the absorption of antibody decreased as the amount of antibody increased above a certain level. It was suggested that a mechanism similar to that observed by Chapman-Andresen (1961) may be involved. This author noted that in

experiments with Amoeba proteus, pinocytosis was arrested by placing the organism in progressively more highly concentrated protein solutions. The possibility that the method of management and dosing may have influenced the absorption efficiency cannot be ignored. The experiments performed by Perry and Watson (1966) were conducted on piglets which were allowed to obtain colostrum from the sow during the experimental period, whereas the piglets used by the previous authors have been removed from the sow for the duration of the experiment.

The effect of the amount of antibody and the timing of dosing on the amount of antibody absorbed has been studied in calves by Schoenaers and Kaeckenbeeck (1964). These authors using a pool of colostrum with a high agglutination titre to E. coli 0.137, fed 200 ml and 600 ml of colostrum to two groups of calves immediately after birth. The resulting agglutination titres obtained from these calves were compared with a third group which was fed 3 meals of 200 ml of colostrum at 6 hour intervals. The calves which received 200 ml and 600 ml immediately after birth developed average agglutination titres of 1/600 and 1/700 respectively. In marked contrast, the calves which received 600 ml divided into 3 equal doses had an average titre of 1/1750. The conclusion drawn from this experiment is that the most active transmission of immunity is attained by administering colostrum in several meals of moderate volume.

In view of the work described above, it was decided to investigate the relationship between the amount of gamma globulin ingested by a calf and the amount absorbed. From this relationship, an estimate of the efficiency of the absorption mechanism might be formed.

Materials and Methods

Management of Calves. Calves were removed from their dams immediately after birth, dried, weighed and put into a cubicle. A blood sample was taken from the jugular vein for estimation of the total serum protein concentration. Approximately 1 gallon of colostrum was taken from the cow and a measured volume was offered immediately to the calf. Calves were allowed to drink to satiation, and the volume ingested was found by measuring the volume left. Colostrum was again offered to the calf at the following feeding time, i.e. morning or later afternoon, and the volume taken was again recorded. Once 15 hours had elapsed since birth, only milk was offered at the next and subsequent feedings. After 72 hours had elapsed, the calf was reweighed, a second blood sample was taken, and then the plasma volume was estimated. This period was chosen in order to allow the absorbed globulins time to equilibrate between the intravascular and extravascular spaces.

Plasma Volume Estimation. Plasma volume estimation was performed using Evans Blue dye (T1824) and using the same technique as described in Section III.

Estimation of Colostrum Globulin Content. A 20 ml sample of the colostrum offered to each calf was taken. From this, whey was made by adding to it a few drops of a commercially available rennet and placing the sample in a water bath at 37°C until adequate clotting had occurred. The whey was separated from the clot and centrifuged to remove small portions of casein. The protein concentration of the whey was measured by the biuret reaction (Varley, 1963).

Using 12 x 2.5 cm cellulose acetate strips (Oxoid Ltd., Southwark Bridge Road, London, SE1), electrophoresis of the whey samples was carried out. A barbitone n-octoate buffer of pH 8.6 was used and a current of 1mA per strip was applied to 2 hours. The strips were fixed by passing through 5% aqueous trichloracetic acid and then placed for 10 minutes in a staining solution containing 0.2% Ponceau S in 3% aqueous trichloracetic acid. After staining, the strip was washed in several changes of 5% aqueous acetic acid until the background was completely white.

The relative proportions of the whey proteins were measured using an integrating densitometer (Chromoscan, Joyce Loebl, Gateshead, England). The strips were placed wet on the white mat backing plate of the sample holder and evaluated by reflectance. Provided that scanning was performed without delay, no artifacts due to the effects of drying were apparent.

The amount of globulin ingested by the calf was calculated by multiplying the volume of colostrum ingested by the concentration of the gamma globulin.

It was realised that the quantity of globulin determined by this calculation was an overestimate because in the process of whey formation, a casein clot is formed which constitutes a significant proportion of the total volume of colostrum ingested. Therefore, an estimate of the relative size of the casein clot was made. To 10 ml samples of colostrum, taken from 12 different cows, a few drops of rennet were added. The colostrum samples were then placed in a water

bath at 37°C and left for several hours until whey had formed. The samples were then centrifuged and the volume of the supernatant whey of each sample was then measured. The results are shown below.

TABLE 4.4

Sample No.	Vol. of Whey (ml)	Sample No.	Vol. of Whey (ml)
1	8.9	7	7.9
2	8.5	8	8.4
3	8.6	9	8.0
4	8.9	10	8.5
5	7.8	11	7.9
6	8.7	12	8.3

The mean volume and standard deviation is 8.3 $\pm$ 0.39 ml. From this small experiment, it would appear that the casein clot formed as a result of the action of rennet, occupies about 17% of the initial volume of colostrum. No estimation was made of the quantity of whey which remained trapped within the casein clot.

Estimation of Absorption Efficiency. The amount of globulin absorbed by the calf was calculated by multiplying the difference in the concentration of the serum proteins before and after colostrum feeding by the plasma volume. When this quantity of globulin is expressed on a bodyweight basis and compared with the quantity ingested by the calf expressed in similar terms, a measure of the efficiency of absorption is found. It is recognised that several factors will tend to make this figure an underestimate, and these will be discussed later.

Results

The results of this investigation are shown in Tables 4.5 and 4.6. The mean volume of colostrum ingested by these calves was 2.96 litres. This volume represents a mean quantity of 214.8 g of globulin. This quantity, when expressed in terms of dosage on a body weight basis, gives a mean dosage rate of 6.18 g of globulin per Kg nody weight. The resultant increase in the mean serum protein concentration of the calves was 1.92 g per 100 ml and the increase in the mean intravascular globulin concentration expressed on a body weight basis was 1.58 g per Kg body weight.

The relationship between the amount of globulin presented and the amount of globulin absorbed is shown in Figure 4.2. The correlation between these two parameters is expressed by the equation $y = 0.16x + 0.6$, where y = amount of globulin absorbed (g per Kg. body weight) and x = amount of globulin presented (g per Kg. body weight). The coefficient of correlation $r = 0.629$.

Discussion

It would appear that in the newborn calf the amount of globulin absorbed is proportional to the amount ingested. This finding would agree with similar observations made in piglets by Olsson (1959 a and b), Payne and Marsh (1962), and to some extent with the findings of Pierce and Smith (1967).

This relationship may be of importance in the explanation of the occurrence of low immune globulin levels in market calves. A minimum quantity of immune globulin, e.g. 50 g, must be absorbed into

TABLE 4.5

Estimation of the Amount of Globulin Ingested by the Newborn Calves

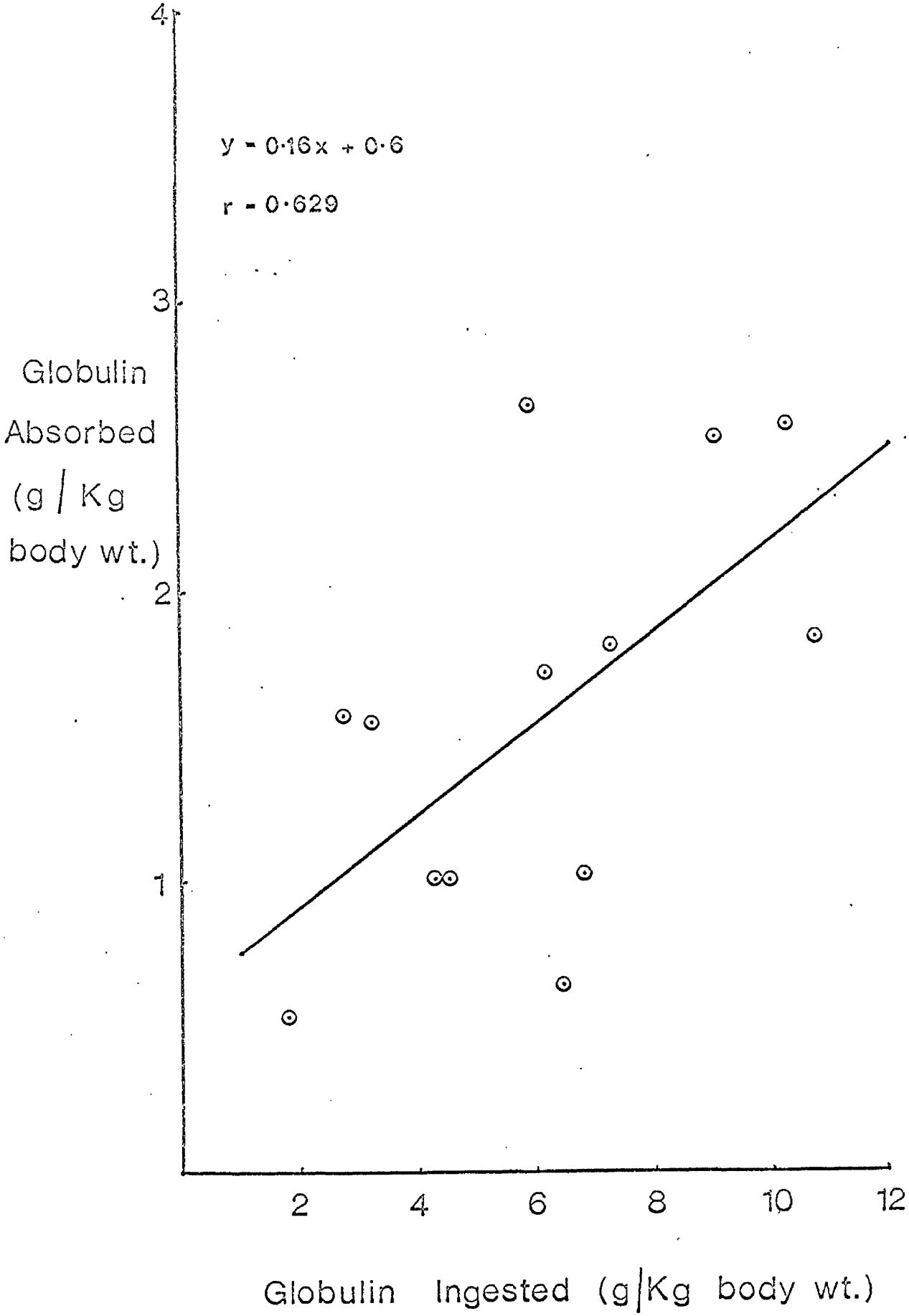
Calf No.	Volume of Colostrum Fed (l.)	Whey Protein Concentration (g/100 m)	% Globulin	Total Globulin Ingested (g)	Weight (Kg)	Dosage of Globulin (g/Kg)
1	2.16	10.0	72.6	157	34.5	4.55
2	3.8	8.9	68	230	33.0	6.96
3	1.65	15.0	79.3	196	45.5	4.30
4	4.2	12.0	68.5	345	33.6	10.26
5	2.2	14.1	78.8	244	37.5	6.50
6	3.9	9.5	72.8	269	43.6	6.16
7	3.7	9.1	74.0	249	34.3	7.25
8	3.8	2.8	55.6	59	31.1	1.8
9	2.38	16.9	72.6	292	32.3	9.04
10	3.4	8.9	72.3	218	31.8	6.85
11	3.0	12.5	78.5	294	27.3	10.76
12	2.5	6.9	65.4	112	40.5	2.76
13	1.8	11.3	62.9	128	40	3.2
Mean	2.96	10.6	70.86	214.8	35.76	6.18

TABLE 4.6

Estimation of the Amount of Globulin Absorbed by the Newborn Calves

Calf No.	Increase in Serum Protein (g/100 ml)	Plasma Volume (l/Kg)	Total Globulin Absorbed (g)	Weight (Kg)	Globulin Concentration (g/Kg)
1	1.3	2.555	33.2	32.7	1.01
2	3.8	2.287	86.9	32.7	2.65
3	1.1	4.064	44.7	44.1	1.01
4	2.4	3.404	81.7	31.6	2.58
5	0.8	3.014	24.1	37.0	0.65
6	1.9	3.955	75.1	43.4	1.73
7	2.5	2.727	68.2	37.3	1.82
8	0.7	2.299	16.1	30.0	0.53
9	2.5	3.289	82.2	32.3	2.54
10	1.5	2.075	31.1	32.7	1.03
11	2.1	2.564	53.8	29.1	1.85
12	1.7	3.585	60.9	38.5	1.58
13	2.7	2.286	61.7	39.1	1.57
Mean	1.92	2.931	55.36	35.4	1.58

Fig. 4.2. The relationship between the amount of globulin ingested and the amount absorbed by the newborn calf.



the circulation to protect a 35 Kg calf from the effects of neonatal disease. This amount is the equivalent of 1.42 g per Kg body weight, and it may be calculated from the regression equation that this amount of absorption would require feeding at the rate of 6.1 g globulin per Kg body weight. Assuming an average whey globulin concentration of 8.0 g per 100 ml, it would require 2.7 litres of colostrum whey to be fed. From observations made on several samples of colostrum, it would appear that whey forms approximately 80% and the curd, 20% of the volume of colostrum. Applying this factor to the estimate of the volume of whey, a volume of 3.2 litres of colostrum would have to be fed within 15 hours of birth.

The average efficiency of absorption in this series of calves is 23%. This is a higher figure than that calculated by Nordbring and Olsson (1958 a and b) and Olsson (1959 a and b) in piglets, although it does come within the upper limit of 25% mentioned by Balfour and Comline (1962) and 30% mentioned by Pierce (1961). The reason for this may lie in the fact that the actual plasma volume of each animal was measured. It has been shown that during the first few days of life in piglets (McCance and Widdowson, 1959) and in calves (Part III of this thesis) that there occurs an expansion of the plasma volume. This expansion has not been taken into consideration by the majority of workers, most of whom have assumed a plasma volume of 5% of total body weight. Taking the findings of McCance and Widdowson (1959) and Ramirez et al. (1963) into consideration, a volume of between 7 and 8% would be more accurate for one-day old piglets,

and from the observations made earlier on calves, a value of 9%. Accordingly the efficiency figures of these workers could be elevated.

A second factor which gives rise to an apparently low absorption efficiency is the fact that only the absorbed globulin which is contained in the intravascular compartment has been measured and no account has been taken of the extravascular pool. The size of this pool in neonatal calves is not yet known, although figures are available for adult cattle. Hansen and Nellsen (1966) using isotopically labelled IgG have found an average extravascular/intravascular ratio of about 0.7. Assuming that this ratio pertains in neonatal calves would mean that in addition to the 50 g of globulin located within the intravascular space, an additional 35 g lies in the extravascular space, and a total amount of 85 g have been absorbed. This would allow the calculated efficiency to rise to 39%.

Finally, if allowance is made for the fact that the casein clot occupies about 17% of the initial volume of colostrum, the calculated efficiency of the absorption mechanism would be about 46%.

Summary

1. The effect of delaying the feeding of colostrum beyond 6 hours after birth results in a diminution of the serum immune globulin levels attainable by the calf.

2. Under the system of management described, the amount of globulin absorbed by a calf is proportional to the amount fed within 15 hours of birth.

3. The calculated efficiency of the absorption mechanism is approximately 46%.

GENERAL SUMMARY AND DISCUSSION

GENERAL SUMMARY AND DISCUSSION

In this thesis it has been shown that in the performance of the zinc sulphate turbidity test there are many factors which may influence the development of the turbidity reaction.

Probably the most important of these are the temperature and pH at which the test is performed. These factors, in addition to other variables, such as the time allowed for the development of the reaction and the ionic strength of the reagent, may be controlled by the adoption of standardised conditions within the laboratory. The influence of variables occurring in the serum sample itself, such as the presence of hemolysis, may be minimised by the use of a filter in the colorimeter and by use of control samples. Other factors, such as the presence of fetuin and the concentrations of alpha and beta globulins and albumin, cannot be controlled, although the influence of albumin would appear to be minimal at physiological levels.

Once a standardised technique for the performance of the test was established and the accuracy of its repeatability determined, the test was calibrated against various fractions of the serum proteins. This calibration correlated the turbidity developed in the test with the concentration of total serum proteins, total globulin, IgG and IgM. The closest correlation was found with the immune globulins.

In Part 2 of the thesis the zinc sulphate turbidity test was used to investigate the changes in the immune globulin content of neonatal calf sera over a three-week period. The results obtained in

this investigation are in agreement with the findings of workers who have used other techniques. The half life of passively acquired immune globulin was calculated as 21.5 days and active production of immune globulin by agammaglobulinaemic calves was detectable between 7 and 14 days of age.

Investigations into the correlation between serum immune globulin concentrations and neonatal mortality confirmed the findings of Foy (1962). Deaths arising from colisepticaemia occurred in calves which showed evidence of having little or no passive immunity, and the deaths arising from effects of neonatal diarrhoea were found in calves with low levels of circulating immune globulin. A two-year survey of the concentration of immune globulin in the sera of neonatal calves which passed through the local market revealed a very pronounced seasonal variation. The period during which the average immune globulin levels were lowest coincided with the period when mortality amongst calves was at its highest.

In view of the importance of a high concentration of circulating immune globulin, an investigation was carried out into two other functions of these globulin molecules. These were the expansion of plasma volume and the increase in buffering capacity. It would appear that during the first three days of life, an expansion of the plasma volume of the calf occurred. The osmotic pressure and plasma sodium concentration decreased in both colostrum and milk fed calves as did the haematocrit. This evidence suggests that the plasma volume expansion may be due predominantly to the effect of dilution rather than an osmotic effect arising from the absorption of high molecular weight globulin molecules.

No difference was detected between the buffering capacity of calves with high and low immune globulin levels. The reason for this may lie in the lack of sensitivity of the technique used. From theoretical calculations the increase which might be expected is relatively small. In addition, the electrocardiographic response of anaesthetised calves to acid infusion would appear to be variable, a fact which is supported by the work of Stewart et al. (1965).

A similarity has been noted between the electrocardiographic changes observed in calves dying as a result of neonatal diarrhoea and those dying from acid infusion. In both these circumstances a reduction in concentration of myocardial potassium has been shown (Fisher and McEwan, 1967). In consequence, it has been suggested that the electrocardiographic changes are a result of interference with the depolarisation mechanism of cardiac muscle.

In Part 4 observations were made on the influence of time of feeding colostrum on the amount of immune globulin absorbed by the calf and an estimate was made of the efficiency of the absorption mechanism. It was found that if feeding was delayed until after six hours had elapsed from birth, a significant decrease in the average serum immune globulin concentration might be expected. Estimations of the efficiency of the absorption mechanism suggest that approximately 46% of the immune globulin presented to the calf intestine is absorbed.

As a result of the findings described in Part I, it would appear that when the zinc sulphate turbidity test is performed under standard conditions on the sera of neonatal calves, it gives an accurate estimate of the immune globulin content of serum. This test therefore may be of considerable value in general veterinary practice because of the correlation which exists between susceptibility to disease and low levels of circulating immune globulins. The outstanding advantages of such a test are the speed with which it can be performed, its relative simplicity in comparison with other methods of estimating immune globulin concentration and the low price of the reagents. Even if a colorimeter is not available, visual estimations of the turbidity are possible. Alternatively, the routine measurement of the serum immune globulin concentration of homebred calves amongst which deaths are occurring may indicate that the management of the newborn calf is at fault, in that colostrum is not being fed soon enough or in great enough quantities.

In view of the results of the survey of the serum immune globulin levels several questions arise. The reasons for the variations in the average concentration are not definitely known although they probably arise as a direct consequence of the management of newborn calves. A field investigation into the levels of serum immune globulin of calves in relation to the type of management would undoubtedly be worth investigation. A point which has been demonstrated in the publications of other workers--Smith (1962), Penhale (1965), Smith et al. (1967)--and which is re-emphasised in

Part 2 is that approximately 10% of calves are markedly hypogammaglobulinaemic even when the natural relationship between the cow and calf is not disturbed and natural suckling is allowed to take place. Two possibilities exist to explain this state. Either this percentage of calves are born with a lack of ability to absorb significant amounts of immune globulins, or that they do not obtain colostrum from the dam sufficiently early or in sufficient quantities. Descriptions of the behavioural relationships of cow and calf, and especially of suckling behaviour, are required if this latter possibility is to be investigated.

The failure to demonstrate significant differences between the plasma volume or buffering activity of calves with high and low levels of circulating immune globulin suggests that the main function of these globulins is the immunological one. The specific nature and action of these antibodies still await elucidation. From the correlation between the serum immune globulin concentration and the subsequent fate of these calves, it can be seen that although many calves with negligible levels of immune globulin died, many did not. This might be explained in terms of lack of challenge by a pathogenic organism and since no measures were taken to ensure an equal and similar challenge to all the animals included in this survey, this criticism must be accepted. However, an immunological comparison of the sera of all these hypogammaglobulinaemic calves may reveal the presence of a protective factor not yet recognised amongst the survivors.

Provided that maximum amounts of colostrum are fed, the amount of immune globulin which is absorbed by a calf is determined by the rate of absorption and the duration of the absorptive ability, and hence the efficiency of the mechanism is dependent on both of these factors. It has been shown by Balfour and Comline (1962) that the amount of gamma globulin absorbed is influenced by the composition of the whey, and Hardy (1968) has demonstrated differences in the rate of absorption as well as the volume and concentration of the thoracic duct lymph as a result of presenting gamma globulin solutions of different composition to the intestine of the newly born calf. The possibility, therefore, exists that the efficiency of the mechanism may be influenced by the chemical composition of the colostrum. Variations in the composition of milk due to breed and diet are well recognised and hence the absorption efficiency calculated in Part 4 may be characteristic only of Ayrshire cattle on winter feeding.

REFERENCES

Adner, P.L. (1957) Acta Soc. Med. upsal. 62, Supplement 6.

Aschaffenburg, R. (1949) Br. J. Nutr. 3, p. 200.

Aschaffenburg, R., Bartlett, S., Kon, S.K., Terry, P., Thompson, S.Y., Walker, D.M., Briggs, C., Cotchin, E. and Lovell, R. (1949a) Br. J. Nutr. 3, p. 187.

Aschaffenburg, R., Bartlett, S., Kon, S.K., Walker, D.M., Briggs, C., Cotchin, E. and Lovell, R. (1949b) Br. J. Nutr. 3, p. 196.

Aschaffenburg, R., Bartlett, S., Kon, S.K., Roy, J.H.B., Walker, D.M., Briggs, C. and Lovell, R. (1951a) Br. J. Nutr. 5, p. 171.

Aschaffenburg, R., Bartlett, S., Kon, S.K., Roy, J.H.B., Walker, D.M., Briggs, C. and Lovell R. (1951b) Br. J. Nutr. 5, p. 343.

Aschaffenburg, R., Bartlett, S., Sears, H.J., Thompson, S.Y., Ingram, P.L., Lovell, R. and Wood, P.C. (1953) Br. J. Nutr. 7, p. 275.

Asplund, J.M., Grummer, R.H. and Phillips, P.H. (1962) J. Anim. Sci. 21, p. 412.

Baglioni, T. and Locatelli, A. (1966) Clinica vet. Milano 89, p. 305.

Baglioni, T., Locatelli, A. and Ruggeri, L. (1964) Archo vet. ital. 15, p. 345.

Balfour, W.E. and Comline, R.S. (1959) J. Physiol. London 148, 77P.

Balfour, W.E. and Comline, R.S. (1962) J. Physiol. London 160, p. 234.

Bamford, D.R. (1967) Proc. Roy. Soc. B. 166, p. 30.

Bangham, D.R., Ingram, P.L., Roy, J.H.B., Shillam, K.W.G. and Terry, R.J. (1958) Proc. Roy. Soc. B. 149, p. 184.

Bardelli, P.C. (1930) Ann. Igiene (sper) 40, p. 675.

Barrick, E.R., Matrone, G. and Osborne, J.C. (1954) J. Proc. Soc. Exp. Biol. Med. 87, p. 92.

Batty, I., Thomson, A. and Hepple, J.R. (1954a) Vet. Rec. 66, p. 249.

Batty, I., Brambell, F.W.R., Hemmings, W.A. and Oakley, C.L. (1954b) Proc. Roy. Soc. B. 142, p. 452.

Bergman, E.N. and Sellers, A.F. (1954) Am. J. Vet. Res. 15, p. 25.

Bergman, F.H., Levine, L. and Spiro, R.G. (1962) Biochim. Biophys. Acta. 58, p. 41.

Blanca, W. (1966) Br. Vet. J. 122, p. 530.

Bjorkman, N. and Bloom, G. (1957) Z. Zellforsch. 45, p. 649.

Bjorneboe, M. (1967) "Gamma globulins. Structure and Control of Biosynthesis." Nobel Symposium 3. Intersclence Publishers.

Bland, J.H. (1963) Clinical Metabolism of Body Water and Electrolytes. W.B. Saunders Company, Philadelphia and London.

Brambell, F.W.R. (1958) Biol. Rev. 33, p. 488.

Brambell, F.W.R. (1966) Lancet No. 7473, p. 1087.

Brambell, F.W.R., Oakley, C.L. and Porter, R.R. (1960) Proc. R. Soc. B. 151, p. 478.

Briggs, C. (1951) Br. J. Nutr. 5, p. 349.

Briggs, C., Lovell, R., Aschaffenburg, R., Bartlett, S., Kon, S.K., Roy, J.H.B., Thompson, S.Y and Walker, D.M. (1951) Br. J. Nutr. 5, p. 356.

Brown, R.D. (1958a) J. Hyg. Camb. 56, p. 427.

Brown, R.D. (1958b) J. Hyg. Camb. 56, p. 435.

Bruner, D.W., Edwards, P.R. and Doll, E.R. (1948) Cornell Vet. 38, p. 363.

Carroll, E.J., Theilen, G.H. and Leighton, R.L. (1968) Am. J. Vet. Res. 29, p. 67.

Chamberlain, A.G., Perry, G.C. and Jones, R.E. (1965) Nature, Lond. 207, p. 429.

Chapman-Andresen, C. (1961) Proc. 1st Int. Conf. on Protozoology, p. 267.

Clark, S.L. (1959) J. Biophys. Biochem. Cytol. 5, p. 41.

Collnet, G., Kaeckenbeeck, A. and Schoenaers, F. (1961) Annls vét méd 105, p. 254.

Comline, R.S., Pomeroy, R.W. and Titchen, D.A. (1953) J. Physiol., Lond. 122, 6P.

Comline, R.S., Roberts, H.E. and Titchen, D.A. (1951a) Nature, Lond. 167, p. 561.

Comline, R.S., Roberts, H.E. and Titchen, D.A. (1951b) Nature, Lond. 168, p. 84.

Cournand, A., Noble, R.P., Breed, E.S., Lauson, H.D., Baldwin, E de F., Pinchot, G.D. and Richards, D.W. (1944) J. Clin. Invest. 23, p. 491.

Dalton, R.G. (1966) Vet. Rec. 79, p. 53.

Dalton, R.G. (1967) Br. Vet. J. 123, p. 48.

Dalton, R.G. and Fisher, E.W. (1961) Br. Vet. J. 117, p. 115.

Dalton, R.G., Fisher, E.W. and McIntyre, W.I.M. (1965) Br. Vet. J. 121, p. 34.

Dalton, R.G., Fisher, E.W. and McIntyre, W.I.M. (1960) Vet. Rec. 72, p. 1186.

de la Huerga, J. and Popper, H. (1950a) J. Lab. Clin. Med. 35, p. 459.

de la Huerga, J., Popper, H., Franklin, M. and Routh, J.I. (1950b) J. Lab. Clin. Med. 35, p. 466.

Deutsch, H.F. (1954) J. Biol. Chem. 208, p. 669.

Deutsch, H.F. and Smith, V.R. (1957) Am. J. Physiol. 191, p. 271.

Discombe, G. (1959) Lancet 1, p. 619.

Discombe, G., Jones, R.F. and Winstanley, D.P. (1954) J. Clin. Path. 7, p. 106.

Dixon, F.J., Talmage, D.W., Maurer, P.H. and Deichmiller, M. (1952) J. Exp. Med. 96, p. 313.

Dixon, F.J., Weigle, W.O. and Vazquez, J.J. (1961) Lab. Invest. 10, p. 216.

Dunne, H.W., Glantz, P.J., Hokanson, J.F. and Bortree, A.L. (1956) Ann. N.Y. Acad. Sci. 66, p. 129.

Earle, I.P. (1935) J. Agric. Res. 51, p. 479.

Edgson, F.A. (1964) Vet. Rec. 76, p. 1351.

Ehrlich, P. (1892) Z. Hyg. InfektionsKrankh. 12, p. 183.

El-Nageh, M.M. (1967a) Annls. Méd. Vét. 111, p. 370.

El-Nageh, M.M. (1967b) Annls. Mßd. Vßt. 111, p. 380.

El-Nageh, M.M. (1967c) Annls. Mßd. Vßt. 111, p. 384.

El-Nageh, M.M. (1967d) Annls. Mßd. Vßt. 111, p. 400.

Famulener, L.W. (1912) J. Infect. Dis. 10, p. 332.

Fennestad, K.L. and Borg-Petersen, C. (1962) J. Infect. Dis. 110, p. 63.

Fey, H. (1962) Schweizer Arch. Tierheilk 104, p. 1.

Fey, H. and Hunyady, G. (1962) Berl. Munch. tierarztl. Wschr. 24, p. 466.

Fey, H. and Margadant, A. (1961) Pathologia Microbiol. 24, p. 970.

Filkins, M.E. and Gillette, D.D. (1966) Proc. Soc. Exp. Biol. Med. 122, p. 686.

Fisher, E.W. (1960) Br. J. Nutr. 14, p. 9.

Fisher, E.W. (1965) Br. Vet. J. 121, p. 132.

Fisher, E.W. and McEwan, A.D. (1967) Br. Vet. J. 123, p. 4.

Gay, C.C. (1965) Bact. Rev. 29, p. 75.

Gay, C.C., Anderson, N., Fisher, E.W., and McEwan, A.D. (1965) Vet. Rec. 77, p. 148.

Gay, C.C., Fisher, E.W. and McEwan, A.D. (1965) Vet. Rec. 77, p. 994.

Gay, C.C., McKay, K.A. and Barnum, D.A. (1964a) Can. Vet. J. 5, p. 248.

Gay, C.C., McKay, K.A. and Barnum, D.A. (1964b) Can. Vet. J. 5, p. 297.

Gay, C.C., McKay, K.A. and Barnum, D.A. (1964c) Can. Vet. J. 5, p. 314.

Gillespie, J.H., Baker, J.A., Burghor, J., Robson, D., and Gilman, B. (1958) Cornell Vet. 48, p. 103.

Gillette, D.D. and Filkins, M.E. (1966) Am. J. Physiol. 210, p. 419.

Gitlin, D. (1957) Ann. N.Y. Acad. Sci. 70, p. 122.

Glantz, P.J., Dunne, H.W., Helst, C.E. and Hokanson, J.F. (1959) Bull. Pa. agric. Exp. Stn. No. 645, p. 22.

Graves, J.H. (1963) J. Immun. 91, p. 251.

Greatorex, J.C. (1954) Br. Vet. J. 110, p. 120.

Grosser, O. (1908) Abl. Physiol. 22, p. 198.

Grosser, O. (1927) Fruhentwicklung, Eihautbildung und Placentation des Menschen und der Saugetiere. Bergmann, Munich.

Gurd, F.R.N. and Goodman, D.S. (1952) J. Am. Chem. Soc. 74, p. 670.

Guyton, A.C. (1961) Textbook of Medical Physiology, 2nd Ed. W.B. Saunders Company, Philadelphia and London.

Halliday, R. (1955) Proc. Roy. Soc. B. 143, p. 408.

Halliday, R. (1957) J. Physiol. Lond. 140, p. 44P

Halliday, R. (1959) J. Endocrin. 18, p. 56.

Hamilton, W.J., Harrison, R.J., and Young, B.A. (1900) J. Anat. Lond. 34, p 1.

Hansard, S.L., Butler, W.O., Comar, C.L. and Hobbs, C.S. (1953) J. Anim. Sci. 12, p. 402.

Hansen, R.G. and Phillips, P.H. (1947) J. Biol. Chem. 171, p. 223.

Harding, S.K., Brunar, D.W. and Bryant, I.W. (1961) Cornell Vet. 51, p. 535.

Hardy, R.N. (1958) J. Physiol. Lond. 194, p. 45P.

Harris, R.C., Andersen, D.H. and Day, R.L. (1954) Pediatrics 13, p. 293.

Hector, A. and Rowatt, R. (1948) J. Dept. Agric. Scot. 28, p. 43.

Hemmings, W.A. (1960) Nature, Lond. No. 4722, p. 399.

Henning, M.W. (1953) Onderstepoort J. vet Res. 26, p. 45.

Hill, K.J. (1956) Q. Jl. Exp. Physiol. 41, p. 421.

Hill, K.J. and Hardy, W.S. (1956) Nature, Lond. 178, p. 1353.

Holman, H.H. (1956) Br. Vet. J. 112, p. 91.

Holman, H.H. and Dew, S.M. (1965) Res . Vet. Sci. 6, p. 245.

Horn, Z. and Kovacs E. (1959) Acta Med. Scand. 164, p. 143.

Howe, P.E. (1921a) J. Biol. Chem. 49, p. 109.

Howe, P.E. (1921b) J. Biol. Chem. 49, p. 115.

Howe, P.E. (1924) J. Exp. Med. 39, p. 313.

Inglis, J.S.S. (1960) Vet. Rec. 72, p. 1174.

Ingram, P.L., Lovell, R., Wood, P.C. Aschaffenburg, R., Bartlett, S., Kon, S.K., Roy, J.H.B. and Sears, H.J. (1953) Proc. XIIIth Int. Dairy Congr. The Hague, 111, p. 1369.

Ingram, P.L., Lovell, R., Wood, P.C., Aschaffenburg, R., Bartlett, S., Kon, S.K., Palmer, J., Roy, J.H.B. and Shillam, K.W.G. (1956) J. Path. Bact. 72, p. 561.

Jamason, E., Alvarez-Tostado, C. and Sortor, H.H. (1942) Proc. Soc. Exp. Biol. and Med., 51, p. 163.

Johnson, P. and Pierce, A.E. (1959) J. Hyg. Camb. 57, p. 309.

Jones, R.A. (1967) Vet. Rec. 81, p. 494.

Jones, R.A. (1968) Vet. Rec. 82, p. 140.

Jordan, L. (1933) Vet. J. 89, p. 202.

Kaeberle, M.L. and Segre, D. (1964) Am. J. Vet. Res. 25, p. 1096.

Kaeckenbeeck, A., Collnet, G. and Schoenaers, F. (1961) Annls. Méd. Vét. 105, p. 197.

Kaeckenbeeck, A., El-Nageh, M. and Schoenaers, F. (1967) Annls. Méd. Vét. 111, p. 391.

Kammeraad, A. (1942) J. Morph. 70, p. 323.

Kerr, W.R. (1956) Vet. Rec. 68, p. 476.

Kerr, W.R. and Robertson, M. (1943) J. Comp. Path. 53, p. 280.

Kerr, W.R. and Robertson, M. (1954) J. Hyg. Camb. 52, p. 253.

Kniazeff, A.J., Rimer, V. and Gaeta, L. (1967) Nature, Lond. 214, p. 806.

Kunkel, H.G. (1947) Proc. Soc. Exp. Biol. Med. 66, p. 217.

Laskowski, M. and Laskowski, M. (1951) J. Biol. Chem. 190, p. 563.

Lecce, J.G. (1966) J. Physiol. Lond. 184, p. 594.

Lecce, J.G. and Morgan, D.O. (1962) J. Nutr. 78, p. 263.

Lecce, J.G., Matrone, G. and Morgan, D.O. (1961) J. Nutr. 73, p. 158.

Little R.B. and Orcutt, M.L. (1922) J. Exp. Med. 35, p. 161.

Long, C. (1961) Biochemists Handbook. E & F. N. Spon Ltd., London.

Loosemore, R.M. (1964) Vet. Rec. 76, p. 1335.

Lovell, R. and Bradford Hill, A. (1940) J. Dairy Sci. 11, p. 225.

McCance, R.A. and Widdowson, E.M. (1956) J. Physiol. Lond. 133, p. 373.

McCance, R.A. and Widdowson, E.M. (1957) Lancet 273, p. 585.

McCance, R.A. and Widdowson, E.M. (1959) J. Physiol. Lond. 145, p. 547.

McCarthy, E.F. and McDougall, E.I. (1953) Biochem. J. 55, p. 177.

McDiarmid, A. (1946) Vet. Rec. 58, p. 146.

McEwen, A.D. (1950) Vet. Rec. 62, p. 83.

Maclagan, N.F. (1948) Br. Med. J. 11, p. 892.

McSherry, B.J. and Grinyer, I. (1954) Am. J. Vet. Res. 15, p. 535.

Manton, V.J.A. (1963) Vet. Rec. 75, p. 1395.

Mason, J.H., Dalling, T. and Gordon, W.S. (1930) J. Path. Bact. 33, p. 783.

Mattisson, A.G.M. and Karlson, B.W. (1964) Third European Regional Conference on Electron Microscopy, p. 431.

Meyer, H. and Steinbach, G. (1965) Mh. Vet. Med. 20, p. 84.

Miller-Ben Shaul, D. (1965) Israel J. Med. Scis. 1, p. 563.

Miller, E.R. Ullrey, D.E., Ackerman, I., Schmidt, D.A., Hoefer, J.A. and Luecke, R.W. (1961) J. Anim. Sci. 20, p. 31.

Miller, E.R., Harmon, B.G., Ullrey, D.E., Schmidt, D.A., Luecke, R.W. and Hoefer, J.A. (1962) J. Anim. Sci. 21, p. 309.

Ministry of Agriculture (1966) Vet. Rec. 78, p. 498.

Moog, F. (1951) J. Exp. Zool. 118, p. 187.

Moog, F. (1953) J. Exp. Zool. 124, p. 329.

Morrill, C.C. (1952) Am. J. Vet. Res. 13, p. 322.

Morris, D. and Steel, E.D. (1967) J. Zool. Lond. 152, p. 257.

Morris, B. and Steel, E.D. (1964) J. Endocr. 30, p. 195.

Mossman, H.W. (1926) Am. J. Anat. 37, p. 433.

Mossman, H.W. (1937) Contr. Embryol. Carneg. Instn. 26, p. 129.

Nagy, L.K. and Hignett, P.G. (1967) Res. Vet. Sci. 8, p. 247.

Nanson, P. and Nellsen, K. (1966) Can. J. Comp. Med. 30, p. 327.

Nelmann-Sørensen, A., Konggaard, S.P. and Kruse, V. (1966) LandøKon. Aarb., p. 347.

Nordbring, F. and Olsson, B. (1957) Acta Soc. Med. upsal. 62, p. 193.

Nordbring, F. and Olsson, B. (1958a) Acta Soc. Med. upsal. 63, p. 25.

Nordbring, F. and Olsson, B. (1958b) Acta Soc. Med. upsal. 63, p. 41.

Olsson, B. (1959a) Nord. Vet. Med. 11, p. 375.

Olsson, B. (1959b) Nord. Vet. Med. 11, p. 441.

Ott, H. (1956) Klin. Wschr. 34, p. 1079.

Ott, H. (1957) Z. ges. exptl. Med. 128, p. 340.

Patterson, D.S.P. (1967) Vet. Rec. 80, p. 260.

Payne, L.C. and Marsh, C.L. (1962a) J. Nutr. 76, p. 151.

Payne, L.C. and Marsh, C.L. (1962b) Fedr. Proc. 21, p. 909.

Payne, E., Ryley, J.W. and Gartner, R.J.W. (1967) Res. Vet. Sci. 8, p. 20.

Penhale, W.J. (1965) Ph.D. thesis, University of Liverpool.

Penhale, W.J. and Christie, G. (1968) Res. Vet. Sci., in press.

Perkins, F.T., Yetts, R. and Galsford, W. (1959) Br. Med. J. I, p. 680.

Perry, G.C. and Watson, J.H. (1967a) Anim. Prod. 9, p. 377.

Perry, G.C. and Watson, J.H. (1967b) Anim. Prod. 9, p. 385.

Persson, S. (1967) Acta Vet. Scand., Supplement 19.

Pierce, A.E. (1955a) J. Hyg. Camb. 53, p. 247.

Pierce, A.E. (1955b) J. Hyg. Camb. 53, p. 261.

Pierce, A.E. (1959) J. Physiol. Lond. 148, p. 469.

Pierce, A.E. (1960) Nature, Lond. 188, p. 940.

Pierce, A.E. (1961a) J. Physiol. Lond. 156, p. 136.

Pierce, A.E. (1961b) Colston Pap. 13, p. 189.

Pierce, A.E., Risdall, P.C. and Shaw, B. (1964) J. Physiol. Lond. 171, p. 203.

Pierce, A.E. and Smith, N.W. (1967a) J. Physiol. Lond. 190, p. 1.

Pierce, A.E. and Smith, N.W. (1967b) J. Physiol. Lond. 190, p. 19.

Pitts, R.F. (1953) Harvey Lect. Series 48, p. 172.

Polson, A. (1952) Onderstepoort J. Vet. Sci. 25, p. 7.

Porter, R.R. (1959) Biochem. J. 73, p. 119.

Ramirez, C.G., Miller, E.R., Ullrey, D.E. and Hoefer, J.A. (1963) J. Anim. Sci. 22, p. 1068.

Reinhold, J.G. (1960) Adv. Clin. Chem. 3, p. 83.

Reinhold, J.G. and Yonan, V. (1956) Clin. Chem. 2, p. 228.

Ricketts, W.E., Sterling, K. and Levine, R.S. (1951) J. Lab. Clin. Med. 38, p. 153.

Roberts, E.J. and Archer, R.K. (1966) Vet. Rec. 79, p. 61.

Roberts, H.E., Worden, A.N. and Rees-Evans, E.T. (1954) J. Comp. Path. 64, p. 283.

Rothschild, M.A., Oratz, M., Franklin, E.C. and Schreiber, S.S. (1962) J. Clin. Invest. 41, p. 1564.

Roy, J.H.B. (1964) Vet. Rec. 76, p. 511.

Roy, J.H.B., Shillam, K.W.G., Hawkins, G.M., Lang, J.H. and Ingram, P.L. (1959) Br. J. Nutr. 13, p. 219.

Rutquist, L. (1958) Am. J. Vet. Res. 19, p. 25.

San Clemente, C.L. and Huddleson, I.F. (1943) Tech. Bull. Mich. (St. Coll.) agric. Exp. Stn., No. 182.

Scatchard, G., Batchelder, A.C. and Brown, A. (1944) J. Clin. Invest. 23, p. 458.

Schneider, L. and Szathmary, J. (1938) Z. ImmunForsch. 94, p. 465.

Schneider, L. and Szathmary, J. (1939) Z. Immunforsch. 95, p. 169 and p. 177.

Schoenaers, F. and Kaeckenbeeck, A. (1958) Annls. Méd. Vét. 102, p. 211.

Schoenaers, F. and Kaeckenbeeck, A. (1963) Annls. Méd. Vét. 107, p. 81.

Schoenaers, F. and Kaeckenbeeck, A. (1964) Annls. Méd. Vét. 108, p. 205.

Shank, R.E. and Hoagland, C.L. (1946) J. Biol. Chem. 162, p. 133.

Shannon, A.D. and Lascelles, A.K. (1966) Aust. J. Biol. Sci. 19, p. 831.

Sharpe, M.B.A. (1966) Res. Vet. Sci. 7, p. 74.

Singh, K.V., Osman, O.A., El Ciey, I.F. and Daz, T.I. (1967) Can. J. Comp. Med. 31, p. 295.

Sisson, T.R.C. and Whalen, L.E. (1960) J. Pediat. 56, p. 43.

Smith, A.N. and Ingram, D.G. (1965) Can. Vet. J. 6, p. 226.

Smith, E.L. and Holm, A. (1948) J. Biol. Chem. 175, p. 349.

Smith, H.W. (1962) J. Path. Bact. 84, p. 147.

Smith, H.W. and Halls, S. (1967a) J. Path. Bact. 93, p. 499.

Smith, H.W. and Halls, S. (1967b) J. Path. Bact. 93, p. 531.

Smith, H.W., O'Neill, J.A. and Simmons, E.J. (1967) Vet. Rec. 80, p. 664.

Smith, R.M. (1934) Vet. Rec. N.S.14, p. 1004.

Smith, T. and Little, R.B. (1922) J. Exp. Med. 36, p. 181.

Smith, T. and Little, R.B. (1924) J. Exp. Med. 39, p. 303.

Smith, V.R. and Erwin, E.S. (1959) J. Dairy Sci. 42, p. 364.

Smith, V.R. and Reed, R.E. and Erwin, E.S. (1964) J. Dairy Sci. 47, p. 923.

Speer, V.C., Brown, H., Quinn, L. and Catron, D.V. (1959) J. Immunol. 83, p. 632.

Spiro, R.G. (1960) J. Biol. Chem. 235, p. 2860.

Starling, E.H. (1896) J. Physiol. Lond. 19, p. 312.

Steck, F. (1961) Pathologia. Microbiol. 24, p. 963.

Steel, E.D. (1965) Ph.D. thesis. University of Nottingham.

Stewart, J.S.S., Stewart, W.K., Morgan, H.G. and McGowan, S.W. (1965) Br. Heart J. 27, p. 490.

Strickland, K.L. (1962) Vet. Rec. 74, p.630.

Tanford, C. (1952) J. Am. Chem. Soc. 74, p. 211.

Ulbrich, F. (1962) Berl. Wiss. Ges. f., Tierarzte, Confer., p. 5.

VanSlyke, D.D., Hastings, A.B., Hiller, A. and Sendroy, J. (1928) J. Biol Chem. 79, p. 769.

VanSlyke, D.D., Wu, H., and McLean, F.C. (1923) J. Biol. Chem. 56, p. 765.

Varley, H. (1963) 'Practical Clinical Biochemistry.' 3rd Ed. William Heinemann Medical Books Ltd., London.

Omissions from List of References

Pedersen, K.O. (1944) Nature Lond. 154, p. 575.

Pedersen, K.O. (1947) J. phys. Chem. 51, p. 164.

Widdowson, E.M. and McCance, R.A. (1956) Clin. Sci. 15, p. 361.

Wood, P.C. (1955) J. Path. Bact. LXX, p. 179.

APPENDIX I

The Effect of Time and Temperature on the Zinc Sulphate Turbidity Reaction

A. Temperature 6°C

	Time (mins)			
Calf No.	15	30	60	120
1	18.0	21.5	22.0	23.0
2	22.0	24.25	24.5	24.5
3	5.75	6.5	7.0	7.75
4	14.0	16.25	17.0	17.5
5	3.25	4.75	5.25	5.75
6	13.5	16.25	17.25	17.75
7	12.0	15.75	17.0	17.75
8	17.0	19.75	19.5	22.0
9	4.75	7.0	7.75	8.0
10	18.25	19.5	20.75	21.75
Mean	12.8	15.1	15.8	16.6
± SE	± 2.01	± 2.14	± 2.13	± 2.19

B. Temperature 20°C

	Time (mins)			
Calf No.	15	30	60	120
1	20.75	23.0	24.25	27.0
2	23.75	25.75	27.0	29.75
3	6.75	8.25	9.25	11.75
4	14.5	17.5	19.0	20.75
5	4.5	5.75	5.75	7.25
6	17.25	21.0	22.25	24.75
7	13.75	16.5	17.5	20.75
8	18.5	22.25	24.75	27.5
9	9.0	11.75	13.0	15.5
10	18.5	22.25	23.5	27.0
Mean	14.7	17.4	18.6	21.2
± SE	± 1.98	± 2.14	± 2.26	± 2.37

The Effect of Time and Temperature on the Zinc Sulphate Turbidity Reaction

C. Temperature 25°C

Calf No.	Time (mins) 15	30	60	120
1	22.5	26.25	32.5	35.25
2	24.75	28.25	30.25	32.0
3	7.75	12.75	15.75	18.75
4	15.75	22.75	24.75	27.25
5	5.0	8.0	9.5	11.75
6	18.75	25.75	29.0	31.75
7	17.75	22.75	25.25	28.25
8	23.25	28.0	30.25	32.75
9	9.75	17.5	20.75	23.75
10	22.5	27.75	31.5	34.75
Mean	16.8	22.0	24.95	27.6
± SE	± 2.22	± 2.08	± 2.38	± 2.5

D. Temperature 31°C

Calf No.	Time (mins) 15	30	60	120
1	34.5	40.0	44.25	45.0
2	34.75	37.75	42.0	43.0
3	29.5	32.75	36.25	38.75
4	22.75	32.25	38.0	40.5
5	20.0	22.75	26.5	29.0
6	36.75	39.0	41.75	43.5
7	31.5	34.25	37.75	41.0
8	36.75	38.75	41.5	44.25
9	26.75	30.25	34.5	37.5
10	36.0	38.75	41.75	44.0
Mean	30.3	34.6	38.4	40.6
± SE	± 1.91	± 1.70	± 1.63	± 1.51

The Effect of Time and Temperature on the Zinc Sulphate Turbidity Reaction

E. Temperature 37°C

	Time (mins)			
Calf No.	15	30	60	120
1	45.5	47.5	47.75	50.75
2	43.75	43.75	44.25	47.25
3	37.75	40.0	41.0	44.75
4	40.75	43.25	45.25	47.5
5	32.25	34.0	36.0	39.0
6	41.5	43.5	43.75	46.5
7	42.25	42.5	43.5	46.25
8	44.0	44.75	46.0	48.5
9	42.5	42.5	44.5	47.5
10	45.75	46.5	48.0	49.75
Mean	41.6	42.8	44.0	46.7
± SE	± 1.27	± 1.18	± 1.10	± 1.02

The Effect of pH on the Zinc Sulphate Turbidity Reaction

Calf No.	Zinc Sulphate Solution (pH 4.7)	Zinc Sulphate Solution + Reinhold Buffer (pH 7.53)	Zinc Sulphate Solution + Kunkel Buffer (pH 7.6)
1	1.5	3.25	0.5
2	3.25	1.75	1.0
3	5.25	0.5	1.0
4	5.25	2.5	1.25
5	9.0	3.5	1.0
6	11.0	5.0	0.5
7	14.5	5.25	1.75
8	14.5	5.0	1.75
9	16.25	5.25	0.75
10	17.0	9.5	1.0
11	19.5	12.0	1.75
12	24.0	9.75	1.25
13	25.5	9.0	1.5
14	28.5	12.0	1.5
15	30.25	12.0	2.0
16	30.5	11.25	1.0
17	32.0	10.75	1.5
18	33.0	13.5	2.5
19	34.75	11.75	1.5
20	37.5	15.5	1.0

The Effect of Ionic Strength on the Zinc Sulphate Turbidity Reaction

Calf No.	Original Turbidity	Ionic Strength ($I \times 10^{-3}$) 4.4 (1/16)*	5.9 (1/8)	8.9 (1/4)	15.0 (1/2)	27.1 (1/1)
1	16.75	0.0	1.25	3.25	4.75	6.5
2	20.25	0.5	3.5	4.0	5.0	5.75
3	22.75	1.0	2.25	2.25	4.5	8.0
4	25.25	0.0	0.0	2.0	4.0	7.25
5	33.25	0.25	1.25	2.0	4.5	8.75
6	37.0	0.5	0.5	1.0	4.5	8.5
7	41.0	1.0	2.0	2.75	6.0	9.0
8	43.5	1.0	1.5	1.0	5.5	7.0
9	45.75	0.25	1.5	2.75	6.25	9.75
10	47.25	1.25	2.0	3.0	5.5	11.25
	Mean	0.5	1.5	2.4	5.0	8.1
	± SE	± 0.12	± 0.30	± 0.30	± 0.23	± 0.51

* Saline dilution

Figures in the table refer to the depression produced from the original turbidity in terms of zinc sulphate turbidity units.

The Effect of Albumin on the Zinc Sulphate Turbidity Reaction

Albumin Added (mg per ml)

Calf No.	0	25	50	100	150	200
1	46.75	45.75	40.5	33.75	29.5	20.25
2	47.25	48.25	45.75	40.75	32.25	21.5
3	46.25	46.25	45.25	38.75	30.75	22.5
4	34.75	34.75	31.25	27.0	20.25	12.0
5	46.5	45.25	44.5	39.5	30.75	25.0
Mean	44.3	44.0	41.5	36.0	28.7	20.25
± SE	± 2.39	± 2.38	± 2.71	± 2.53	± 2.15	± 2.20

Albumin Added (mg per ml)

Calf No.	0	2.5	5.0	10	20	30	40
1	46.5	44.5	46.5	46.5	43.75	45.5	44.5
2	47.0	45.0	46.0	46.5	47.5	48.5	46.0
3	45.5	47.0	46.0	46.0	47.0	46.0	45.5
4	34.25	32.75	33.25	35.25	33.75	33.75	32.5
5	45.25	45.25	44.25	44.75	44.75	46.25	44.25
Mean	43.7	42.9	43.2	43.8	43.4	44.0	42.5
± SE	± 2.38	± 2.57	± 2.51	± 2.16	± 2.49	± 2.61	± 2.53

The Effect of Adding Bovine Gamma Globulin to Hypogammaglobulinaemic Calf Serum

Globulin Added (mg per ml)

Calf No.	0	0.25	0.5	1.0	2.0	3.0	4.0
1	1.0	3.0	6.25	13.0	26.25	36.75	46.25
2	0.5	3.25	6.75	14.25	28.5	34.75	44.75
3	1.0	2.75	6.5	13.25	26.75	37.25	44.5
4	0.5	4.5	7.5	16.0	27.5	38.5	47.0
5	1.0	4.0	6.25	14.5	27.25	36.25	46.0
Mean	0.8	3.5	6.7	14.2	27.25	36.7	45.7
± SE	±0.11	±0.32	±0.23	± 0.53	± 0.37	± 0.61	± 0.40

Globulin Added (mg per ml)

Calf No.	0	0.15	0.31	0.62	1.25	2.5	3.75	5.0
6	1.25	3.0	5.25	9.0	16.25	32.75	40.75	48.25
7	1.25	2.5	4.25	8.75	16.0	30.0	38.75	48.0
8	0.25	1.5	3.0	7.0	14.75	30.25	39.25	48.75
9	0.5	1.25	3.5	7.75	14.75	30.25	38.75	48.75
10	1.25	3.0	3.0	7.5	15.75	29.75	40.25	50.75
Mean	0.9	2.25	3.8	8.0	15.5	30.6	39.5	48.9
± SE	±0.21	±0.37	±0.42	±0.37	± 0.31	± 0.56	± 0.40	± 0.48

THE CORRELATION BETWEEN THE ZINC SULPHATE TURBIDITY AND THE TOTAL SERUM PROTEIN OF NEONATAL CALVES

Calf No.	$ZnSO_4$ Turbidity	Total Serum Protein (g per 100 ml)	Calf No.	$ZnSO_4$ Turbidity	Total Serum Protein (g per 100 ml)
1	0.7	4.4	26	27.7	7.6
2	2.4	4.4	27	28.5	6.25
3	3.5	4.7	28	28.7	6.25
4	5.7	4.5	29	29.8	6.4
5	6.3	4.6	30	29.8	6.6
6	7.5	4.9	31	30.8	6.75
7	7.9	4.4	32	32.1	7.4
8	8.9	4.5	33	32.5	6.5
9	9.3	5.3	34	34.5	7.7
10	9.4	4.9	35	35.25	7.7
11	10.3	4.75	36	35.5	7.7
12	10.8	5.25	37	36.0	8.3
13	11.1	5.25	38	36.6	7.1
14	11.9	5.9	39	37.25	7.9
15	12.1	5.4	40	39.25	8.0
16	12.3	5.0	41	40.5	7.8
17	12.8	4.7	42	41.25	8.8
18	14.7	5.0	43	41.5	8.9
19	16.6	5.4	44	41.75	8.8
20	17.5	5.0	45	42.5	9.0
21	24.1	5.9	46	44.1	8.0
22	25.0	6.0	47	44.5	8.7
23	25.2	6.9	48	47.5	9.4
24	27.1	6.3	49	48.5	9.0
25	27.2	6.1	50	49.5	9.6

THE CORRELATION BETWEEN THE $ZnSO_4$ TURBIDITY AND THE TOTAL SERUM GLOBULIN OF NEONATAL CALVES

Calf No.	$ZnSO_4$ Turbidity	Globulin (g per 100 ml)	Calf No.	$ZnSO_4$ Turbidity	Globulin (g per 100 ml)
1	0.7	2.0	26	23.1	2.52
2	2.4	2.18	27	23.3	5.34
3	2.8	1.88	28	23.5	3.01
4	3.4	2.2	29	24.1	3.30
5	3.5	2.08	30	25.0	3.77
6	5.7	2.40	31	25.2	4.58
7	6.3	2.28	32	27.1	3.56
8	7.5	2.76	33	27.2	3.24
9	7.9	2.70	34	27.7	5.00
10	8.9	2.25	35	28.0	4.13
11	9.3	3.73	36	28.5	4.09
12	9.4	2.57	37	28.7	4.27
13	10.3	2.64	38	29.8	4.43
14	10.8	3.03	39	29.8	4.54
15	11.1	2.41	40	30.8	4.70
16	11.9	3.71	41	32.1	5.09
17	12.1	2.95	42	32.5	5.05
18	12.3	3.09	43	33.2	5.50
19	12.8	2.00	44	34.2	5.16
20	14.7	2.77	45	36.6	4.57
21	15.6	3.88	46	39.6	6.76
22	16.6	3.05	47	41.8	4.91
23	17.5	3.08	48	42.5	6.53
24	17.7	2.42	49	44.1	5.91
25	18.5	2.64	50	49.5	7.03

THE CORRELATION BETWEEN THE ZINC SULPHATE TURBIDITY TEST AND THE SERUM IMMUNE GLOBULIN CONCENTRATION OF NEONATAL CALVES

Calf No.	$ZnSO_4$	IgG	IgM	IgG + IgM
1	10.5	7.1	0.83	7.93
2	15.25	12.6	1.85	14.45
3	7.5	4.5	0.53	5.03
4	9.5	9.8	1.4	11.2
5	1.5	0.9	0.12	1.02
6	7.0	5.7	0.25	5.95
7	9.25	7.1	1.25	8.35
8	4.5	4.7	0.25	4.95
9	9.75	6.3	0.41	6.71
10	4.5	1.9	0.36	2.26
11	5.25	0.4	0.18	0.58
12	13.5	6.8	0.23	7.03
13	3.5	2.6	0.13	2.73
14	7.0	6.3	0.49	6.79
15	4.0	2.2	0.24	2.44
16	11.0	8.5	0.29	8.79
17	0.5	0	0	0
18	1.75	0	0	0
19	17.0	8.5	0.24	8.74
20	3.0	0.7	0.20	0.9
21	17.5	11.9	0.76	12.66
22	7.5	5.6	0.80	6.4
23	1.0	0.2	1.08	1.28
24	36.0	34.0	6.7	40.7
25	27.75	25.0	1.6	26.6
26	35.5	30.0	2.65	32.65
27	20.75	17.0	0.49	17.49
28	37.25	26.5	1.95	28.45
29	24.5	19.0	2.05	21.05
30	27.5	34.0	3.15	37.15
31	23.75	26.8	1.08	27.88
32	41.5	47.0	2.8	49.8
33	23.5	23.0	1.66	24.66
34	31.75	26.5	1.72	28.22
35	27.25	20.5	3.7	24.2
36	29.0	28.0	2.3	30.3
37	34.0	39.0	3.15	42.15
38	25.5	26.5	1.66	28.16
39	34.5	34.0	3.15	37.15
40	35.25	32.0	4.2	36.2

THE CORRELATION BETWEEN THE ZINC SULPHATE TURBIDITY TEST AND THE SERUM IMMUNE GLOBULIN CONCENTRATION OF NEONATAL CALVES (cont'd)

Calf No.	$ZnSO_4$	IgG	IgM	IgG + IgM
41	39.5	34.0	2.85	36.85
42	22.5	25.0	1.23	26.23
43	39.25	42.0	4.05	46.05
44	29.25	23.3	2.45	25.75
45	36.0	34.0	5.4	39.4
46	29.75	25.1	5.6	30.7
47	31.0	19.1	0.58	19.68
48	45.5	40.0	4.9	44.9
49	24.0	21.5	1.95	23.45
50	32.25	31.5	2.3	33.8
51	36.75	35.0	3.9	38.9
52	24.75	18.0	2.1	20.1
53	31.0	20.0	6.0	26.0

APPENDIX II

CHANGES IN ZINC SULPHATE TURBIDITY OVER A PERIOD OF 21 DAYS

Class I — Initial turbidity 0-5

Calf No.	Day 0	7	14	21
1	0	0.75	4.5	7.0
2	1.75	4.0	6.5	9.5
3	4.25	7.75	6.75	8.5
Mean ± SE	2.0 ± 1.2	3.2 ± 1.2	5.9 ± 0.7	8.3 ± 0.7

Class II — Initial turbidity 5-15

Calf No.	Day 0	7	14	21
4	10.0	8.0	7.0	7.5
5	10.0	8.75	10.25	11.0
6	11.25	8.75	8.75	9.0
Mean ± SE	10.4 ± 0.4	8.5 ± 0.25	8.7 ± 0.9	9.2 ± 1.0

Class III — Initial turbidity 15-25

Calf No.	Day 0	7	14	21
7	18.75	15.25	12.75	11.5
8	18.75	17.75	16.75	15.0
9	19.5	18.25	15.0	14.0
Mean ± SE	19.0 ± 2.5	16.4 ± 1.6	14.8 ± 1.1	13.5 ± 1.0

Class IV — Initial turbidity 25-35

Calf No.	Day 0	7	14	21
10	25.25	20.75	12.75	12.0
11	30.25	24.5	19.25	16.5
12	35.0	27.75	25.25	20.0
Mean ± SE	30.2 ± 2.8	24.3 ± 2.0	19.1 ± 3.6	16.2 ± 2.3

Class V — Initial turbidity 35-45

Calf No.	Day 0	7	14	21
13	40.5	37.0	29.5	21.75
14	41.25	29.75	26.75	18.5
15	41.5	32.75	26.0	19.5
Mean ± SE	41.0 ± 0.3	33.2 ± 2.1	27.4 ± 1.0	19.9 ± 0.9

THE RELATIONSHIP BETWEEN SERUM IMMUNE GLOBULIN CONCENTRATION AND NEONATAL MORTALITY

Immune Globulin (ZST Units)	No. of Calves	Septicaemias (%)	Deaths from other causes (%)
0 - 10	182	30.7	29.1
10 - 20	81	2.4	18.5
20 - 30	61	-	6.5
30 - 40	54	-	3.7
40 - 50	28	-	-
50 - 60	8	-	-
60 - 70	1	-	-

Serum Immune Globulin Concentrations of Neonatal Market Calves

July, 1964

Calf No.	Immune Globulin (ZST Units)	Calf No.	Immune Globulin (ZST Units)	Calf No.	Immune Globulin (ZST Units)
1	6.5	5	18.0	9	37.0
2	7.5	6	27.5	10	39.25
3	7.75	7	28.0	11	47.0
4	8.75	8	35.75	12	48.75

Number of calves = 12
Average Zinc Sulphate Turbidity = 25.8
Relative Distribution

<10	<20	<30	<40	<50	<60	<70
33.3%	8.3%	16.7%	25%	16.7%	-	-

August, 1964

Calf No.	Immune Globulin (ZST Units)	Calf No.	Immune Globulin (ZST Units)	Calf No.	Immune Globulin (ZST Units)
1	3.5	8	34.5	15	48
2	8.0	9	38.0	16	49
3	15.5	10	38.5	17	53
4	18.0	11	41.5	18	57.5
5	27.0	12	43.5	19	58
6	31.0	13	46	20	64
7	32.5	14	47		

Number of calves = 20
Average Zinc Sulphate Turbidity = 35.7
Relative Distribution

<10	<20	<30	<40	<50	<60	<70
10%	10%	5%	25%	30%	15%	5%

September, 1964

Calf No.	Immune Globulin (ZST Units)	Calf No.	Immune Globulin (ZST Units)	Calf No.	Immune Globulin (ZST Units)
1	4.5	3	41	5	48.5
2	32.0	4	44	6	58.5

Number of calves = 6
Average Zinc Sulphate Turbidity = 38
Relative Distribution

<10	<20	<30	<40	<50	<60	<70
16.6%	0	0	16.6%	50.0%	16.6%	

Serum Immune Globulin Concentrations of Neonatal Market Calves

October, 1964

Calf No.	Immune Globulin (ZST Unit)	Calf No.	Immune Globulin (ZST Unit)	Calf No.	Immune Globulin (ZST Unit)
1	0	15	20.0	29	34.5
2	0.5	16	21.5	30	35.0
3	1.5	17	22.0	31	35.5
4	3.75	18	24.0	32	36.5
5	5.0	19	24.75	33	37.0
6	7.0	20	25.5	34	38.75
7	8.75	21	26.0	35	38.75
8	10	22	27.0	36	41
9	10.5	23	27.75	37	44
10	11.0	24	29.0	38	48
11	11.5	25	30.0	39	49.5
12	13.5	26	30.5	40	60.0
13	15.0	27	32.25		
14	16.5	28	33.0		
1	0	15	9.0	29	29.5
2	0	16	9.5	30	30.5
3	0.5	17	10.5	31	32.5
4	0.75	18	11.5	32	33.0
5	1.0	19	12.0	33	33
6	2.5	20	13.0	34	35.5
7	3.0	21	13.5	35	36.0
8	4.0	22	14.75	36	37.0
9	5.0	23	16.0	37	37.75
10	5.75	24	17.75	38	41.5
11	6.75	25	20.5	39	45
12	7.0	26	23.5	40	47.5
13	8.0	27	25.0		
14	8.75	28	26.0		
1	0.5	7	9.0	13	21.75
2	0.75	8	9.5	14	24.5
3	2.5	9	10.0	15	25.75
4	4.5	10	12.25	16	28.5
5	6.0	11	14.0	17	31.0
6	7.5	12	19.5		

Number of calves = 97
Average Zinc Sulphate Turbidity = 19.8
Relative Distribution

<10	<20	<30	<40	<50	<60	<70
32%	19.5%	19.5%	21%	7.0%	0%	1%

Serum Immune Globulin Concentrations of Neonatal Market Calves

November, 1964

Calf No.	Immune Globulin (ZST Units)	Calf No.	Immune Globulin (ZST Units)	Calf No.	Immune Globulin (ZST Units)
1	0.3	8	5.9	15	12.1
2	0.9	9	7.8	16	12.8
3	0.9	10	8.4	17	13.0
4	2.1	11	9.8	18	13.8
5	2.7	12	9.8	19	18.6
6	3.3	13	9.9	20	24.3
7	4.1	14	11.1		

Number of Calves = 20
Average Zinc Sulphate Turbidity = 8.5
Relative Distribution

<10	<20	<30	<40	<50	<60	<70
65%	30%	5%				

December, 1964

No Samples

January, 1965

12th January

Calf No.	Immune Globulin (ZST Units)	Calf No.	Immune Globulin (ZST Units)	Calf No.	Immune Globulin (ZST Units)
1	0.1	8	2.9	15	8.3
2	0.2	9	3.1	16	9.0
3	0.6	10	3.3	17	9.0
4	0.6	11	3.7	18	9.3
5	1.3	12	4.7	19	15.0
6	1.9	13	5.7	20	17.3
7	2.1	14	6.6		

Serum Immune Globulin Concentrations of Neonatal Market Calves

January, 1965 (continued)

19th January

Calf No.	Immune Globulin (ZST Units)	Calf No.	Immune Globulin (ZST Units)	Calf No.	Immune Globulin (ZST Units)
1	0.3	8	2.6	15	8.8
2	0.8	9	4.0	16	9.5
3	0.9	10	5.0	17	9.7
4	1.3	11	5.8	18	12.9
5	1.7	12	7.6	19	17.3
6	1.8	13	7.9	20	18.2
7	2.3	14	8.1		

Number of Calves = 40
Average Zinc Sulphate Turbidity = 5.8
Relative Distribution

<10	<20	<30	<40	<50	<60	<70
87.5%	12.5%					

February, 1965

2nd February

Calf No.	Immune Globulin (ZST Units)	Calf No.	Immune Globulin (ZST Units)	Calf No.	Immune Globulin (ZST Units)
1	0.0	8	2.1	15	5.3
2	0.0	9	2.9	16	5.3
3	0.1	10	3.4	17	5.5
4	0.9	11	3.8	18	7.0
5	1.0	12	4.3	19	16.3
6	1.2	13	4.6	20	17.1
7	1.2	14	4.6		

Number of Calves = 20
Average Zinc Sulphate Turbidity = 4.3
Relative Distribution

<10	<20	<30	<40	<50	<60	<70
90%	10%					

Serum Immune Globulin Concentrations of Neonatal Market Calves

March, 1965

23rd March

Calf No.	Immune Globulin (ZST Units)	Calf No.	Immune Globulin (ZST Units)	Calf No.	Immune Globulin (ZST Units)
1	0.0	8	2.2	15	9.7
2	0.0	9	4.0	16	11.0
3	0.8	10	4.1	17	11.3
4	0.9	11	6.0	18	12.6
5	1.5	12	6.4	19	14.6
6	1.6	13	6.5	20	21.2
7	1.7	14	9.3		

Number of Calves = 20
Average Zinc Sulphate Turbidity = 6.2
Relative Distribution

<10	<20	<30	<40	<50	<16	<70
75%	20%	5%				

April, 1965

6th April

Calf No.	Immune Globulin (ZST Units)	Calf No.	Immune Globulin (ZST Units)	Calf No.	Immune Globulin (ZST Units)
1	0.0	8	3.8	15	10.4
2	0.4	9	4.1	16	12.1
3	1.0	10	4.4	17	13.3
4	1.1	11	4.4	18	15.4
5	1.8	12	6.2	19	17.1
6	2.6	13	10.1	20	17.9
7	3.6	14	10.2		

19th April

1	0.0	8	5.5	15	14.5
2	0.5	9	7.0	16	16.0
3	1.5	10	8.5	17	26.0
4	2.0	11	10.5	18	27.0
5	3.0	12	11.5	19	32.0
6	3.5	13	13.5	20	33.0
7	5.5	14	14.0		

Serum Immune Globulin Concentrations of Neonatal Market Calves

April, 1965 (continued)

26th April

Calf No.	Immune Globulin (ZST Units)	Calf No.	Immune Globulin (ZST Units)	Calf No.	Immune Globulin (ZST Units)
1	0.0	8	2.9	15	10.0
2	0.0	9	3.0	16	10.3
3	0.2	10	3.9	17	11.1
4	0.4	11	4.8	18	17.8
5	0.9	12	5.6	19	23.2
6	2.0	13	7.3		
7	2.6	14	7.5		

Number of Calves = 59
Average Zinc Sulphate Turbidity = 8.2
Relative Distribution

<10	<20	<30	<40	<50	<60	<70
61%	30.5%	5%	3.5%			

May, 1965

11th May

Calf No.	Immune Globulin (ZST Units)	Calf No.	Immune Globulin (ZST Units)	Calf No.	Immune Globulin (ZST Units)
1	0.3	13	4.6	25	12.3
2	0.4	14	4.9	26	14.4
3	0.6	15	5.1	27	14.8
4	0.9	16	5.1	28	15.3
5	1.6	17	5.6	29	15.6
6	1.6	18	6.1	30	20.9
7	1.7	19	6.1	31	25.4
8	1.8	20	6.6	32	32.5
9	1.9	21	7.4	33	34.3
10	2.1	22	8.2	34	34.8
11	2.2	23	8.9		
12	4.0	24	11.5		

Serum Immune Globulin Concentrations of Neonatal Market Calves

May, 1965 (continued)

18th May

Calf No.	Immune Globulin (ZST Units)	Calf No.	Immune Globulin (ZST Units)	Calf No.	Immune Globulin (ZST Units)
1	0	16	13.5	31	27.0
2	0.5	17	14.2	32	30.5
3	0.7	18	16.0	33	31.0
4	0.8	19	16.2	34	31.5
5	1.5	20	16.4	35	32.4
6	3.8	21	17.0	36	34.2
7	3.7	22	17.4	37	35.5
8	5.0	23	18.5	38	36.0
9	5.75	24	18.7	39	40.0
10	8.5	25	19.4	40	42.0
11	8.5	26	19.8	41	44.0
12	9.0	27	20.5	42	48.0
13	9.5	28	21.6	43	52.0
14	9.7	29	24.2		
15	11.8	30	26.4		

25th May

1	0.3	15	7.0	29	24.3
2	0.3	16	7.3	30	24.5
3	0.7	17	8.4	31	25.0
4	0.9	18	9.4	32	25.0
5	1.5	19	12.4	33	25.2
6	1.6	20	13.8	34	25.2
7	1.6	21	15.7	35	27.7
8	2.9	22	17.0	36	29.2
9	3.6	23	18.0	37	30.5
10	4.8	24	20.0	38	31.0
11	5.0	25	20.2	39	33.7
12	5.0	26	21.5	40	34.9
13	6.0	27	22.3		
14	6.9	28	22.8		

Number of calves = 117
Average Zinc Sulphate Turbidity = 15
Relative Distribution

<10	<20	<30	<40	<50	<60
47%	19.7%	17%	12%	3.5%	0.8%

Serum Immune Globulin Concentrations of Neonatal Market Calves

June, 1965

6th June

Calf No.	Immune Globulin (ZST Units)	Calf No.	Immune Globulin (ZST Units)	Calf No.	Immune Globulin (ZST Units)
1	1.0	15	9.5	29	34.0
2	3.0	16	13.0	30	35.5
3	4.0	17	13.5	31	36.0
4	4.0	18	16.5	32	36.0
5	4.5	19	17.5	33	37.0
6	4.5	20	20.5	34	39.0
7	5.0	21	24.0	35	40.5
8	5.0	22	25.0	36	42.0
9	6.0	23	27.0	37	43.0
10	6.5	24	28.0	38	49.0
11	7.0	25	28.5	39	51.0
12	7.0	26	29.0	40	51.0
13	8.0	27	29.5		
14	9.0	28	32.5		

22nd June

1	2.0	12	23.5	23	37.5
2	4.5	13	24.0	24	40.0
3	5.0	14	24.5	25	41.0
4	5.0	15	25.5	26	41.5
5	5.5	16	27.0	27	42.5
6	7.0	17	29.0	28	44.0
7	9.5	18	31.0	29	44.5
8	17.0	19	32.0	30	45.0
9	18.0	20	33.5	31	50.0
10	21.5	21	34.0	32	52.0
11	22.0	22	36.0		

Number of Calves = 72
Average Zinc Sulphate Turbidity = 24.4
Relative Distribution

<10	<20	<30	<40	<50	<60	<70
37.5%	10.0%	20.0%	17.5%	10.0%	5.0%	

Serum Immune Globulin Concentrations of Neonatal Market Calves

July, 1965

No Samples

August, 1965

9th August

Calf No.	Immune Globulin (ZST Units)	Calf No.	Immune Globulin (ZST Units)	Calf No.	Immune Globulin (ZST Units)
1	2.8	11	16.6	21	29.8
2	6.3	12	17.5	22	29.8
3	7.5	13	17.7	23	30.8
4	8.9	14	18.5	24	32.1
5	10.3	15	23.1	25	32.5
6	10.8	16	23.5	26	36.6
7	11.1	17	25.0	27	41.8
8	12.1	18	27.2	28	44.1
9	12.8	19	28.5	29	44.8
10	14.9	20	28.7	30	54.8

Number of Calves = 30
Average Zinc Sulphate Turbidity = 23.4
Relative Distribution

<10	<20	<30	<40	<50	<60
13.3%	33.3%	26.7%	13.3%	10%	3.3%

Serum Immune Globulin Concentrations of Neonatal Market Calves

September, 1965

7th September

Calf No.	Immune Globulin (ZST Units)	Calf No.	Immune Globulin (ZST Units)	Calf No.	Immune Globulin (ZST Units)
1	0.8	10	23.0	19	34.1
2	2.1	11	23.4	20	34.8
3	3.0	12	23.5	21	36.0
4	5.5	13	26.0	22	39.3
5	6.9	14	27.2	23	40.0
6	8.2	15	27.5	24	43.3
7	8.2	16	28.7	25	48.9
8	11.3	17	30.7		
9	14.2	18	33.2		

22nd September

1	0.7	17	9.3	33	27.1
2	0.9	18	9.4	34	27.7
3	1.0	19	11.9	35	28.0
4	1.2	20	12.3	36	29.8
5	1.9	21	15.6	37	30.1
6	2.4	22	16.0	38	33.2
7	2.7	23	16.3	39	34.2
8	3.4	24	17.7	40	34.4
9	3.5	25	20.6	41	39.6
10	3.9	26	21.2	42	40.7
11	3.9	27	22.5	43	41.0
12	4.6	28	23.3	44	41.8
13	5.3	29	24.1	48	42.5
14	5.7	30	24.7	46	49.2
15	7.5	31	25.2	47	50.5
16	7.9	32	25.6		

Number of Calves = 72
Average Zinc Sulphate Turbidity = 20.6
Relative Distribution

<10	<20	<30	<40	<50	<60
34.7%	11.1%	26.4%	15.3%	11.1%	1.4%

Serum Immune Globulin Concentrations of Neonatal Market Calves

October, 1965

18th October

Calf No.	Immune Globulin (ZST Units)	Calf No.	Immune Globulin (ZST Units)	Calf No.	Immune Globulin (ZST Units)
1	1.0	8	16.5	15	36.5
2	3.5	9	17.5	16	38.0
3	4.0	10	20.5	17	39.5
4	6.0	11	21.5	18	42.0
5	8.0	12	28.5	19	47.5
6	9.0	13	30.0	20	51.5
7	15.0	14	35.0		

25th October

1	0.0	15	8.5	29	31.5
2	0.5	16	9.5	30	34.0
3	1.5	17	13.0	31	35.0
4	3.0	18	13.5	32	36.5
5	3.5	19	15.5	33	37.5
6	4.5	20	18.0	34	38.5
7	4.5	21	19.0	35	40.0
8	4.5	22	19.5	36	40.0
9	5.0	23	22.5	37	42.0
10	5.0	24	23.5	38	42.0
11	5.5	25	29.0	39	42.5
12	5.5	26	29.5	40	44.0
13	7.0	27	30.0		
14	7.5	28	30.0		

Number of Calves = 60
Average Zinc Sulphate Turbidity = 21.2
Relative Distribution

<10	<20	<30	<40	<50	<60
36.7%	15%	11.6%	21.7%	13.3%	1.7%

Serum Immune Globulin Concentrations of Neonatal Market Calves

November, 1965

2nd November

Calf	Immune Globulin	Calf	Immune Globulin	Calf	Immune Globulin
1	0.0	11	9.0	21	16.75
2	1.3	12	10.0	22	18.0
3	2.5	13	11.5	23	20.5
4	4.0	14	12.0	24	20.75
5	4.0	15	12.75	25	21.0
6	4.5	16	14.5	26	22.75
7	6.5	17	15.25	27	26.0
8	7.0	18	16.25	28	30.0
9	8.0	19	16.5	29	50.25
10	9.0	20	16.5		

8th November

1	1.0	8	7.5	15	19.0
2	3.0	9	8.5	16	29.0
3	3.5	10	9.0	17	30.0
4	4.0	11	11.5	18	33.5
5	4.5	12	15.5	19	34.0
6	4.5	13	16.0	20	40.5
7	5.0	14	18.0		

22nd November

1	0.25	11	3.25	21	13.0
2	0.25	12	3.25	22	13.8
3	0.5	13	4.25	23	14.0
4	1.0	14	4.25	24	15.25
5	1.0	15	4.25	25	16.0
6	1.5	16	8.5	26	18.8
7	1.5	17	8.5	27	20.8
8	2.2	18	9.75	28	27.0
9	2.3	19	12.0		
10	2.5	20	12.3		

Number of Calves = 77
Average Zinc Sulphate Turbidity = 12.1
Relative Distribution (%)

<10	<20	<30	<40	<50	<60
49.3%	32.5%	10.4%	5.2%	1.3%	1.3%

Serum Immune Globulin Concentrations of Neonatal Market Calves

December, 1965

6th December

Calf No.	Immune Globulin (ZST Units)	Calf No.	Immune Globulin (ZST Units)	Calf No.	Immune Globulin (ZST Units)
1	0.0	14	4.0	27	11.5
2	0.0	15	4.5	28	12.5
3	0.0	16	4.5	29	15.0
4	0.0	17	4.5	30	15.0
5	0.5	18	5.0	31	15.5
6	0.5	19	5.5	32	17.0
7	1.0	20	6.0	33	17.5
8	1.0	21	6.5	34	18.5
9	1.5	22	8.0	35	20.0
10	1.5	23	8.5	36	20.5
11	2.5	24	9.0	37	23.0
12	3.0	25	9.0	38	27.0
13	3.0	26	11.0	39	37.0

Number of Calves = 39
Average Zinc Sulphate Turbidity = 9.0
Relative Distribution (%)

≤10	≤20	≤30	≤40	<50	<60
66.6%	20.5%	10.3%	2.6%		

January, 1966

7th January

Calf No.	Immune Globulin (ZST Units)	Calf No.	Immune Globulin (ZST Units)	Calf No.	Immune Globulin (ZST Units)
1	0.0	15	2.65	29	6.75
2	0.0	16	3.0	30	9.25
3	0.25	17	3.0	31	9.5
4	0.25	18	3.0	32	10.25
5	0.25	19	3.75	33	11.0
6	0.5	20	3.75	34	11.75
7	0.7	21	4.0	35	12.0
8	1.75	22	4.5	36	12.5
9	1.75	23	5.5	37	14.75
10	2.0	24	6.0	38	19.0
11	2.5	25	6.25	39	20.25
12	2.5	26	6.5	40	32.0
13	2.5	27	6.75		
14	2.5	28	6.75		

Number of Calves = 40
Average Zinc Sulphate Turbidity = 6.3
Relative Distribution (%)

≤10	≤20	≤30	≤40	<50	<60
77.5%	17.5%	2.5%	2.5%		

Serum Immune Globulin Concentrations of Neonatal Market Calves

February, 1966

1st February

Calf No.	Immune Globulin (ZST Units)	Calf No.	Immune Globulin (ZST Units)	Calf No.	Immune Globulin (ZST Units)
1	0.25	4	2.0	7	3.75
2	1.25	5	2.5	8	12.5
3	1.5	6	2.75	9	13.5

8th February

1	0.5	15	4.25	29	9.75
2	0.5	16	4.5	30	10.0
3	1.0	17	5.0	31	11.0
4	2.0	18	5.0	32	11.0
5	2.0	19	6.0	33	12.0
6	2.0	20	6.0	34	12.5
7	2.0	21	6.5	35	13.0
8	2.25	22	7.25	36	13.0
9	2.25	23	7.5	37	14.0
10	2.5	24	7.5	38	19.0
11	2.75	25	8.25	39	22.0
12	3.5	26	8.25	40	39.0
13	3.5	27	8.5		
14	4.0	28	9.0		

15th February

1	0.0	10	3.0	19	8.0
2	0.0	11	3.0	20	8.75
3	0.25	12	3.75	21	12.5
4	0.75	13	4.00	22	14.0
5	1.25	14	4.75	23	16.75
6	2.0	15	5.25	24	25.0
7	2.0	16	6.0	25	44.5
8	2.2	17	7.0		
9	2.5	18	8.0		

Number of Calves = 74
Average Zinc Sulphate Turbidity = 7.2
Relative Distribution (%)

<10	<20	<30	<40	<50	<60	<70
75.7%	19.0%	2.7%	1.3%	1.3%	-	

Serum Immune Globulin Concentrations of Neonatal Market Calves

March, 1966

21st March

Calf No.	Immune Globulin (ZST Units)	Calf No.	Immune Globulin (ZST Units)	Calf No.	Immune Globulin (ZST Units)
1	1.0	9	1.75	17	5.0
2	1.25	10	2.0	18	5.0
3	1.25	11	2.25	19	6.5
4	1.25	12	2.75	20	10.25
5	1.5	13	3.75	21	10.25
6	1.75	14	3.75	22	10.5
7	1.75	15	4.0	23	12.5
8	1.75	16	4.75	24	16.25

Number of Calves = 24
Average Zinc Sulphate Turbidity = 4.7
Relative Distribution (%)

<10	<20	<30	<40	<50	<60
76%	20%	4%	-	-	-

April, 1966

18th April

Calf No.	Immune Globulin (ZST Units)	Calf No.	Immune Globulin (ZST Units)	Calf No.	Immune Globulin (ZST Units)
1	0.25	8	5.5	15	12.5
2	0.25	9	6.3	16	15.8
3	1.8	10	6.5	17	17.5
4	3.5	11	7.0	18	18.3
5	3.75	12	7.5	19	25.3
6	3.8	13	8.3	20	30.5
7	5.0	14	10.8		

26th April

1	1.5	8	4.5	15	15.0
2	1.5	9	5.0	16	16.0
3	2.0	10	6.0	17	20.5
4	2.0	11	6.5	18	21.5
5	2.0	12	7.5	19	23.5
6	3.5	13	7.5	20	37.5
7	4.0	14	14.5		

Number of Calves = 40
Average Zinc Sulphate Turbidity = 9.8
Relative Distribution (%)

<10	<20	<30	<40	<50	<60
65%	20%	10%	5%	-	-

Serum Immune Globulin Concentrations of Neonatal Market Calves

May, 1966

2nd May

Calf No.	Immune Globulin (ZST Units)	Calf No.	Immune Globulin (ZST Units)	Calf No.	Immune Globulin (ZST Units)
1	0.0	11	3.5	21	7.0
2	1.0	12	4.0	22	9.0
3	1.0	13	4.0	23	9.5
4	1.5	14	4.5	24	10.5
5	2.0	15	4.5	25	12.5
6	2.5	16	4.5	26	15.0
7	2.5	17	6.0	27	18.5
8	3.0	18	6.5	28	38.0
9	3.0	19	7.0	29	40.5
10	3.5	20	7.0	30	41.5

24th May

1	1.0	9	14.25	17	24.5
2	4.5	10	14.75	18	27.5
3	6.5	11	15.5	19	27.75
4	7.25	12	19.25	20	35.5
5	7.5	13	20.5	21	36.0
6	9.0	14	20.75	22	37.25
7	9.75	15	23.5	23	41.5
8	12.0	16	23.75		

31st May

1	0.0	8	10.0	15	19.5
2	0.25	9	10.0	16	25.25
3	1.75	10	10.25	17	30.25
4	2.0	11	11.25	18	35.25
5	2.75	12	11.25	19	40.5
6	4.25	13	18.75	20	41.25
7	9.75	14	18.75		

Number of Calves = 73
Average Zinc Sulphate Turbidity = 13.7
Relative Distribution

<10	<20	<30	<40	<50	<60
50.7%	23.3%	10.9%	8.2%	6.8%	-

Serum Immune Globulin Concentrations of Neonatal Market Calves

June, 1966

17th June

Calf	Immune Globulin	Calf	Immune Globulin	Calf	Immune Globulin
1	1.0	5	21.75	9	31.75
2	18.75	6	25.5	10	34.0
3	19.5	7	27.25		
4	21.75	8	29.0		

20th June

1	7.75	5	22.5	9	35.25
2	15.75	6	29.25	10	36.0
3	19.5	7	29.75	11	39.25
4	20.75	8	34.5	12	39.5

Number of Calves = 22
Average Zinc Sulphate Turbidity = 25.4
Relative Distribution

<10	<20	<30	<40	<50	<60
9.0%	15.2%	41.0%	31.8%	-	-

APPENDIX III

APPENDIX 3.1

Changes in the Heart Rate, Blood Pressure and Blood pH Observed During the Infusion of Isotonic Hydrochloric Acid

Calf No. 1

Time (mins.)	Heart Rate (per min.)	Blood Pressure (mm Hg)	pH
-5	185	140/130	7.44
0	-	-	-
17	170	145/134	-
38	150	142/126	-
53	165	142/126	6.84
60	160	150/135	-
75	135	196/164	<6.6
84	80	175/150	-
87	55	145/125	-
88	24	95/65	-

Calf No. 2

Time (mins.)	Heart Rate (per min.)	Blood Pressure (mm Hg)	pH
-12	220	162/140	7.35
0	220	156/132	-
35	150	136/120	7.2
69	175	142/126	7.12
103	185	158/140	7.12
136	165	150/132	7.07
166	165	160/138	6.95
193	145	184/154	6.84
203	120	190/144	6.84
209	105	198/150	<6.6
214	85	178/142	-
215	56	159/135	-
217	20	88/62	-
220	-	-	-

Calf No. 3

Time (mins)	Heart Rate (per min)	Blood Pressure (mm Hg)	pH
-10	140	120/90	7.4
0	130	105/78	7.46
20	125	122/94	7.29
45	140	104/80	7.25
68	135	110/86	7.22
80	190	84/66	7.21
92	160	142/122	7.08
103	160	104/82	6.96
115	150	168/134	6.87
128	100	196/145	6.78
135	59	180/126	-

Calf No. 4

Time (mins)	Heart Rate (per min)	Blood Pressure (mm Hg)	pH
-10	176	153/132	7.34
0	157	154/132	-
27	171	164/138	-
47	193	168/148	-
75	176	148/130	-
99	187	156/136	-
105	150	184/152	-
114	140	200/164	-
121	70	122/106	-
125	47	102/86	-
129	61	124/100	-
134	78	200/150	-
141	62	142/115	-
142	46	116/88	-
143	24	114/84	-

Calf No. 5

Time (mins)	Heart Rate (per min)	Blood Pressure (mm Hg)	pH
-10	155	160/130	7.38
0	-	-	-
8	160	166/134	7.24
19	140	144/120	7.01
26	135	138/118	-
63	110	132/112	6.98
78	120	124/110	6.95
93	160	170/136	6.80
106	150	180/144	6.65
124	135	186/150	<6.6
128	135	198/160	-
141	115	200/160	-
161	82	184/154	-
164	75	174/148	-
166	59	146/120	-
167	36	122/100	-

Calf No. 6

Time (mins)	Heart Rate (per min)	Blood Pressure (mm Hg)	pH
0	145	102/88	-
22	145	112/98	-
45	145	114/98	-
72	135	124/104	-
100	130	154/122	-
115	110	170/130	-
133	70	148/114	-
135	62	144/110	-
148	50	142/106	-
150	44	134/96	-
157	18	80/30	-
160	10	45/10	

Calf No. 7

Time (mins)	Heart Rate (per min)	Blood Pressure (mm Hg)	pH
-10	-	-	7.42
0	145	100/80	-
16	120	106/90	-
38	100	94/74	6.91
44	100	100/78	-
68	105	100/76	6.84
91	83	108/88	-
96	67	116/92	6.63

Calf No. 8

Time (mins)	Heart Rate (per min)	Blood Pressure (mm Hg	pH
-10	150	112/96	7.42
0	145	112/96	7.42
8	125	130/110	7.18
17	120	144/118	7.05
30	100	194/140	-
43	100	194/140	-
47	105	175/136	-
51	105	175/135	6.68
57	110	193/140	-
63	105	194/144	-
66	100	144/125	-
74	75	59/48	-
77	30	52/16	-

Calf No. 9

Time (mins)	Heart Rate (per min)	Blood Pressure (mm Hg)	pH
-10	240	142/120	7.38
0	240	142/120	7.35
45	222	140/120	7.30
81	205	128/110	7.06
105	200	112/92	7.04
135	175	124/104	6.97
153	93	160/125	6.80
164	43	170/125	6.70
170	40	110/75	6.60
183	33	78/40	-
185	-	-	-

Calf No. 10

Time (mins)	Heart Rate (per min)	Blood Pressure (mm Hg)	pH
-10	130	148/118	-
0	115	120/100	7.38
25	110	106/90	-
30	110	104/84	7.13
60	120	96/76	7.0
85	115	102/80	6.92
100	105	112/90	6.88
105	105	108/86	6.79
113	100	145/118	-
123	95	162/130	-
125	95	158/128	-
126	78	160/130	6.61
130	78	186/146	<6.6
135	35	200/135	-
138	33	180/130	-
140	30	116/94	-
141	18	88/54	-
142	12	65/20	-

APPENDIX IV

Immuno Globulin Concentrations (ZST Units) of Calves Fed at Different Times after Birth

0-6 Hrs.	6-12 Hrs.	12-18 Hrs.
6.0	0.5	0.1
6.25	3.2	1.0
9.5	4.3	2.2
10.5	8.0	3.0
11.5	8.5	3.1
13.5	10.7	4.8
15.75	13.4	5.1
17.25	15.2	6.0
17.75	18.3	6.3
18.5	20.9	7.5
18.75	23.0	7.6
19.25		13.1
20.25		13.6
20.5		20.0
21.0		27.2
23.0		
25.75		
29.0		
30.0		
30.0		
31.5		
37.25		
39.5		
Mean $\pm$ S.E.:		
20 $\pm$ 1.84	11.45 $\pm$ 2.23	8.0 $\pm$ 1.87