BODY FLUIDS AND DIARRHOEA IN NEONATAL CALVES

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Preface

Diarrhoea is a very common condition affecting the newborn of many mammals and is a frequent cause of death. The investigation of some aspects of this condition in calves is the basis of the studies presented herein.

These studies evolved from problems encountered when rearing large numbers of neonatal calves in the University of Glasgow Veterinary Hospital. Many of these calves became affected with diarrhoea and some died. The clinical appearance of these calves simulated that frequently observed in calves of a similar age on farms in the west of Scotland.

It was important to rear as many calves as possible and a part of the studies described herein was concerned with methods of preventing diarrhoea by methods of management and the use of antibiotics and a sulphonamide. Clinical observations were made on these calves and it appeared that diarrhoea caused changes in the body fluids.

Comparatively little is known about the volume and composition of the body fluids in healthy neonatal calves or of the effects of diarrhoea on the body fluids.

In order to assess the effects of diarrhoea on the body fluids of the calf, it was necessary, in the first instance, to define some parameters of the volume and composition of the body fluids of healthy neonatal calves. Having determined these parameters in healthy calves, a study was then made of the effects of diarrhoea on some of these **patameters**.

The studies of diarrhoea and body fluids in neonatal calves are presented in three parts. The first part describes the clinical appearance of the condition observed in the calves and the efficacy of management,

antibiotics and a sulphonamide in mitigating the incidence and severity of the condition. The second part describes the determination of the volumes of the principal body fluid compartments in healthy calves. The third part is concerned with the composition of blood and plasma in neonatal calves when healthy and when affected with diarrhoea. Some determinations were also made of the effects of diarrhoea on haematocrit, plasma volume and total body water.

In order to relate the studies presented herein to those of other workers, it was necessary to ascertain the extent of previous studies and the relevant literature has been reviewed and is presented separately with each of the three parts of this study.

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PART I

CLINICAL OBSERVATIONS ON DIARRHOEA IN NEWBORN CALVES AND THE EFFECTS OF SOME ANTIBIOTICS, A SULPONAMIDE AND METHODS OF FEEDING ON THE INCIDENCE AND SEVERITY OF DIARRHOEA AND MORTALITY RATE

CALF DIARRHOEA

Introduction and literature review

Some attempts have been made to assess the mortality rate and the causes of the death of young calves in the United Kingdom.

Jordan (1933) and Smith (1934) investigated the calf mortality rate and the cause of death on some farms in Ayrshire. These investigations, like many subsequent ones, were made on the female calf population only, owing to the practical difficulties involved in recording the male calf population since male calves were frequently sold and left the farms at an early age.

Jordan found that, of 784 female calves born, 169 or 22% died shortly after birth. Smith found the average mortality rate of newborn female calves on 52 farms was 20%.

Both Jordan and Smith found that the calf mortality rate was highest during the early spring months of the year. In these investigations the causes of death were not always recorded but diarrhoea occurred in a large proportion of calves.

In 1933-1937, Lovell and Bradford Hill (1940) investigated the female calf mortality rate on 335 dairy farms in England and Wales and on 47 farms in Scotland. The farms in England and Wales were selected as being representative of the cattle population in different regions. The 47 farms investigated in Scotland included 34 farms in Ayrshire which had been previously surveyed by Jordan (1933) and Smith (1934).

On the English and Welsh farms, 25,000 calves were born. One thousand, two hundred and fifty one of these calves, or approximately 5%, died before they were 6 months old. In England and Wales, one herd in five

had a mortality rate greater than 10% and in one herd in fourteen the mortality rate was greater than 16%. In the Scottish herds, the average mortality rate was over 11% and in one third of the herds the mortality rate was over 16%.

Lovell and Bradford Hill (1940) found that half the deaths occurred within the first week of life, two thirds within the first fortnight and three quarters within the first month of life. They also observed that the mortality rate was highest in the early spring months of the year. The causes of death were not always recorded in this survey but diarrhoea was listed as a frequent cause of death.

Hector and Rowat (1948) found the mortality rate in 886 calves, born between the months November and April on some farms in Dumfriesshire, was 6.8% during the first 60 days of life. They observed that the mortality rate was highest during the first week of life and that the overall mortality rate was higher in February, March and April. The cause of death of these calves was not always determined but diarrhoea was frequently observed.

Withers (1952 and 1953) carried out a survey on 35 dairy herds and 9 beef herds in England, Wales and southern Scotland. On these farms, 4,380 calves were born and 322 or 7.4% of them died before they were 6 months old. In England and Wales the average mortality rate was 6% but in Scotland it was over 11%. Like the previous investigators, Withers found that the mortality rate was highest in the early spring months of the year. He also observed that approximately half the calves that died did so within the first week of life, two thirds within the first fortnight and three quarters within the first month of life. The causes of death were investigated in more detail in this survey and it was recorded that the commonest single cause of deaths appeared to be infection with E coli (28% of the deaths). This

organism was associated with a condition characterised by either a septicaemia or diarrhoea, the former occurring most frequently in the calf less than a week old and the latter in the slightly older calf. Diarrhoea, which was believed to be primarily nutritional in aetiology, was associated with the death of 5.8% of the calves.

Withers also concluded that about three quarters of the calves which became ill actually died. He refers to 115 calves which became ill and eventually recovered. Eighty-one of these calves were affected with diarrhoea and some calves were affected on more than one occasion. The majority of these calves were less than a month old when affected with diarrhoea.

An analysis of the results of post-mortem examinations of calves sent to Veterinary Investigation Centres from November 1959 to October 1961 was published in the Veterinary Record, Volume 74, 1964. It was recorded that septicaemia and gastro-enteritis were the most frequent causes of death in calves up to the age of one month. Approximately 63% of the calves examined were said to have died from these causes. This article does not define "gastro-enteritis" but it is presumably indicative of diarrhoea, although, as described later, diarrhoea in neonatal calves is not always characterised pathologically by gastro-enteritis. It is also questionnable if the diagnosis of an E. coli septicaemia made on the calves in this investigation was always a valid one. It has been shown by Briggs (1950) that E. coli rapidly invades the tissues shortly after death. Since this survey was made primarily on dead calves submitted to Veterinary Investigation Centres, it is possible that this diagnosis may be erroneous. A similar criticism can be made of the diagnosis of septicaemia as a cause of death in calves in the survey carried out by Withers (1952 and 1953) since his

information was also based on post-mortem examinations carried out at Veterinary Investigation Centres.

From the results obtained in these surveys, it appears that the mortality rate of calves during the first months of life is probably between 5 and 10% for the country as a whole. The mortality rate is highest within the first month of life and diarrhoea is apparently a frequent cause of death. Diarrhoea also appears to affect a large number of calves which do not always die from the condition. The mortality rate appears to vary during the year, the highest mortality rate being in the early spring months of the year. The mortality rate of calves also appears to be greater in the northern regions than the southern regions of the United Kingdom.

The aetiology of diarrhoea in neonatal calves has not been clearly defined and it appears that several independent factors may be implicated. The activity of infectious agents, particularly Escherichia coli, many aspects of the husbandry of the preparturient cow and the neonatal calf, and also variations in the individual calf's susceptibility to the condition, have been considered in the aetiology. In some instances it may be that one particular aetiological factor is responsible for the condition but in others it is possible that several of the factors may be involved at the same time.

The clinical signs of the disease in neonatal calves associated with E. coli infection, also known as colibacillosis, and that associated with faulty feeding, or so-called "Dietetic Diarrhoea", were described by Blood and Henderson (1963) as follows.

Peracute colibacillosis: Affected newborn animals collapse and die in as short a time as 2 to 6 hours. Outstanding clinical signs include coma, sub-normal temperature, a cold clammy skin, pale mucosae, wetness around the mouth, collapse of the superficial veins, slowness and irregularity

of the heart, mild convulsive movements and periodic apnoea. No diarrhoea is evident.

Septicaemic colibacillosis: This form of the disease is most common during the first 4 days of life. The illness is acute, varying in duration from 24 - 96 hours. There are no diagnostic clinical signs. Affected animals are depressed and weak, anorexia is complete, there is a marked increase in heart rate and, although the temperature may be high initially, it falls rapidly to sub-normal levels when diarrhoea and sometimes dysentery appears. Post septicaemic localisation may cause arthritis, pneumonia or meningitis.

Enteric colibacillosis: This form of the disease is most common in calves during the first 3 weeks of life, and particularly during the first week. The faeces are watery or pasty and usually chalk white to yellow in colour, and occasionally streaked with blood. Defaecation is frequent and the faeces have an offensive rancid smell. There is usually a systemic reaction with a temperature up to 105°F and an increase in pulse rate. The animal ceases to drink and is dull and listless and rapidly becomes dehydrated. There may be abdominal pain on palpation, sometimes tenesmus is evident and the back may be arched. Without treatment, death usually occurs in 3 to 5 days.

Blood and Henderson (1963) describe the post-mortem findings in these calves as follows. In cases of peracute and septicaemic colibacillosis, there may be no gross lesions and diagnosis may depend on the isolation of bacteria from the abdominal viscera and, in the latter, from the heart blood. In less severe cases, there may be subserous and submucosal petechial haemorrhages and a degree of enteritis and gastritis is usually present. Occasionally, fibrinous exudates may be present in the joints and serous

cavities. There may be pneumonia and meningitis. In enteric colibacillosis, gastro-enteritis is the only visible lesion.

Blood and Henderson (1963) describe diarrhoea of dietetic origin in calves as follows. The condition is manifest by the passage of soft fluid faeces varying in colour from white to yellow green, depending on the composition of the diet. Although affected calves lose weight rapidly and have a gaunt appearance, the appetite is good and the demeanour, pulse rate and temperature normal. If the condition is prolonged, dehydration may occur. They also state that dietetic diarrhoea may be complicated by bacterial infection.

In the British Veterinary Association handbook, 'The Husbandry and Diseases of Calves', a distinct differentiation is not made between the clinical signs of diarrhoea associated with bacterial and dietetic causes. It is also stated that, in some cases, post-mortem examination of calves which die from diarrhoea show no significant findings and there are no characteristic lesions in this disease. It is recorded that signs associated with shock or septicaemia may be found in some calves and that pneumonia, splenic enlargement, peritonitis and petechial haemorrhages may be found in some calves. Gastro-enteritis is not mentioned as a post-mortem finding.

The activity of infectious agents in the aetiology of neonatal calf diarrhoea has been widely investigated. Attention has been principally directed at the role of bacteria, and, in particular, Escherichia coli, in the disease. To a lesser extent, the importance of virus like micro-organisms has been studied.

E. coli was first implicated in the aetiology of diarrhoea by Jensen (1893) in Denmark. This bacterium has also been associated with disease in the newborn of other species including man, horse, sheep and pig. In all these

species, E. coli infection may take the form of a septicaemia-toxaemia or of an intestinal disease characterised by diarrhoea - Blood and Henderson (1963).

The literature on the role of E. coli in calf diarrhoea was reviewed by Lovell (1955), Fey (1955) and Gay (1964).

From these reviews it is apparent that there is some confusion regarding the exact role and pathogenesis of E. coli in neonatal diarrhoea of calves and also of other newborn mammals. Blood and Henderson (1963) state that, until relatively recently, E. coli was not commonly accepted as a primary pathogen and its presence in many diseases was ascribed to its activity as a secondary invader after primary lesions had been established by other agents. They further state that it is now apparent that certain serotypes of E. coli can initiate disease but it is probable that the organism can fill both roles.

The reasons for some of the confusion regarding the role of E. coli and its pathogenicity are apparent from the studies referred to later. There are several possible explanations why the role of E. coli has not been clearly understood, including the fact that E. coli is ubiquitous in the environment of the calf and is normal inhabitant of the calf's intestine. Consequently, this organism is invariably isolated from calves affected with diarrhoea although the primary cause of the condition may be due to faulty management or feeding of the calf. In addition, many workers made bacteriological examinations of calves which had been dead for some time, and as Briggs (1950) has shown that E. coli rapidly invades the tissues of the dead animal, it is questionnable if the results obtained by such workers and described as septicaemic deaths are always valid. With the development of serological and 'phage techniques to identify specific strains of E. coli, it was shown that in some outbreaks of diarrhoea some strains appeared more pathogenic than others. It was frequently found, however, that such strains of E. coli which were

implicated as the cause of disease could be isolated also from healthy calves. Attempts to prove the pathogenicity of E. coli by satisfying Koch's Postulates have met with variable success as described later.

Christiansen (1917) attempted to classify E. coli isolated from calves by morphological, biochemical and serological techniques but found it impossible to distinguish between strains isolated from diarrhoeic and healthy calves. Lovell and Hughes (1935) and Lovel (1937) examined by serological methods strains of E. coli isolated from healthy and diarrhoeic calves and found the same strains occurred in both groups of calves. They also found that more than one strain of E. coli was often associated with diarrhoea. Wramby (1948) examined strains of E. coli from a large number of calves and he, too, found the same strains occurred in both healthy and diarrhoeic animals. Using the method of phagetyping, Smith (1960) investigated diarrhoea in 18 herds and also in experimental animals. The results obtained were variable and in 1960 he wrote, 'The marked similarity of the distribution of phage types in faeces before, during and after the diarrhoea period, the multiplicity of the types and the changes of dominant type during the diarrhoea period, considered together with the close similarity, quantitatively and qualitatively, between the E. coli population of the healthy and diarrhoeic calves, made one wonder what part E. coli was playing in the disease'. In view of his findings, he suggested that the investigation of the bacteriology of calf diarrhoea should be extended to include the whole bacterial flora of the calf's alimentary tract.

He has shown that E. coli, Cl. Welchi and streptococci were first bacteria to appear in the faeces of healthy calves after birth. Within a few days, bacteroides and lactobacilli colonised the faeces in large numbers and were more numerous than the other bacteria. Smith found it was not unusual for the bacteroides to outnumber the E. coli by 100 to 1. The Cl. Welchi population

of the faeces was found to decline rapidly after the first few days of life and these organisms were present in only small numbers after the first or second week of life. The E. coli population of the faeces was found to decrease about a fortnight after birth and at the age of one month the E. coli population of the faeces might be about a million bacteria per gram of faeces compared with a population of 10,000 million bacteria per gram at the age of one week. Smith also carried out bacterial counts on the faeces of calves before, during and after they were affected with diarrhoea but was unable to demonstrate a constant difference in either the type or number of bacteria. Occasionally he noticed, like Van Pelt, Johnson and Plastridge (1953), that the E. coli population of the faeces decreased before the onset of diarrhoea.

In some instances, outbreaks of neonatal diarrhoea of calves have been related to the isolation of specific 'pathogenic' strains of E. coli. Such outbreaks were described by Glantz, Dunne, Heist and Hohanson (1959), Gay (1962) and Smith (1958).

Attempts to reproduce colibacillosis using strains of E. coli isolated from diarrhoeic calves have met with variable success. The disease has been reproduced in susceptible calves by Jensen (1913), Glantz et al. (1959), Schoenaers and Kaeckenbeeck (1958) and Gay (1962). In some of these and in other studies, the ability to reproduce the disease was apparently dependent on whether the experimental calves had been given colostrum. Other workers, such as Poels (cited by Jensen 1913), Smith and Orcutt (1925), McEwen (1950), Van Pelt et al. (1953), Williams, Hagan and Carpenter (1920), failed to reproduce the disease in susceptible calves.

It has also been suggested, on the basis of field observations, that particular bacterial strains may increase in virulence during an outbreak of disease in calves, Briggs (1951). Blood and Henderson (1963) also state that

DIARRHOEA AND BODY FLUIDS IN NEONATAL CALVES

SUMMARY

Diarrhoea is a very common clinical condition of neonatal calves. This study is concerned with an investigation of some aspects of this condition.

The study evolved from problems encountered when rearing large numbers of calves in the University Veterinary Hospital. Many of these calves became affected with diarrhoea and some died. This provided an opportunity to make a clinical study of calf diarrhoea and to investigate the efficacy of some methods of preventing diarrhoea. From the clinical studies it was concluded that diarrhoea had effects on the body fluids. In view of the known importance of these effects in infants affected with diarrhoea and the limited knowledge on this subject in diarrhoeic calves, it was decided to investigate these effects. In order to appreciate the effects of diarrhoea on the body fluids it was mandatory to know something about the volume and composition of the body fluids in healthy calves. As little was known of the volume and composition of the body fluids in healthy calves some parameters were determined prior to an investigation of the effects of diarrhoea.

The study is divided into three parts.

Part I.

Clinical observations on diarrhoea in newborn calves. The effects of some antibiotics, a sulphonamide and methods of feeding on the incidence and severity of diarrhoea and on mortality rate.

In the introduction present knowledge of the incidence, aetiology, clinical signs, pathogenesis, effects, therapy and prophylaxis of diarrhoea in neonatal calves has been reviewed. From this review it was apparent that although this subject has been extensively investigated by previous workers, there were many aspects which had not been convincingly elucidated.

Ten groups of calves were used in this study. The number of calves in each group ranged from 20 - 50. The total number of calves studied was 280.

From clinical studies on these calves it was apparent that diarrhoea was a clinical sign common to two recognised clinical conditions. These were designated as the 'acute' and 'chronic' conditions and simulated respectively the acute septicaemia colibacillosis and the chronic enteric colibacillosis described by previous workers. The chronic condition also simulated in many respects the dietetic diarrhoea of calves described by previous workers. An extensive bacteriological examination was not carried out in this investigation but an E.coli septicaemia was detected in calves dying of both conditions. It was considered that the pathogenesis of these two conditions differed. Calves affected with the acute condition appeared to die from the direct effects of the bacteria. Those dying from the chronic condition were considered to die from the effects of prolonged diarrhoea. The majority of calves were affected with the chronic condition. The most outstanding clinical signs in these animals apart from diarrhoea was loss of weight and emaciation. It was on the evidence of these and some other clinical signs that it was suspected that diarrhoeic calves had serious derrangements of the body fluids.

The aetiology of the diarrhoea in the experimental calves appeared to be associated with three factors, bacterial activity, diet and seasonal variations in the calves susceptibility.

A series of ten experiments was carried out to determine the effects of different prophylactic procedures in preventing diarrhoea. In each experiment a group of ten control calves was compared with one or more groups of ten calves which were subjected to a prophylactic procedure. It was considered that the method of interpreting the results of these experiments was most important in view of the varied aetiology of diarrhoea in calves and its pathogenesis. The results obtained in these experiments showed that antibiotics could effectively reduce the incidence and severity of diarrhoea in calves. They could also reduce the mortality rate. It was also shown that the effects of antibiotics on body weight appeared to be mediated through their effects in controlling diarrhoea.

Part II.

The volumes of the body fluid compartments in healthy calves

In the introduction, the physiology of the body fluids has been described and also the principles of measuring the volumes of the body fluid compartments. Previous studies on the body fluids in cattle have been reviewed.

The volumes of the principle body fluid compartments in healthy neonatal calves were determined by the single injection dilution method. Details of the solutes used, the number of calves studied and the results obtained are shown below.

	As % Body Weight	No. Measurements
Plasma volume (T.1824)	6.6 ± 0.9	67
Blood volume (T.1824)	11.0 ± 2.0	40
Extracellular fluid volume (Thiosulphate)	24.2 ± 2.6	10
Total Body Water (Urea)	73.6 ± 6.4	29

Part III.

The composition of blood and plasma in healthy and diarrhoeic calves.

The effects of diarrhoea on haematocrit, plasma volume and body water.

In the introduction previous studies on the effects of diarrhoea in calves have been reviewed.

The concentrations of some blood and plasma constituents were determined in 60 healthy calves many of which later became affected with diarrhoea. The effects of diarrhoea on plasma volume and haematocrit were investigated in 30 of these calves. The effects of diarrhoea on total body water were studied in a few other calves. From these studies it was concluded that under the present experimental conditions, diarrhoeic calves became either hypotonically or isotonically dehydrated. It was also found that many diarrhoeic calves became uraemic. Very few diarrhoeic calves were found to be hypovolaemic as indicated by either an increased haematocrit or decreased plasma volume. It was found that the loss in weight in diarrhoeic calves was due to a reduction in both total body solids and body water.

On the basis of these results and also those in the first and second parts of this study, suggestions have been made for the correction of the effects of diarrhoea on the body fluids.

outbreaks of diarrhoea may occur with strains of E. coli of low virulence if the protective antibodies against the particular antigen are present in only small amounts in the colostrum given to the calf.

Several hypothesis have been made to explain the mechanism by which E. coli causes diarrhoea in calves but these hypothesis have not been conclusively proved experimentally.

Smith and Orcutt (1925) found that they could culture far more colon bacilli from the upper parts of the alimentary tract in diarrhoeic than healthy calves. These workers were of the opinion that the bacteria formed films over the intestinal epithelium which interfered with the function of the epithelium, caused an intoxication and this led to diarrhoea.

Lovell (1955) prepared an endotoxin from a strain of E. coli isolated from diarrhoeic calves. When injected into healthy calves, it caused a copious discharge of froth from the nose and mouth, exaggerated respiration and death one to two hours later. From this work, he concluded that endotoxin may cause diarrhoea indirectly by producing intestinal and other metabolic disorders. Later, Ingram and Lovell (1960) found that lipopolysaccharide endotoxins of E. coli and Salmonella have the same order of toxicity for mice and there was no apparent difference between endotoxins of E. coli isolated from diarrhoeic calves and those isolated from other bovine sources. They also stated that the virulence of a special strain is probably an attribute of the living organism and emphasis is shifting from toxicity to the relationship of enzymes and their substrates.

The possible pathogenesis of the diarrhoea in calves was outlined by Roberts, Worden and Rees-Evans (1954) as follows. At 3 to 10 days of age, it is possible that the appearance of blood and spots of mucus in the faeces of normal calves is due to the shedding of the neonatal epithelium which was permeable to the colostral proteins. If the bacterial population of the

intestine at the time shedding of the epithelium occurs is high, the epithelium becomes inflamed and enteritis results. Most diarrhoeic calves do not, however, have a typical enteritis (Jarrett 1964) and it is questionnable if this theory of Roberts and his co-workers is applicable to every case.

Blaxter and Wood (1953) hypothesised that diarrhoea develops because "a massive bacterial activity leads to an increase in the number of smaller molecules such as steam volatile acids, thereby increasing the osmotic activity in the lumen of the gut. An infiltration of water in response to the high osmotic pressure then takes place. The water infiltration is accompanied by electrolytes and finally the irritating watery mass is explosively discharged from the colon. Acid irritation of the mucosa, increased motor activity, loss of protective mucus and partial invasion of the gut wall are possibly contributing factors in the later stages but dependent on the primary fact that absorption from the small intestine is faulty providing a substrate in the lower intestine so that coliform and other bacteria normally inhabiting the area may proliferate. It is their metabolic products which induce the change".

Recent studies on the pathogenicity of endotoxins produced by E. coli and other gram negative bacteria in diseases of man and other species may explain the role of E. coli in diarrhoea of neonatal calves.

Braude (1964) described how endotoxins, isolated from recognised pathogenic and non-pathogenic strains of gram negative bacteria, both produced identical reactions when injected into man, dog, rat, mouse and guinea pig; though the nature of the reaction varied in different species. In this respect, these observations concur with those previously made by Ingram and Lovell (1960) regarding the pathogenicity of endotoxins of E. coli and Salmonellae isolated from cattle.

Braude (1964) stated that the reaction to endotoxin simulated the

tuberculin reaction and is basically an anaphylactic response. It appears paradoxical that the endotoxin antigen and its corresponding antibody can react in such a way to produce disease. This paradox has not been entirely explained and Braude listed several hypothesis that have been suggested. One hypothesis was that the type of response to endotoxin depended on the relative amounts of antigen to antibody. If the amount of antibody exceeded the amount of antigen, it protected the animal against the reaction; if the reverse applied, then the reaction occurs. It is interesting to compare this hypothesis with the observation of Blood and Henderson (1963) that the strains of E. coli of low pathogenicity may cause infection because, although their virulence is low, protective antibodies may be at such a low level in the colostrum that invasion may occur.

Braude (1964) described how the liver and spleen can excrete endotoxin and their ability to do so is enhanced by repeated injections of small doses of endotoxin over a number of days. Substances other than antibody are believed to be involved and a substance has been isolated which can break down antibody into smaller molecules. He also described how cortisone has been shown to have protective action in an animal injected with endotoxin whereas adrenalin potentiated the response.

The peracute form of colibacillosis in neonatal calves described by Blood and Henderson (1963) and in the British Veterinary Association handbook, 'Calf Husbandry and Disease', and the description of the response to the intravenous administration of endotoxin in the calf by Lovell (1955), simulate in many respects the description of the generalised acute endotoxin and anaphylactic responses observed in other species by Braude (1964).

It has been shown that some serotypes of E. coli produce potent endotoxins which cause marked pyrexia, hypotension and collapse in man, Weil

and Miller (1961) and in animals, Sandstedt (1960). Blood and Henderson (1963) stated that in young animals endotoxin may be absorbed from the intestine and this may be a possible explanation of the syndrome seen in peracute cases of the disease. In these cases there is no gross septicaemia; at the most, there is a limited bacteraemia with bacteria recoverable from the mesenteric lymph nodes. They further state that the principal difficulty in the determination of the pathogenesis of this form of the disease is that associated with isolation and purification of the toxins. Because of the common association of individual serotypes of E. coli with specific forms of colibacillosis, they believe that there are a number of endotoxins capable of causing such conditions as acute hypotension, damage to the vascular endothelium, leading to plasma transudation, and also enteritis.

In the rat and pig localised endotoxin reactions have been described by Braude (1964) and Buxton and Thomlinson (1961). It is possible that similar reactions may occur in calves and cause diarrhoea. In the rat, endotoxins reaction causes haemostasis of the intestinal vessels and intraluminal haemorrhages. Haemostasis in the intestinal vessels could lead to impaired absorption and hence to diarrhoea.

Intraluminal intestinal haemorrhages are not regularly a feature of calf colibacillosis but do occur, quite frequently, in calf salmonellosis (Blood and Henderson 1963) and it is interesting to speculate if, in these diseases, an endotoxin response occurs in calves comparable to that in rats.

An additional feature of endotoxin reaction in other species described by Braude (1964), which may explain the pathogenesis of diarrhoea in calves, is the liberation of serotonin and histamine. These substances promote smooth muscle contraction, Keel and Neile (1961). It is possible that, if they were produced in excess by the calf with an endotoxin reaction, they may affect the

motility of the intestine and the blood flow through the mesenteric vessels, and thus interfere with normal intestinal function, causing diarrhoea.

The possible role of viruses in calf diarrhoea was first investigated in the U.S.A. by Baker (1943). He isolated a virus from calves affected with pneumonia and successfully transmitted this virus through mice into calves and also from calf to calf. This virus seemed to be mainly a cause of pneumonia although Baker observed that many of his experimental calves also developed diarrhoea.

Baker and York (1951) isolated Miyag wanella bovis, a member of the psittacosis-lymphogranuloma group, and thought this organism may be a cause of diarrhoea. Baker et al. (1944) also found that the virus of 'Virus Diarrhoea', isolated from affected adult cattle, caused diarrhoea in calves. Also, in America, Brandly and McClurkin (1954), and Moll and Brandly (1954, 1955 and 1955a) isolated a pneumo-enteritis virus. This virus caused a disease characterised by diarrhoea in the early stages and a slight or marked pneumonia in the later stages. The pathological lesions observed in this disease were not confined to any particular organ but were of a generalised nature and included congestion, degeneration, vascular damage and oedema of various organs including the intestinal wall, lungs, kidney and liver.

The role of viruses in the aetiology of calf diarrhoea in the United Kingdom has not been elucidated. It would be not unexpected that viruses may play a part in aetiology of calf diarrhoea either directly or indirectly in so much as they would lower the calves' resistance to alimentary disturbances and bacterial infection. Two outbreaks of mucosal disease have been seen in very young calves by the author. In these outbreaks the disease simulated the typical syndrome of colibacillosis described by Blood and Henderson (1963). Detailed examination of these calves showed the characteristic mucosal disease

ulcers in the oral cavity and interdigital space described by Dow, Jarrett and McIntyre (1956).

The importance of feeding milk correctly in relation to the development of diarrhoea in neonatal calves is widely assumed, although, in fact, there is little experimental evidence to support this assumption. The factors thought to be of particular importance are the frequency of feeding and the amount, composition and temperature of the milk (British Veterinary Association handbook, 'Calf Husbandry and Disease').

In surveys of calf mortality previously referred to, it was found that the incidence of diarrhoea was less in herds where the calves were allowed to suckle their dams than in herds where the calves were reared artificially and fed from a bucket. Allowing calves to suckle their dams is advocated as a method of controlling calf diarrhoea by Inglis (1960) but, in his experience, does not always control the condition. Cowie (1964) recorded a 7% mortality rate from diarrhoea in 72 suckled calves compared with a 12% mortality rate in 8 bucket fed calves. He also observed that 7 out of 19 calves (36%) given a milk substitute, died.

It is interesting to compare the feeding behaviour of calves which are allowed to suckle their dams with that of calves reared artificially and fed from a bucket in relation to the frequency and the speed of feeding and the amount consumed.

Walker (1950) observed that newborn calves allowed to suckle their dams fed, on the average, at five hourly intervals on the first day and at three hourly intervals on subsequent days. The time spent at each feed varied from 2 - 25 minutes, and, of this time, the actual period spent sucking varied from 10 seconds to 10 minutes. The total amount of milk consumed was slightly more than the usual bucket fed ration of 1 lb. of milk per 10 lb. of the

calf's body weight. Occasionally Walker observed that the calves suckled more milk in one feed than they would normally consume when fed from a bucket. None of the calves observed by Walker developed diarrhoea and they showed a steady increase in body weight. In other studies, Walker (1962) and Walker (1963) found that calves with their dams, suckled on the average 4 - 5 times a day.

In contrast to the behaviour of naturally suckled calves, the artificially reared calf is usually fed only twice a day and is consequently given more milk at each meal than the suckled calf normally consumes. The rate at which the bucket fed calf consumes the milk is apparently greater than in the suckled calf. The experimental calves in the present study often drank the three pints of milk provided at each meal in less than one minute.

Blaxter, Hutcheson, Robertson and Wilson (1951) observed that diarrhoea developed in calves fed the whole daily allowance of milk in one feed and they suggested that overfeeding may account for the high incidence of diarrhoea in pail fed calves compared with naturally suckled calves. Ingham, Meade and Berry (1930), in contrast to Blaxter et al. (1951), stated that feeding too little milk often leads to diarrhoea. Roy (1959), discussing the aetiology of calf diarrhoea, stated "that without a doubt the main predisposing cause of calf diarrhoea is the quality and quantity of milk given after the colostrum feeding period".

In an attempt to simulate the natural feeding pattern of the suckled calf, the practice of feeding artificially reared calves from the bucket three or more times a day has been advocated, Inglis (1960). The only known experimental evidence on the value of this practice is that described by Sheehy (1948). He carried out an experiment on a small number of calves and concluded that feeding three times daily compared with twice daily was of some

advantage but this practice did not by any means eliminate diarrhoea in the calves.

In view of the limited experimental evidence on the effect of feeding calves three times daily compared with twice daily as a means of controlling diarrhoea, an experiment was carried out to determine if this practice was of value.

It would appear not unlikely that the rapid ingestion of a large volume of milk by the calf could lead to diarrhoea for several reasons. In man, mass peristalsis in the ileum and the colon may be initiated taking food into the stomach, the so called gastro-ileal reflex, McDowall (1955). It is possible that if the calf has an excessive volume of milk in its abomasum, this reflex may be greater than normal and diarrhoea may occur. Alternatively, if a large amount of milk is ingested, some may overflow from the abomasum into the duodenum before it has been properly digested in the stomach and this may be a stimulus to peristalsis and the rapid emptying of the abomasal contents into the duodenum. In man, it has been suggested that the presence of acid in the duodenum (originating from gastric secretions) tends to close the pylorus and thus prevent the stomach emptying too rapidly, Wright (1947). The presence in the duodenum of milk, which has not been adequately acidified and digested in the abomasum, may provide an abnormal substrate for subsequent intestinal digestion and possibly a medium suitable for bacterial multiplication which initiate diarrhoea.

The British Veterinary Association handbook, 'The Husbandry and Diseases of Calves', also suggested that the formation of hard milk curds in the calf's abomasum can lead to abomasal and duodenal irritation and hence to the rapid passage of ingesta through the alimentary tract.

Little is known of the effects of milk composition on the incidence of diarrhoea in calves. The British Veterinary Association handbook, 'The Husbandry

and Diseases of Calves', stated that outbreaks of diarrhoea have been observed in calves when the cows, from which they are receiving milk, changed from the summer diet to the winter diet. The introduction of fish meals and beans into the diets of the cows has also been associated with outbreaks of diarrhoea in their calves. It is also stated that diarrhoea occurs in calves if their dams are grazing pastures whichhave been heavily dressed with nitrogenous fertilisers or if fed an unusually high protein diet. Milk with a high butter fat content and the milk from cows in late lactation has also been implicated as a cause of diarrhoea in calves. The above observations are presumably based on experiences in the field for there is little known experimental evidence to prove the importance of the composition of the milk in relation to diarrhoea in neonatal calves. Shanks (1950) encountered diarrhoea in calves whose dams were grazing a luscious pasture. He succeeded in reproducing the condition in a small number of calves in controlled experimental conditions and, from this, concluded that substances in the milk can initiate diarrhoea in calves. Cowie (1964) recorded a 36% mortality rate from diarrhoea in calves fed milk substitute compared with a 12% mortality rate in calves fed cow's milk from a bucket.

Because of the limited experimental evidence on this subject, an experiment was carried out to determine if diarrhoea occurred more frequently in calves fed a diet of cow's milk than in others fed a reconstituted milk.

The importance of housing and environment in relation to calf diarrhoea has been investigated by some workers. Erb, Gilden, Goodwin, Millard and Murdoch (1951) reared calves in an open shed in which the temperature fell to -20°F. and found the mortality rate in these calves was less than in other calves raised in conventional calf houses. Murley and Culvahouse (1958) and Hofman and Schwark (1958) also described the successful rearing of calves kept under very cold conditions.

Wood (1955) studied the bacterial population in calf houses used over long periods and concluded that there was a build-up of infection in such premises which led to an increased disease incidence. Smith (1958) made a similar observation.

Withers (1952-53) found that good housing and standards of hygiene were usually associated with low calf mortality rate. There were, however, several exceptions to this finding and he did observe that, in some herds with apparently good housing, there was a high indicence of diarrhoea and, conversely, in some poor houses there was a low mortality rate. McEwen (1950) reared 9 calves on clean dry abundant bedding and 6 calves on a poor thin bed over a cold cement floor and found that there was no difference in the incidence of disease in the two groups.

The incidence of diarrhoea in newborn calves appears to be related to variations in the calf resistance and susceptibility which can be affected both before and after birth.

Sheehy (1948), Fraser (1953) and Mackintosh (1953) stated that calves which are born to ~~calves~~ cows which are not correctly fed before parturition are more susceptible to disease and have emphasised the importance of providing adequate and correct nutrition of the pregnant cow. These workers do not give any experimental evidence in support of these statements, nor do they specify what they consider to be suitable diet. In view of the marked decline in human infant mortality rate, associated with the improvement of the diet and pre-natal care of the pregnant mother, Shryock (1948), it would be expected that attention to the diet of the pregnant cow is important in relation to the health of the calf.

The importance of vitamin A is one aspect of the calf and cow's nutrition which has been investigated in some detail. The importance of this vitamin in relation to the incidence of calf diarrhoea was well illustrated by

Stewart and McCallum (1938). They obtained statistically significant results on 206 calves to show that calves born to mothers whose colostrum had a high vitamin A content were less susceptible to diarrhoea than calves born to cows whose colostrum had a low vitamin content. They also showed that cow's livers had low vitamin A contents in the early spring months of the year and suggested this may be related to the higher incidence of diarrhoea observed at that time of year. It has since been shown that Vitamin A occurs in ester and alcohol forms and that the form of vitamin fed to the dam can affect the calf's Vitamin A status through either the placenta or colostrum, Barron (1942), Thomas (1947) and Wise (1946). The alcohol form of the vitamin which occurs in green food does not cross the placental barrier and hence does not affect the newborn calf's liver content. Antepartum feeding of the alcohol form of the vitamin or carotene does, however, raise the colostral levels of vitamin A. The ester form occurs in fish oils and can cross the placental barrier and raise the foetal liver content. Although the calf can receive some vitamin A via the placenta, Moore and Berry (1957) have shown that the plasma vitamin concentration is low in the newborn calf but increases considerably following ingestion of colostrum which contains 10 - 100 times the vitamin A content of normal milk. The effect on the incidence of diarrhoea in the newborn calf of giving vitamin A to both the preparturient cow and the newborn calf has been investigated.

Speilman, Eaton, Loosli and Turk (1949) found that the incidence of diarrhoea was significantly lower in calves whose dams had been supplemented with either the ester or alcohol forms of the vitamin for one month prior to calving. In 16 calves born from cows which were not supplemented, diarrhoea occurred on an average on 4.5 ± 0.9 days. Sixteen calves, whose dams had been given vitamin A supplements, had diarrhoea on the average of 0.3 ± 0.1 days.

Jacobsen, Converse and Moore (1949) found, in experiments using 52

calves, that Jersey calves given no vitamin A supplement had diarrhoea on the average for 12 days during the first 90 days of life. Other calves given a vitamin A supplement had diarrhoea on the average on 9 days. Some Friesian calves given vitamin A supplement had diarrhoea on the average on 2.5 days, whereas other calves, which were not given a supplement, had diarrhoea on the average 7 days. In this experiment, 2 of the 23 calves given vitamin A supplement died from diarrhoea or pneumonia before they were 90 days old. Six of the 24 calves, which were not given the vitamin A supplement, died over a similar period.

Other workers have found that vitamin A supplementation had no effect on the incidence of calf diarrhoea. Hibbs and Krauss (1947) and Nevens and Kendall (1947) found that supplements containing vitamins A, C and D and also niacin and nicotinic acid, were of no apparent value in preventing diarrhoea when administered to calves which had been given colostrum. Blakemore, Davies, Burge Moore and Sellers (1948), Esh, Sutton Hibbs and Krauss (1948) and Aschaffenburg, Bartlett, Sears, Thompson, Ingram, Lovell and Wood (1953) all found that the administration of vitamin A to calves did not significantly reduce the incidence of diarrhoea in animals deprived of colostrum. The conflicting evidence on the effects of vitamin A on the incidence of calf diarrhoea is possibly because the aetiology of the condition is complex and attention to only one facet of the disease may not necessarily be sufficient to eliminate it in all instances.

The functions of vitamin A in man and other mammals are recognised in association with its effect on sight, bone development, growth rate, structure of epithelia and in protecting against infection (Moore 1957). How vitamin A protects against infection in some species, including the calf, has not been elucidated. In vitamin A deficient rats the mucous secreting cells of the

intestine atrophy and the tips of the villi necrose and masses of bacteria are found infiltrating the lumena of the intestinal glands Keele and Neil (1961). They also describe the effects of vitamin A deficits on the resistance of mice to infection and it is interesting to speculate if a similar condition occurs in vitamin A deficient calves. Mice fed a vitamin A deficient diet were given measured doses of mouse typhoid bacteria, as were also mice fed the same ration supplemented with vitamin A. The mortality rate of the vitamin A deficient mice ranged from 80-100% whereas the mortality rate in the supplemented mice ranged from 10-20%. The physical appearance of both groups of mice was similar and the authors concluded that subclinical avitaminosis A may nevertheless lead to the death of an animal due to a lowered resistance to infection.

It may be expected that any vitamin or mineral deficit, or current infection such as pneumonia, would affect the calf's health and make it more susceptible to diarrhoea. How important other vitamin or mineral deficits, apart from avitaminosis A, are in newborn calves is not known. It would appear not unlikely that such deficits may occur, particularly in calves born to cows fed a winter type ration and it is possible that these deficits may be related to the higher incidence of the disease, observed in calves born during the winter and early spring months of the year. It is perhaps relevant that avitaminosis E is most frequently observed in the United Kingdom in the early spring months of the year and avitaminosis D in calves which are kept inside, British Veterinary Association handbook, 'The Husbandry and Diseases of Calves'.

The importance of water soluble vitamins in relation to the health of the newborn calf is not known but it is considered that they may be of unrecognised significance. Water soluble vitamins are synthesised by the adult ruminant animal but the newborn calf is unable to do so and acquires these vitamins from its mother, Lundquist and Phillips (1943). They have shown that

the concentration of vitamin C in the calf's blood falls immediately after birth and rises again when the calf begins to ruminate. It is interesting to speculate if calves born during the winter to cows which are fed a winter type ration deficient in fresh green vegetable matter, may, in fact, be deficient in vitamin C. Although the importance of vitamin C to the calf is not known, it is interesting that, in man, avitaminosis C is associated with an increased fragility of the capillaries leading to intestinal haemorrhages, Best and Taylor (1945). If a similar condition does occur in calves, it may offer an explanation for the blood seen in the faeces of some healthy and diarrhoeic animals.

The importance of the water soluble vitamins of the B and K groups in relation to calf diarrhoea is not known but it has been suggested that avitaminosis K may explain the blood-stained faeces seen in newborn calves, Christian and Segard (1953) and Anderson, Dupre and LaMaster (1952). Nevens and Kendall (1947), as previously described, included some B vitamins in a supplement fed to calves and were of the opinion that avitaminosis B may be of importance in the young calf.

Pneumonia is one of the most common pathological conditions encountered in calves of any age, British Veterinary Association handbook, 'The Husbandry and Diseases of Calves', and because of the wide distribution of this condition, it would be expected that the effects of this disease may lower the calves' resistance and increase their susceptibility to diarrhoea. It is also of interest that the viruses isolated by Baker (1943), Brandly and McClurkin (1954) and Moll and Brandly (1954, 1955 and 1955a) in the U.S.A. were all associated with the production of pneumonia in calves as well as diarrhoea. No comparable viruses have as far as it is known been isolated from calves in the United Kingdom.

Unlike man, transplacental transmission of antibodies does not occur in bovine animals and the passive immunity of the newborn calf depends on the

acquisition of colostral antibodies, Blood and Henderson (1963). The intestinal mucosa of the newborn calf is permeable to colostral antibodies for about 30 hours after birth and the passive immunity gained persists for a few weeks after birth, at which stage the calves' own immunogenic mechanisms become active, Comline, Tichen and Roberts (1951), McCarthy and McDougall (1953).

The importance of colostrum to the newborn calf was first recognised by Smith and Little (1922) and in 1930 Smith demonstrated the presence of specific antibodies to E. coli in the colostrum.

Aschaffenburg, Bartlett, Kon, Terry, Thompson, Walker, Briggs, Cotchin and Lovell (1949) have shown that colostrum can be divided into fatty and aqueous fractions. The fatty fraction contains vitamin A and its importance has been previously discussed. The aqueous fraction contains the immune lactoglobulins. These are antibodies produced by the calf's dam against the prevalent pathogens in her environment. The importance of colostrum for the survival of the young calf is well illustrated by the experimental results obtained by Ingram, Lovell, Wood, Aschaffenburg, Bartlett, Kon, Plamer, Roy and Shillam (1956). In their study, 225 calves were given colostrum and, of these, 166, or 74%, survived the 3 week experimental period. One hundred and three calves were deprived of colostrum and, of these, only 9 or 9% survived. These workers also carried out an investigation to determine if there was an association between the calf's survival and the type of antibodies in the colostrum. They found that 45 of the 59 colostrum fed calves which died did not receive antibodies in the colostrum against the type of bacteria isolated from them at post mortem. From 66 of the 94 calves deprived of colostrum which died, bacteria were isolated against which antibodies were present in the colostrum fed to the other calves.

Blakemore and Garner (1956) have shown that the peak serum concentration

of maternal globulins occurs about 3 weeks before parturition. This is of interest in view of the practice of 'prepartum milking' and its possible effects on the amount of antibody the calf subsequently receives through the colostrum. Hill, Widowson and Maggs (1950) demonstrated that milking cows for 2-3 days before parturition had little effect on the composition of colostrum or on the health of the calf. Turner (1931), Keyes, Reid, Bechdel and Williams (1944) and Aschaffenburg, Bartlett, Kon, Roy, Walker, Briggs and Lovell (1951) found that calves were more susceptible to diarrhoea if their dams had been milked for several days before calving. It would thus appear that extensive prepartum milking may have an undesirable effect on the composition of colostrum and consequently on the health of the newborn calf.

Although most farmers are aware of the importance of feeding the newborn calf colostrum, it is debatable if they feed colostrum correctly to the calf and if failure to do so may, in part, explain the difference in the incidence of diarrhoea in suckled and bucket fed calves. Farmers usually feed their calves only twice daily and it has been personally observed that very often the newborn calf is not fed until several hours after birth and that less colostrum is given during the first 2 days of life than the calf would normally drink if left with its mother, Walker (1950). It is possible that, if the calf is fed only twice daily, there will be periods between meals when the intestine becomes relatively empty of colostral antibodies and this may permit the multiplication of intestinal bacteria. Although the calf's intestine is permeable to colostral antibodies for only the first 2 days of life, Comline et al. (1951), the cow appears to secrete globulin in the milk for at least a week after parturition, Rowlands, Roy, Sears and Thompson (1953). It is common farming practice to give the calf its mother's milk for the first 2 or 3 days after parturition and when the cow's secretion is no longer like colostrum in

appearance, to put this "milk" in with that collected from other cows in the herd and then to feed the newborn calf on this "bulk" milk. By so doing, it is possible that the newly born calf is deprived of additional antibodies which, in the natural state, would provide a local immunity to bacteria in the intestinal lumen.

Attempts have been made to increase the newborn calf's immunity to infection by several methods. Dollahite (1939) prepared a hyperimmune serum by injecting a cow with cultures of bacteria isolated from diarrhoeic calves. This serum was given in 3 doses of 100 ml. to 58 newborn calves, of which 4 or 6.8% died. Sixty five control calves were not given thisserum and, of these, 15 or 23.1% died.

Thorp and Shigley (1942) prepared a hyperimmune serum from coliform organism isolated from diarrhoeic calves and claimed that the serum was of some value in treating calf diarrhoea. In their experiments, no untreated control groups of calves were compared with the treated calves and hence it is impossible to accurately assess their claims. Wise and Coarsey (1945) found that a combination of hyperimmunization of the preparturient dam by repeated administration of an autogenous bacterin and the injection of the calf with polyvalent antibacterial caused a slight reduction in the incidence of diarrhoea. The results obtained in this experiment are not very convincing as 59% of the untreated calves had diarrhoea compared with 42% of the treated calves.

Sellers, Smith and Pook (1962) prepared a vaccine from strains of E.coli isolated from the faeces of diarrhoeic calves and also a control vaccine prepared from a non-pathogenic staphylococcus. These vaccines were administered subcutaneously to the calves' dams approximately 1 month and again 2 weeks before parturition. Seventeen (17.5%) of the 97 calves born to E. coli vaccinated dams were affected with diarrhoea compared with 19 (19.4%) of the 93 calves born to

dams which were given the control vaccine. From this experiment, the authors concluded that vaccination of the preparturient cow was an ineffective method of preventing diarrhoea in the calf.

Udall (1954) stated that transfusion of the calf with 200-300 ml. of plasma or blood from an adult cow was a more effective treatment than the use of hyperimmune serum.

Christian (1952) and Anderson, DuPre and LaMasters (1952) described the successful treatment of calf diarrhoea with 'serum solids' plus vitamin K. The serum solids were prepared by defibrinating ox blood and separating the serum which was then carefully dried. Christian fed 232 calves colostrum alone and, of these, 21% were affected with diarrhoea and 5.6% died. Colostrum and oral serum solids plus vitamin K were given to 322 calves and, of these, 6.6% were affected with diarrhoea and 0.6% of them died.

Prior to the 1940's, the treatment of neonatal calf diarrhoea was largely based on attempts to limit intestinal irritation and bacterial activity by the use of absorbant-protectants such as kaolin and chalk and intestinal antiseptics such as brilliant green. Such mixtures are still prescribed for the treatment of neonatal calf diarrhoea though there is no known experimental evidence to show their value. Since the 1940's, the sulphonamides, antibiotics and nitrofurans have been widely used to treat and occasionally to prevent neonatal diarrhoea. Surprisingly, there is little good experimental evidence to show how effective these drugs really are.

Udall (1949) gave 10 calves 8 gms. of phthalysulphathiazole daily by mouth for the first 3 days of life and 4 gms. daily for the next 4 days. None of the calves which received the sulphonamide died although, in a control group of 10 untreated calves, 5 died of diarrhoea.

Thorp and Shigley (1942) described the treatment of 40 diarrhoeic

calves with oral sulphaguanidine. The dose given was as high as 17.5 gms. per day to some calves. Only one of the 40 treated calves died but, since they did not describe the mortality rate in untreated control calves, it is difficult to assess the value of their therapy.

In a later study, Thorp (1943) treated 57 newborn calves affected with diarrhoea using 5 different sulphonamides in varying doses. The sulphonamides were given orally. Of the 56 diarrhoeic calves, 6 died but, again, no untreated control calves were studied so that the value of this therapy cannot be assessed.

In a further comparative study of the therapeutic values of different sulphonamides, Thorp et al. (1944) treated 45 diarrhoeic calves with oral sulphasuxidine and 16 diarrhoeic calves with oral sulphathalidine. Four of 45 calves receiving sulphasuxidine died and one of the 16 calves receiving sulphathalidine also died. Untreated control groups of calves were not described in this study either.

Wise and Anderson (1943) administered varying doses of sulphathalidine by mouth to newborn calves affected with diarrhoea. They found that only 4 out of 38 (i.e. 10.5%) calves which received treatment died whereas 26 out of 91 (i.e. 28.6%) calves which were not receiving treatment died.

Voelker and Jacobson (1953) found that oral doses of 40-80 mgms. of penicillin, given daily, had no effect on the incidence of diarrhoea in 8 calves which received the antibiotic when compared with 8 untreated control calves.

Henderson and McKay (1949) described the use of streptomycin given orally in ¼ gm. doses as a prophylactic and therapeutic for calf diarrhoea. Their investigation was made on a number of farms and, though they reported favourable results with streptomycin, it is impossible to make a real assessment of the value of streptomycin since no untreated control calves were used for comparison.

Fox (1952) and Kastelic et al. (1950) also reported that oral streptomycin was effective in treating diarrhoeic calves but they did not compare the incidence with untreated control calves so, again, it is impossible to make a real assessment of the value of streptomycin.

Bortree et al. (1952) treated 58 diarrhoeic calves with oral chlortetracycline and, of these calves, 51 responded immediately to treatment and 5 gradually. Two calves did not respond to treatment and one died. No untreated control animals were studied in this experiment. In another investigation, 67 calves were given 500 mgms. of chlortetracycline by mouth at birth and, of these calves, 40 remained healthy and 27 developed diarrhoea. The affected calves were given additional doses of chlortetracycline. Twenty-one of the 27 diarrhoeic calves responded to a single oral dose of 500 mgms. of chlortetracycline. Six of the affected calves needed additional treatment and one calf eventually died. No untreated control calves were described in this study so that it is impossible to assess the value of chlortetracycline as a prophylactic or therapeutic.

Roy et al. (1955) observed a reduced mortality rate and an increased body weight in colostrum deprived calves which were given daily doses of 240 mgms. of chlortetracycline by mouth. Of 20 treated calves, only 2 died compared with 10 untreated control calves of which 4 died.

Pearson (1954) administered 2 oral doses of 500 mgms. of oxytetracycline to 41 clinical cases of calf diarrhoea and, of these, 40 recovered completely within 36 hours of the start of treatment. He also administered 2 oral doses of 500 mgms. of tetracycline to calves within the first 36-48 hours of life. This experiment was carried out on 6 farms and included 21 calves. Although specific groups of untreated control calves were not kept in this study, Pearson did observe that untreated calves on the same farms were affected with diarrhoea.

Lassiter (1955), in a review on the many studies that have been made of

the value of different antibiotics as a food additive, came to the following conclusions. Oxytetracycline and chlortetracycline are the only antibiotics on which adequate study has been made to warrant valid conclusions. These antibiotics, when fed as supplements, appear to reduce the incidence of calf diarrhoea, increase the food consumption and improve the overall condition of the animal. The recommended daily dose of these antibiotics as a food additive is 15-20 mgms./100 lb. body weight, which is about 1/25th of the usual therapeutic dose. Lassiter also describes how the growth rate of calves can be stimulated by 10-30% during the first 4 months of life by feeding a supplement.

Clark Osborne et al. (1959) found diarrhoea ceased after 5 days' treatment in 15 out of 21 diarrhoeic calves, which were given the recommended therapeutic dose of neomycin by mouth. They also found that diarrhoea ceased in 18 out of 21 calves which were given the recommended therapeutic dose of furamazone by mouth for the same period. Diarrhoea ceased in only 2 of the 21 untreated control animals which were studied over the same period. Three of the 21 calves given furamazone died compared with 6 of those given neomycin and 6 which were given no treatment.

Spectacular results were obtained by Henry and Blackburn (1957) using furamazone to treat diarrhoeic calves. They bought large numbers of diarrhoeic calves at a market and sorted them by random selection into 2 groups. The calves in one group were given 1 gm. of furamazone daily by mouth until they either died or recovered. The calves in the other group were given no treatment. Of 63 calves which were treated, only 3 (i.e. 4.7%) died, whereas 21 (i.e. 87.5%) of the 24 untreated calves died.

Smith and Crabb (1960) investigated the effects of the commonly used therapeutics on the faecal coliform count of calves. They found that, when a single dose of 0.5-1.0 gms. of streptomycin was given orally to healthy calves, the number of E. coli in the faeces dropped from 10^8 to 10 organisms per gram.

A single dose of 0.5 to 1.0 gm. of neomycin also caused a very marked fall in the number of E. coli in the faeces. A single oral dose of 0.5 to 1.0 gm. of both chlortetracycline and oxytetracycline and a single oral dose of 10 gms. of sulphadimidine and phthalysulphathiazole caused some reduction in the E. coli count of the faeces, but the effects were not so marked as with the streptomycin and neomycin. A single oral dose of 0.5 to 1.0 gm. of chloramphenicol, of 1 gm of furazolidone and of 1 gm. of furamazone had no effect on the faecal coliform count. When the oral doses of these drugs were administered over a number of days, it was found that streptomycin and neomycin had a very marked depressant effect on faecal bacterial counts, and that the effects of the tetracyclines and sulphonamides were more marked than when they were given in single doses. Only a slight reduction was observed in the faecal count with repeated doses of oral chloramphenicol and nitrofurans given over a period of days. It was also observed that these drugs were more effective in reducing the faecal coliform count if they were given orally than when given parenterally.

The efficiency of therapeutics to limit the E. coli population is partly related to the ability of the bacteria to develop resistance to the drug. Smith and Crabb (1960) have shown that the E. coli population in a calf's intestine may change from a predominantly sensitive one to one which is resistant within 24-48 hours of the administration of the therapeutic. Smith (1958) investigated the faecal flora from 537 calves and found that, on farms where it is common practice to give chlortetracycline supplements, as many as 84% of the calves had chlortetracycline resistant strains of intestinal bacteria.

The biochemical and physiological effects of diarrhoea in neonatal calves have been investigated to a limited extent. Three groups of workers, Blaxter and Wood (1953), McSherry and Grinyer (1954) and Roy, Shillam, Lang and Ingram (1959) have investigated this aspect of the disease and their

observations can be summarised as follows. Diarrhoeic calves lose an excess of water and electrolytes in their faeces. The plasma electrolyte concentration of diarrhoeic calves may change to include both hypo and hyperelectrolytaemia. Diarrhoeic calves may also be affected with a metabolic acidosis and hyperglycaemia. As a result of their observations, one group of workers carried out a limited investigation on fluid and electrolyte replacement therapy of the diarrhoeic calf. Some suggestions are also made for replacement therapy in diarrhoeic calves in the British Veterinary Association handbook, 'The Husbandry and Diseases of Calves'. A detailed description of these works is presented in Part II of this study.

From this review, it can be concluded that diarrhoea in neonatal calves is a complex problem. Its aetiology has not been adequately defined although such factors as the activity of infectious agents, errors of husbandry and variations in the calf's resistance to the disease have been implicated. Neither has the pathogenesis of the condition, or its physiological and biochemical effects, been completely elucidated. It is also apparent that the treatment of the condition has largely been directed at limiting the activity of intestinal bacteria and to a lesser extent by attention to the feeding and management of the newborn calf.

Diarrhoea in the newborn calf poses many unanswered problems and the object of the present study was to investigate some aspects of the condition.

The situation at the University Veterinary Hospital, were large numbers of calves were being reared, afforded an opportunity to make a clinical appraisal of the condition, and also to investigate in controlled experiments, using large numbers of calves, the effects of methods of management and the use of antibiotics and a sulphonamide on the incidence and severity of the disease.

Observations were made of the effects of the condition on the calf's

body weight, rectal temperature, pulse rate, general physical condition and demeanour. Post mortem examinations were also made of calves which died.

From the part of the previous review which described current practices on the treatment of calf diarrhoea, using antibiotics and sulphonamides, it is apparent that, despite the widespread use of these substances, there is limited evidence based on controlled experiments using large numbers of calves to indicate their efficacy. A study was therefore carried out to investigate the efficacy of the antibiotics and sulphonamide frequently used to control neonatal diarrhoea of calves in the field.

In the present study, it was decided to administer these substances every day during the experimental period to the calves, irrespective of whether the calves were healthy or affected with diarrhoea. The dose of antibiotic or sulphonamide administered daily was the therapeutic dose recommended by the manufacturers. Thus, ineffect, the calves were given the recommended therapeutic dose of the antibiotics or sulphonamide as a prophylactic when they were healthy and the recommended therapeutic dose when they were affected with diarrhoea. This procedure was adopted in view of the fact that diarrhoeic calves may apparently die either from the direct effects of the bacteria, such as septicaemia or endotoxaemia, or from the effects of the diarrhoea itself, such as dehydration. By administering the antibiotics and sulphonamide in the way described, the results obtained should theoretically provide a better indication of the efficacy of these substances in controlling the bacterial infection associated with diarrhoea than would be the case if they were used solely as a therapeutic since some treated calves could theoretically die from the effects of the diarrhoea per se despite the fact that the antibiotics may have effectively limited the bacterial population.

In addition, it was also possible, by administering the antibiotics daily as a prophylactic, to investigate their effects on the growth rate of the calves.

MATERIALS AND METHODS

Experimental animals

Two hundred and eighty Ayrshire bull calves were used as experimental animals. They were purchased from Paisley Market over the years 1956-1960 and were typical examples of the thousands of dairy calves sold annually through this and other markets in the West of Scotland. In this area it is customary for farmers to send bull calves to market within the first week of life. The history of the calves before they were purchased was not known but it is usual to feed some colostrum to such newborn calves. Experimental calves were selected on the basis of 3 qualities as follows: (1) They were lively and bright (2) They were of a fairly uniform size (3) They were not affected with diarrhoea, as evidenced by careful examination of their hindquarters for the absence of faecal staining.

Experimental procedure

The procedure adopted in each of the 10 experiments carried out was identical and can be summarised as follows.

A lorry load of calves was brought from Paisley Market to the Veterinary Hospital and all the calves turned loose in a large room. The required number of suitable calves was then selected. The number used in each experiment ranged from 20-50 animals. The selected calves were sorted into groups each of 10 animals. In the first 7 experiments calves were sorted into groups by random selection and in the last 3 experiments on a random weight basis.

In each experiment one group was given no treatment and acted as controls, while other groups were given the various treatments. When the calves had been sorted into groups they were moved from the large room and each calf housed in a seperate calf hut.

The experiment proper began the following morning and lasted for the next 14 days. During this period the calves in each test group were given daily doses of an antibiotic, or were managed in a different way from the control group. Daily records were kept during the experiment of the type of faeces passed by each calf. The calves were also weighed at intervals during the experiment.

Calf feeding.

Apart from calves in some groups in experiments 8 and 10, all calves were fed 3 pints of ostermilk[x] twice daily. In experiment 8 one group of calves was fed 2 pints of ostermilk 3 times daily. In experiment 10, 15 calves were fed 3 pints of ostermilk twice daily and the other 15 calves fed 3 pints of cows milk twice daily. The ostermilk was dissolved in warm water at a concentration of 1½ lbs. of dried powder per gallon of warm water. Its composition was similar to that of cows milk as shown in Table [redacted].A

Table [redacted] A

Comparative composition of ostermilk and cows milk

% Composition	Ostermilk	Cows milk
Carbohydrate	4.9	4.8
Fat	3.5	3.9
Protein	3.4	3.4
Salt	0.7	0.7
Dry Matter	13.0	12.9

x Ostermilk II. Glaxo Laboratories Ltd. Middlesex, England

Care was taken to feed the ostermilk and cows milk at approximately body temperature. Calves were fed between 8.0 a.m. and 9.0 a.m. and 4.0 p.m. and 5 p.m. One group of calves in experiment 8 were also fed between 12.00 a.m. and 1.00 p.m. Each calf was provided with a seperate feeding bucket. The buckets were washed with cold water after each feed but not sterilized. Apart from those in the first 3 experiments, any calf which refused to drink the ostermilk or milk provided at each meal was fed by stomach tube. The tube used was made of soft rubber and had an external diameter of about 1.5 cms. The tube was passed orally and sufficiently far into the calf's oesophagus so that the tip of the tube was estimated to be at the cardiac sphincter. The ostermilk or milk was slowly administered through a funnel attached to the tube.

Calf housing

Calves were housed seperately in galvanized huts approximately 6' x 4' x 5' in size. Before each experiment the huts were cleaned and disinfected. A thick bed of oat straw was provided and every second day the huts were cleaned out, but not disinfected, and fresh bedding provided. The huts were dry and free from draughts but were poorly insulated and the temperature inside them was only a few degrees warmer than outside. During severe frosty weather the huts were thatched with a layer of straw and this helped to raise the inside temperature by a few degrees.

Weighing calves

In experiment 1 the calves were weighed weekly and in other experiments on alternate days. Calves were weighed about 2 hours after the morning meal and their body weights determined to the nearest pound.

Determination of rectal temperature and heart rate

Rectal temperature and heart rates were determined daily of all calves in experiment 2 and of occasional calves in other experiments. These observations were made 2-3 hours after the morning meal. Rectal temperatures were determined with a clinical thermometer and heart rates by auscultation with a stethoscope.

Administration of antibiotics and sulphonamide

Calves which were receiving treatment were given it each day for the 14 day experimental period. The daily dose was divided into halves, one of which was given at the time of the morning feed and the other at the evening feed. Treatment was administered either by mouth as a tablet or in a drench or by intramuscular injection. For convenience, full details of the doses and routes of administration of the different treatments are listed with the tables of results obtained in each experiment. These results are shown later in this thesis.

Classification of calf faeces

During the fortnight of each experiment, a daily record was kept of the type of faeces passed by each calf. The calves were examined about an hour after the morning feed, at which time most had defaecated or did so when disturbed. Faeces were classified into four categories as follows:

Normal Faeces

These were firm in consistency and well-formed, often quite dark in colour and small in volume, and were passed daily of every second day.

Diarrhoea 1 Faeces

These were partly fluid and of an increased volume compared with normal faeces and were passed once or twice daily.

Diarrhoea 2 Faeces

In this category the faeces were fluid and contained only a few solid pieces and were markedly increased in volume. They often smelt offensively and were passed at frequent intervals during the day.

Diarrhoea 3 Faeces

These were completely fluid in character, of copious volume and evil smelling. They were passed very frequently during the day and often dribbled from the rectum of the recumbent calf.

In Figure A is shown a typical record from an experiment of the faeces passed by a group of ten calves.

Figure A Example of record of faeces passed by group of calves during an experiment

FROM 19.1.60 TO 1.2.60														
CALF	1	2	3	4	5	6	7	8	9	10	11	12	13	14	TOTAL
63			D2	X	X	X	X	X	X	X	X	X	X	X	12
65							D3	D3	D2	D2					4
69							D2								1
59					D1	D1		D2		D1		D1			5
56			D2	D3	X	X	X	X	X	X	X	X	X	X	12
49						D1	D2	D3	D1	D2	D1	D1			7
50				D3	D3	D3	D3	D3	D3	D3	X	X	X	X	11
46		D1			D1		D3	D3	D3		D3				6
57				D3	D3	D3	D3	D3	X	X	X	X	X	X	11
66						D2	D3	D2	D2	D2					5
TOTAL				D1=10				D2=12				D3=21			
DEATH 431								GRAND TOTAL 74							

Presentation and analysis of the effects of antibiotics, a sulphonamide and different methods of feeding on the incidence and severity of diarrhoea, mortality rate and body weight.

In the presentation and analysis of results, the following criteria were recorded for each group of calves.

1. The number of calves that became affected with diarrhoea.
2. The number of calves that died.
3. The number of days on which surviving calves* passed each of the following types of faeces, D1, D2 and D3, expressed as a percentage of the total number of days on which the type of faeces passed by these calves was recorded during the experiment.
4. The total number of days on which surviving calves passed any type of diarrhoeic faeces, expressed as a percentage of the total number of days on which the type of faeces passed by these calves was recorded during the experiment.
5. The mean difference between the weights of all surviving calves when determined at the start and finish of the experiment.
6. The number of days on which each calf that died passed any type of diarrhoeic faece before it died.
7. The difference between the weight of each calf that died when determined at the start of the experiment and at death.

A statistical analysis of the effects of antibiotics, a sulphonamide and different methods of feeding on the incidence of diarrhoea and mortality rate was carried out using a method suggested by Dr. S. Sylvie of the Mathematics Department, Glasgow University.

For each calf was calculated the proportion 'p'. Where 'p' equals,

* The term 'surviving calves' has been used to describe calves which lived throughout the 14 day experimental period.

" the number of days during the experiment on which the calf passed any type of diarrhoeic faece, divided by, the number (i.e. 14) of days in the experiment.

Death was considered to be as serious as 14 days continuous diarrhoea and when a calf died a value of p = 1 was used.

For each calf a value $\sin^{-1}\sqrt{p}$ was found from statistical tables. Two analyses were made to determine if there was a difference between methods of assessing significance of treatment. Significance of treatment was assessed (a) as its effect on the incidence of diarrhoea in surviving calves and (b) as its effect on diarrhoea incidence and mortality rate in all calves in a group.

Means and standard deviations of the value $\sin^{-1}\sqrt{p}$ were calculated (a) from the data on surviving calves and (b) from the data on all calves in a group. A 't' test was used to determine the statistical significance of the differences between both mean values of the treated group and both mean values of the corresponding control group.

The effect of treatment on weight changes were analysed statistically using a 't' test. The mean weight changes of surviving calves in treated groups were compared with the mean weight changes of surviving calves in the corresponding control groups. The weight changes of calves which died were not included in these analyses as the calves did not consume the same amount of milk over the experimental periods as the surviving calves.

Clinical signs and post-mortem lesions observed in experimental calves

Although it was observed that, in individual calves, the clinical signs and the course of the condition varied, the overall form of the condition appeared to be consistent in both treated and untreated calves in the 10 successive experiments. Post-mortem examinations of calves which died confirmed the impression that the form of the condition was similar throughout this study. As described later, some of the treatments administered reduced the severity of the disease in groups of calves. In these calves the condition was similar to that which occurred in the less severely affected calves given no treatment or those given a treatment which was not effective.

Every one of the 100 untreated calves used in the 10 control groups in the experiments became affected with diarrhoea at some stage during the investigation. The large majority of these calves were affected with a condition characterised by a period of diarrhoea which lasted for at least 3 days. A small proportion of calves were affected with a more acute condition and died within a period of two days of showing signs of ill health.

The large majority of calves, which developed the condition, became affected during the first week of the experiment and the condition reached its maximum severity and highest incidence between the 6th and 11th days. By the 14th day of the experiment, the majority of surviving calves were showing signs of recovery and passing normal, or slightly diarrhoeic, faeces. The number of days on which calves were affected with diarrhoea ranged from 1 to 13. Details of the number of days on which calves in each group were affected with diarrhoea are given later in this section. The calves were usually affected with diarrhoea on consecutive days but occasionally a calf had diarrhoea, recovered and then relapsed.

It was observed that a small proportion of calves passed the 'meconium'

type of faeces during the first few days of the experiment. These faeces were yellow-green-brown in colour and smelt offensively. Lengths of desquamated epithelium were present in some faeces.

Normally formed and meconial stools, which had a superficial smear of frank blood and mucous, were passed by a number of calves during the first few days of the experiment. Such faeces were passed by calves which remained healthy throughout the experiment and by others which developed diarrhoea. There was no apparent association between this type of stool and the occurrence of diarrhoea.

The type of faeces passed by healthy and diarrhoeic calves, and the frequency of defaecation, has been previously referred to in the classification used to assess the effects of treatment on diarrhoea. On visual examination of the diarrhoeic stools, there was no other evidence of either frank or occult blood.

When affected with diarrhoea, the calves often strained during and immediately after defaecation, to an apparently greater extent than healthy calves when defaecating. Some calves, which were physically very weak and affected with the severest type of diarrhoea, were observed to dribble faeces spontaneously from the rectum without obvious active movements of defaecation.

The mortality rates in different groups of calves varied. Details of the mortality rate in each group in the 10 successive experiments are given later in this section.

Of the 100 untreated control calves in these experiments, 27 died. The mortality rate in individual groups of calves ranged from 0 - 60% and, as described later, the mortality rate showed an apparent seasonal variation.

Approximately two thirds of these control calves died between the 5th and 11th days of the experiment (mean 8th $\pm$ 3 day of experiment). Two thirds of the calves which died were affected with diarrhoea for 2 to 6 days before death (mean 4 $\pm$ 2 days diarrhoea). A small proportion of calves died after having

diarrhoea on only one or two days. Other calves did not die until they had been affected with diarrhoea for 10 or more days.

Diarrhoeic calves lost weight. The amount of weight lost was apparently related to the number of days on which the calf had diarrhoea. The effect of diarrhoea on weight was evident by comparing the changes in weight of control calves which died with those of 11 control calves which were not seriously affected with diarrhoea. These 11 control calves were affected with diarrhoea on 4 days or less. They were selected for comparison as none of the control calves was unaffected with diarrhoea. These calves maintained an almost constant mean body weight during the experiment, as shown by the results given in Table A1. The difference was determined between the weight at the start of the experiment and at death of each control calf which died. The mean loss in weight of these calves was 8 ± 6 lb. The mean loss is equivalent to approximately 12% of the initial body weight. In Figure B are shown the weight losses of untreated calves which died, plotted against the number of days of diarrhoea. From the distribution of points in this graph, it can be seen that loss in weight was apparently related to the period of diarrhoea.

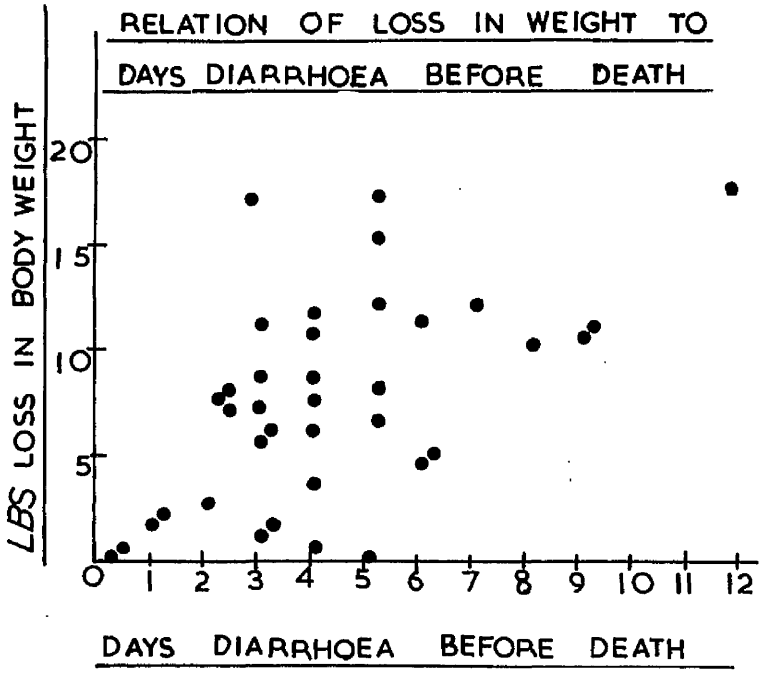
Further evidence of the effects of diarrhoea on weight is presented in Part III. These results show that, in diarrhoeic calves, the weight loss was statistically significant when compared with healthy calves. The results also illustrate that the loss in weight was related to the period of diarrhoea.

The loss of weight of diarrhoeic calves was evident in the emaciated appearance of the animals. There was an obvious reduction in the muscle masses of the shoulder, rump and thighs. The size of the abdomen also appeared to be smaller in diarrhoeic calves than in healthy animals.

Variations were observed in the demeanour and drinking behaviour of calves. When first admitted to the Veterinary Hospital, some calves would not

Figure B

Correlation of the losses in body weight of calves and the number of days on which they were affected with diarrhoea before death.



readily feed from a bucket, possibly because they had previously suckled cows. The majority of these calves soon learned, however, to feed from a bucket.

It was sometimes observed that calves were mentally depressed, physically less active and did not feed with enthusiasm the day before they developed diarrhoea. These changes in behaviour seemed to occur more frequently in calves which subsequently died of the acute form of the condition.

When affected with diarrhoea, some calves showed, so far as it was possible to judge objectively, no apparent change in demeanour. These calves behaved 'normally' in response to the various environmental stimuli associated with feeding, weighing, dosing, bleeding and clinical examination. Other diarrhoeic calves appeared obtunded and did not react so readily in response to the above stimuli. In some instances, it was possible that this 'mental depression' may have been related to, or possibly confused with, the fact that calves were physically weak. Some calves, and in particular those which had been affected with diarrhoea for prolonged periods, became 'sleepy' and finally comatose for several hours before death. In these calves, the faculties of smell, sight and hearing appeared to be depressed and they did not react to stimuli. The calves were also physically incoordinate and unable to stand or walk. Other calves, and in particular those which died of the acute form of the condition, showed abnormal nervous signs before death. These signs included incoordination, opisthonus, vigorous paddling movements and hyperextension of the limbs, fine muscular tremours, chewing movements of the jaws and nystagmus.

Clinical signs such as grinding of the teeth, periodic 'painful' bellowing, a stiff posture, arched back and kicking at the abdomen, which have been associated with abdominal pain, Blood and Henderson (1963), were very occasionally observed in diarrhoeic calves.

When healthy, the calves were keen to feed. They usually stood waiting

Table A1

Mean and standard deviation of the body weights of 11 control calves affected with 4 days or less diarrhoea.

Day of Experiment	1	3	5	7	9	11	13
Mean Wt. lbs.	75	76	77	75	74	75	75
S. D. lbs ±	7	8	7	7	7	7	7

In Table . are shown the rectal temperatures and heart rates of calves when passing faeces of different types.

Table B

Correlation of rectal temperatures and heart rates with the type of faeces passed.

Type of faeces	Normal Faeces.	D 1 Faeces.	D 2 Faeces.	D 3 Faeces.
Temperature ' F.	102.0±0.63	102.1±0.66	102.4±0.57	102.5±0.59
	(136)	(41)	(45)	(23)
Heart Rate/min	95.8±14.6	95.8±14.1	90.7±15.1	92.3±12.6
	(131)	(39)	(46)	(29)

(The figures in parenthesis are the number of observations made.)

at the pen doors when the milk buckets were rattled at feeding time. They drank greedily and usually consumed the 3 pints of milk provided at each meal in about a minute. Differences from this behaviour were observed in diarrhoeic calves.

Some diarrhoeic calves showed little reaction when presented with a bucket of milk, and therefore appeared to have no appetite but were, in fact, keen to drink when assisted. Some started to drink as soon as their muzzles were dipped into the milk. Others, which had previously drunk unaided from the bucket, had to be additionally stimulated to feed by putting fingers into the calf's mouth. Once these calves appeared to realise that milk was available, they drank readily.

Some calves, which were physically active and mentally alert, appeared to recognise the bucket of milk but showed little or no interest in drinking. Some drank only a small proportion of the milk and others appeared to have a complete aversion to drinking. Even when coaxed and stimulated by putting fingers in their mouths, these calves would not willingly drink. This behaviour appeared to be most common in calves which had been affected with diarrhoea for long periods. Interestingly, these calves, which would not willingly drink, had lost weight and were often showing the clinical signs which, as described later, have been associated with dehydration. It was this type of feeding behaviour which most often necessitated the use of the stomach tube to force feed the calves. The other calves fed by this method were those which were physically too weak to drink of their own accord.

It was noticed, when feeding calves through a stomach tube, that, as soon as milk was poured down the tube, and presumably entered either the rumen or abomasum, the calves made sucking and swallowing movements. This reaction was observed in calves which had previously shown no response to the introduction of fingers into their mouths or to the insertion of the stomach tube.

Diarrhoeic calves had changes in the texture of the skin which have been associated with dehydration, Blood and Henderson (1963). In these calves, the skin appeared to lose its normal pliability and, when picked up into a fold and then released, often retained the 'folded' appearance for some time. In a normal healthy calf, the skin is loose and resilient and rapidly returns to its natural contours if a fold is picked up and then released. It is considered questionable, however, if this clinical sign is necessarily always associated with dehydration. Comparable changes in the resilience of skin have been observed in calves which were in poor condition from causes other than diarrhoea. In such calves, there was no clinical evidence of dehydration since they were allowed water ad libatum and were not apparently losing excess water through the faeces or other routes. A change in the pliability of the skin has also been observed in calves which died of the peracute and acute septicaemic form of colibacillosis. In these animals, the course of the disease is so rapid that there would seem to be little opportunity for marked dehydration to occur.

It was observed that, in diarrhoeic calves, the hair lost its normal sheen, tended to stand stiffly and to lie in irregular planes. Alopecia was observed in many calves. A loss of facial hair, which appeared to correspond to the depth the muzzle was dipped into the milk when feeding, was observed in both healthy and diarrhoeic calves. In the latter, there was often a loss of hair over the tail, medial surface of the thighs and perineum. This alopecia was apparently distributed over areas stained with diarrhoeic faeces. On the areas where the hair was lost, the underlying skin appeared slightly hyperaemic but there was no ulceration. In some instances, the exposed skin was covered with a crust of dried milk or faeces. Whether the crust included serous exudate is not known. The cause of this alopecia is unknown. It was possibly a localised allergic response to milk.

Enopthalmus (sinking of the eyeball into the orbital fossa) was observed in many calves affected with diarrhoea. The exact significance of this condition is considered to be questionable. Blood and Henderson (1963) and the British Veterinary Association handbook, 'The Husbandry and Diseases of Calves', state that this condition is the result of dehydration leading to a reduction in the size of the eyeball. Enopthalmus has been frequently observed in emaciated cattle. In these animals there was no reason to suspect that dehydration was present. The enopthalmus in such animals is believed to be due to a reduction in the amount of peri-orbital fat.

It is possible that, in calves affected with diarrhoea for long periods, enopthalmus may be due to either or both dehydration and catabolism of peri-orbital fat. Enopthalmus has, however, been observed in calves affected with the peracute and acute septicaemic form of colibacillosis. In view of the acute course of this form of the disease, it is questionable if enopthalmus could be attributed to either dehydration or catabolism of peri-orbital fat. In these animals it is possible that enopthalmus may be due to a decrease in the size of the eyeball associated with a reduced blood supply to the organ as a result of circulatory failure. On digital palpation, it was found impossible to assess if there was either a reduction in the size of the eyeball or the intra-ocular pressure in calves with enopthalmus associated with either the acute or protracted form of colibacillosis. It is therefore considered that in calves affected with colibacillosis, enopthalmus may not necessarily be a sign of dehydration.

Dryness of the mouth and tongue is a clinical sign of dehydration in man, Bland (1963). This sign was not observed in diarrhoeic calves. In cattle, secretion of saliva is a continuous process, Lewis (1961) and it is possible that, even in a dehydrated calf, the secretion is maintained and this clinical sign would not be present.

Rectal temperatures and pulse rates were determined on large numbers of calves. The results obtained are shown in Table B. It was found that there was no significant difference between the mean rectal temperatures and pulse rates of calves which passed normal faeces and those which passed different types of diarrhoeic faeces.

The 'normal' ranges of temperature and pulse rate were considered as equivalent to two standard deviations on either side of the mean values of these parameters for calves passing normal faeces. On this basis, the normal ranges for calves were 100.5° - 103.5°F for rectal temperature and 66 - 134 beats per minute for pulse rate.

Rectal temperatures below this range were frequently observed in calves for several hours before death. Temperatures below and at the lower end of this range were also observed in calves which had been affected with diarrhoea for long periods. The extremities of the limbs, ears and skin of these animals were palpably colder than those of healthy calves.

It was occasionally observed that the temperature was sometimes slightly above normal in calves which died of the acute condition. Marked pyrexia was not, however, a clinical feature of the disease and none of the calves had a temperature greater than 105°F.

Pulse rates below and at the lower end of the normal range were observed in calves shortly before death and in calves which had been affected with diarrhoea for long periods. In these animals, the pulse volume often appeared to be reduced and, in some, it was found impossible to detect a radial or femoral pulse. In calves affected with the acute condition, the pulse rate was sometimes slightly above the normal range but there was no obvious increase in pulse volume.

Difficulty was often experienced when taking blood samples from the jugular veins of dying calves, or those which had been affected with diarrhoea for long periods. In these animals, the jugular vein did not fill rapidly or become

very distended when the vein was compressed at the base of the neck.

An attempt was made to correlate respiratory rates and diarrhoea but this was not successful. It was found that calves sniffed and licked while their respiratory rates were being measured and, because of this, it was considered that the results obtained were not a reliable indication of the respiratory rate. Clinical signs indicative of pneumonia, such as an obvious tachypnoea, hyperpnoea, dyspnoea and coughing, were not observed in any of the experimental calves apart from a very few exceptions. These observations on the absence of any clinically apparent pneumonia were confirmed by post mortem examinations of animals which died. The pneumonia that was observed in a small number of calves was apparently associated with the inhalation of milk.

Abnormal respiration was observed in some diarrhoeic calves shortly before death. These calves had periods of apnoea which lasted for about half a minute followed by a few deep gasping respiratory movements. When making these gasping movements, the calf often breathed through the mouth and emitted a sigh.

On post mortem examination of calves, which died, it was found that the majority showed no macroscopic lesions apart from emaciation and diarrhoeic faeces in the rectum. Macroscopic enteritis was not observed. In a small proportion of calves, which had died of the acute form of the condition, inflammatory lesions were observed in the meninges and joints. In about 5 calves, ulcerative lesions were observed in the abomasum or rumen. These ulcers were usually quite shallow and confined to the superficial layers of the viscera. They were typically oval or circular in shape and measured up to two inches in diameter. The margins of the ulcers were well defined. Around some ulcers there was a secondary fibrosis. The significance of these ulcers is not known but it is possible that some may have been of traumatic origin following intubation. Similar ulcers have been observed in other calves which were not intubated. In

some of these animals, the ulcers had actually perforated and there was an associated peritonitis.

Oral, oesophageal and interdigital ulcers, indicative of mucosal disease, were not observed in any of the experimental calves.

Blood, faecal and tissue samples were taken for bacteriological examination from most calves which died. Faecal samples were taken from many healthy and diarrhoeic calves and blood samples from a few calves affected with diarrhoea. E. coli was invariably isolated from the faeces. The organism was also isolated from the blood and tissues of many dead calves. Since some of these calves had been dead for several hours before they were sampled, the isolation of bacteria from blood and viscera could not necessarily be taken as indicative of death from septicaemia, Briggs (1951). The bacterium was also isolated from the blood of a few calves affected with both the acute and chronic conditions shortly before death but, again, in view of Briggs' observations, this finding was not necessarily indicative of septicaemia as the primary cause of death.

Facilities were not available to carry out a comprehensive survey of the bacteriology of all calves, or to serotype the bacteria isolated. The bacteriological samples taken were cultured to determine if Salmonella bacteria were present in the calves. These organisms were never isolated.

Seasonal variations in the severity of diarrhoea in control groups of calves

The 10 experiments in this series were carried out at different times of year over a period of 4 years. In all the experiments, the management and feeding of the control groups of calves was identical.

All the control calves became affected with diarrhoea. The number of days on which surviving control calves had diarrhoea varied in different experiments. In Experiment 2, the 8 surviving calves had diarrhoea on 36% of the days on which they were observed. In Experiment 9, the 6 surviving calves had diarrhoea on 76% of the days. The mortality rates in different control groups of calves also varied. In Experiment 4, none of the calves died but, in Experiment 7, 6 calves died.

Seasonal variations in the incidence of diarrhoea have been described by previous workers and the following analysis was carried out to determine if a similar variation occurred in the present study. In Table C are shown the experimental results for the 10 control groups of calves arranged in sequence according to the time of year when they were determined. In this table are shown, for each control group, the number of calves affected, the number of calves which died and the mean value for the surviving calves of $\sin^{-1}\sqrt{p}$. This last figure is a measure of the incidence of diarrhoea.

As previous workers have found that the incidence of diarrhoea was higher in the early spring months of the year than at other times, the results obtained on the 10 groups of control calves in the present study were divided into 2 sections. Five experiments, Nos. 1, 5, 7, 8 and 9, were carried out in the months January, February and March, and the other 5, Nos. 2, 3, 4, 6 and 10, were carried out in June and October. In Table C are shown the mean values for the mortality rate and for $\sin^{-1}\sqrt{p}$ of each section.

Since all calves in each group developed diarrhoea, there was no

Table C

Seasonal variations in diarrhoea in control groups of calves

Experiment number	Date	Calves affected	Calves died	Mean $\sin^{-1}\sqrt{p}$ living calves
8	18.1.60	10	4	0.604
5	26.1.59	10	3	0.636
7	10.2.59	10	6	0.749
9	8.3.60	10	4	1.086
1	19.3.56	10	2	0.914
Mean Section I			3.8 ± 2.2.	0.758±0.21
4	3.6.58	10	0	0.898
6	4.6.58	10	2	0.924
10	7.6.60	10	2	0.827
2	8.10.56	10	1	0.628
3	22.10.57	10	3	0.906
Mean Section II			1.6 ± 1.3	0.841±0.13

evidence that the calves had a seasonal resistance to the development of the disease. In order to determine if there was a statistically significant difference in the severity of the disease in the 2 sections, a 't' test was carried out to compare the sectional mean values of $\sin^{-1}\sqrt{p}$. There was no significant difference. From this result, it would appear that there was no seasonal variation in the severity of the disease in affected calves which survived.

The mean mortality rate in the first section of calves was more than twice that of the second section. A 't' test was carried out to compare the mean mortality rates of the 2 sections. The mean mortality rate of the calves studied in January, February and March was significantly higher at the 25% level than the mortality rate of calves studied in June and October.

The effects of some antibiotics, a sulphonamide and methods of feeding on the incidence and severity of diarrhoea, mortality rate and body weight

The results obtained in the ten experiments are presented in the following ten tables. The interpretations of these results and the conclusions that can be drawn from them are considered in the 'Discussion' of this Part of the study.

The antibiotics and sulphonamide administered in these experiments were veterinary preparations dispensed by Pharmaceutical Houses for the treatment of calf diarrhoea.

They were as follows:

Oral streptomycin, 'Streptovex'. Glaxo Laboratories Ltd.

Oral streptomycin I, 'Streptovex I'. Glaxo Laboratories Ltd.

Parenteral streptomycin, 'Strepolin 33'. Glaxo Laboratories Ltd.

Oral Neomycin, 'Neomin Oral Liquid'. Glaxo Laboratories Ltd.

Oral oxytetracycline, 'Terramycin Animal Formula Tablets'. Pfizers Ltd.

Parenteral oxytetracycline, 'Terramycin Injectable Solution'. Pfizers Ltd.

Oral penicillin, 'Penicillin G Tablets'. D.C.L. Biochemicals Ltd.

Oral chlortetracycline, 'Aureomycin Soluble Oblets'. Cyanamid of Great Britain Ltd.

Oral chloromycetin, 'Chloromycetin Vetrettes'. Parke Davis & Co. Ltd.

Oral phthalylsulphatiazole, 'Thalazole'. May & Baker Ltd.

D

Experiment I Date: 19.3.56

The effect of oral streptomycin and parenteral streptomycin on the incidence and severity of diarrhoea, mortality rate and body weight

Experimental Procedure

Four groups each of ten calves.

Group I Controls, given no treatment.
Group II Given 0.5 gms. streptomycin twice daily by mouth.
Group III Given 0.5 gms. streptomycin I twice daily by mouth.
Group IV Given 0.5 gms. streptomycin twice daily by intramuscular injection.

Results

	Group I	Group II	Group III	Group IV
Number of calves affected with diarrhoea	10	9	10	10
Number of calves that died	2	0	1	0
% expt. days surviving calves passed D1 faeces	20	23	24	25
% expt. days surviving calves passed D2 faeces	11	12	12	15
% expt. days surviving calves passed D3 faeces	31	1	2	13
% expt. days surviving calves passed diarrhoeic faeces	62	36	38	53
Mean value $\sin^{-1}\sqrt{p}$ for surviving calves	0.914	0.608	0.658	0.815
S.D. ±	0.288	0.315	0.186	0.184
Significance of difference between above mean and the control group mean P =		0.1	0.1	0.5
Mean value $\sin^{-1}\sqrt{p}$ for all calves (including dead)	1.046	0.608	0.749	0.816
S.D. ±	0.37	0.31	0.34	0.19
Significance of difference between above mean and the control group mean P =		0.02	0.1	0.3
Mean weight (lb.) change of surviving calves during expt.	-3.7	-1.7	-0.1	-1.5
S.D. ±	4.4	7.0	8.5	6.2
Significance of difference between above mean and the control group mean P =		n.s.	n.s.	n.s.

Weight difference and number of days diarrhoea before death of calves that died were not recorded in this experiment.

n.s. = not significant.

E

Experiment 2 Date: 8.10.56

The effect of oral streptomycin and parenteral streptomycin on the incidence and severity of diarrhoea, mortality rate and body weight

Experimental Procedure

Three groups each of ten calves.

Group I Controls, given no treatment.
Group II Given 0.5 gms. streptomycin twice daily by mouth.
Group III Given 0.5 gms. streptomycin twice daily by intramuscular injection.

Results

	Group I	Group II	Group III
Number of calves affected with diarrhoea	10	10	10
Number of calves that died	1	0	0
% expt. days surviving calves passed D1 faeces	13	17	20
% expt. days surviving calves passed D2 faeces	18	13	17
% expt. days surviving calves passed D3 faeces	6	9	8
% expt. days surviving calves passed diarrhoeic faeces	37	39	45
Mean value $\sin^{-1}\sqrt{p}$ for surviving calves	0.628	0.674	0.733
S.D. ±	0.266	0.176	0.264
Significance of difference between above mean and the control group mean P =		n.s.	n.s.
Mean value $\sin^{-1}\sqrt{p}$ for all calves (including dead)	0.722	0.674	0.733
S.D. ±	0.38	0.18	0.26
Significance of difference between above mean and the control group mean P =		n.s.	n.s.
Mean weight (lb.) change of surviving calves during expt.	4.0	3.1	4.0
S.D. ±	7.0	2.8	3.8
Significance of difference between above mean and the control group mean P =		n.s.	n.s.

In group I, one calf died after two days' diarrhoea and showed a loss of weight of 2½ lb.

n.s. = not significant.

F

Experiment 3 Date: 22.10.57

The effect of oral neomycin and oral oxytetracycline on the incidence and severity of diarrhoea, mortality rate and body weight

Experimental Procedure

Three groups each of ten calves.

Group I Controls, given no treatment.
Group II Given 250 mgms. of neomycin twice daily by mouth.
Group III Given 250 mgms. of oxytetracycline twice daily by mouth.

Results

	Group I	Group II	Group III
Number of calves affected with diarrhoea	10	10	7
Number of calves that died	3	1	0
% expt. days surviving calves passed D1 faeces	16	25	9
% expt. days surviving calves passed D2 faeces	22	21	7
% expt. days surviving calves passed D3 faeces	23	20	1
% expt. days surviving calves passed diarrhoeic faeces	61	66	17
Mean value $\sin^{-1}\sqrt{p}$ for surviving calves	0.906	0.948	0.337
S.D. $\pm$	0.200	0.183	0.298
Significance of difference between above mean and the control group mean P =		n.s.	0.001
Mean value $\sin^{-1}\sqrt{p}$ for all calves (including dead)	1.107	1.011	0.337
S.D. $\pm$	0.35	0.26	0.28
Significance of difference between above mean and the control group mean P =		n.s.	0.001
Mean weight (lb.) change of surviving calves during expt.	-2.1	+0.4	+3.8
S.D. $\pm$	6.5	3.4	5.3
Significance of difference between above mean and the control group mean P =		n.s.	0.1

In Group I, three calves died after 6, 9, and 2 days' diarrhoea and showed a loss of weight of 4½, 15 and 8 lb. respectively.

In Group II, one calf died after 9 days' diarrhoea and showed a loss in weight of 10½ lb.

n.s. = not significant.

9

Experiment 4 Date: 3.6.58

The effect of oral oxytetracycline on the incidence and severity of diarrhoea, mortality rate and body weight

Experimental Procedure

Two groups each of ten calves.

Group I Controls, given no treatment.
Group II Given 250 mgms. of oxytetracycline twice daily by mouth.

Results

	Group I	Group II
Number of calves affected with diarrhoea	10	7
Number of calves that died	0	1
% expt. days surviving calves passed D1 faeces	24	10
% expt. days surviving calves passed D2 faeces	25	5
% expt. days surviving calves passed D3 faeces	12	0
% expt. days surviving calves passed diarrhoeic faeces	61	15
Mean value $\sin^{-1}\sqrt{p}$ for surviving calves	0.898	0.311
S.D. $\pm$	0.266	0.290
Significance of difference between above mean and the control group mean P =		0.001
Mean value $\sin^{-1}\sqrt{p}$ for all calves (including dead)	0.900	0.437
S.D. $\pm$	0.233	0.290
Significance of difference between above mean and the control group mean P =		0.01
Mean weight (lb.) change of surviving calves during expt.	-3.4	+0.4
S.D. $\pm$	3.7	3.9
Significance of difference between above mean and the control group mean P =		0.1

In Group II, one calf died after 3 days' diarrhoea and showed a loss of weight of 2 lb.

H

Experiment 5 Date: 4.6.58

The effect of parenteral oxytetracycline on the incidence and severity of diarrhoea, mortality rate and body weight

Experimental Procedure

Two groups each of ten calves.

Group I Controls, given no treatment.
Group II Given 250 mgms. oxytetracycline by intramuscular injection, twice daily.

Results

	Group I	Group II
Number of calves affected with diarrhoea	10	10
Number of calves that died	2	2
% expt. days surviving calves passed D1 faeces	24	16
% expt. days surviving calves passed D2 faeces	19	24
% expt. days surviving calves passed D3 faeces	20	16
% expt. days surviving calves passed diarrhoeic faeces	63	56
Mean value $\sin^{-1}\sqrt{p}$ for surviving calves	0.924	0.853
S.D. ±	0.160	0.255
Significance of difference between above mean and the control group mean P =		n.s.
Mean value $\sin^{-1}\sqrt{p}$ for all calves (including dead)	1.057	0.997
S.D. ±	0.31	0.38
Significance of difference between above mean and the control group mean P =		n.s.
Mean weight (lb.) change of surviving calves during expt.	-1.2	-1.9
S.D. ±	4.9	4.9
Significance of difference between above mean and the control group mean P =		n.s.

In Group I, two calves died after 5 and 3 days' diarrhoea and showed a loss of weight of 0 and 2 lb. respectively.

In Group II, two calves died after 4 and 3 days' diarrhoea and showed a loss of weight of 6 and 2 lb. respectively.

n.s. = not significant.

I

Experiment 6 Date: 26.1.59

The effects of oral oxytetracycline on the incidence and severity of diarrhoea, mortality rate and body weight

Experimental Procedure

Two groups each of ten calves.

Group I Controls, given no treatment.
Group II Given 250 mgms. of oxytetracycline twice daily by mouth.

Results

	Group I	Group II
Number of calves affected with diarrhoea	10	9
Number of calves that died	3	1
% expt. days surviving calves passed D1 faeces	19	9
% expt. days surviving calves passed D2 faeces	10	6
% expt. days surviving calves passed D3 faeces	6	3
% expt. days surviving calves passed diarrhoeic faeces	35	18
Mean value $\sin^{-1}\sqrt{p}$ for surviving calves	0.636	0.394
S.D. ±	0.157	0.201
Significance of difference between above mean and the control group mean P =		0.05
Mean value $\sin^{-1}\sqrt{p}$ for all calves (including dead)	0.916	0.511
S.D. ±	0.46	0.42
Significance of difference between above means and the control group mean P =		0.1
Mean weight (lb.) change of surviving calves during expt.	-10.1	-8.3
S.D. ±	2.9	3.0
Significance of difference between above mean and the control group mean P =		n.s.

In Group I, three calves died after 3, 8 and 0 days' diarrhoea and showed a loss of weight of 17, 10 and 0 lb. respectively.

In Group II, one calf died 0 days' diarrhoea and showed no loss of weight.

J

Experiment 7 Date: 19.2.59

The effect of oral penicillin and oral chlortetracycline on the incidence and severity of diarrhoea, mortality rate and body weight

Experimental Procedure

Three groups each of ten calves.

Group I Controls, given no treatment.
Group II Given 250,000 I.U. penicillin G twice daily by mouth.
Group III Given 250 mgms. chlortetracycline twice daily by mouth.

Results

	Group I	Group II	Group III
Number of calves affected with diarrhoea	10	8	10
Number of calves that died	6	4	3
% expt. days surviving calves passed D1 faeces	21	13	12
% expt. days surviving calves passed D2 faeces	16	4	6
% expt. days surviving calves passed D3 faeces	9	13	6
% expt. days surviving calves passed diarrhoeic faeces	46	32	20
Mean value $\sin^{-1}\sqrt{p}$ for surviving calves	0.749	0.524	0.477
S.D. ±	0.126	0.403	0.306
Significance of difference between above mean and the control group mean P =		n.s.	0.2
Mean value $\sin^{-1}\sqrt{p}$ for all calves (including dead)	1.263	0.838	0.916
S.D. ±	0.4	0.6	0.77
Significance of difference between above mean and the control group mean P =		0.1	n.s.
Mean weight (lb.) change of surviving calves during expt.	-4.7	-2.9	-5.3
S.D. ±	1.7	3.6	4.6
Significance of difference between above mean and the control group mean P =		n.s.	n.s.

In Group I, six calves died after 7, 5, 5, 3, 6 and 3 days' diarrhoea and showed a loss of weight of 12, 17, 7, 8, 11 and 6 lb. respectively.

In Group II, four calves died after 6, 2, 5 and 4 days' diarrhoea and showed a loss of weight of 5, 8, 12 and 2 lb. respectively.

In Group III, three calves died after 4, 4 and 8 days' diarrhoea and showed a loss of weight of 12, 8 and 14 lb. respectively.

K

Experiment 8 Date: 18.1.60

The effect of oral chlortetracycline and feeding three times daily on the incidence and severity of diarrhoea, mortality rate and body weight

Experimental Procedure

Three groups each of ten calves.

Group I Controls, given no treatment and fed twice daily on three pints ostermilk.
Group II Given 250 mgms. chlortetracycline twice daily by mouth and fed twice daily on three pints ostermilk.
Group III Fed three times daily on two pints ostermilk.

Results

	Group I	Group II	Group III
Number of calves affected with diarrhoea	10	8	10
Number of calves that died	4	0	4
% expt. days surviving calves passed D1 faeces	12	9	19
% expt. days surviving calves passed D2 faeces	12	18	6
% expt. days surviving calves passed D3 faeces	9	7	3
% expt. days surviving calves passed diarrhoeic faeces	33	44	18
Mean value sin^{-1}p for surviving calves	0.604	0.371	0.724
S.D. $\pm$	0.181	0.279	0.213
Significance of difference between above mean and the control group mean P =		0.02	n.s.
Mean value sin^{-1}p for all calves (including dead)	1.061	0.371	0.989
S.D. $\pm$	0.467	0.275	0.525
Significance of difference between above mean and the control group mean P =		0.01	n.s.
Mean weight (lb.) change of surviving calves during expt.	-1.6	-1.7	-7.5
S.D. $\pm$	3.8	2.0	5.2
Significance of difference between above mean and the control group mean P =		0.05*	n.s.

*significantly less weight change.

n.s. = not significant.

L

Experiment 9 Date: 8.3.60

The effect of oral chloromycetin and oral phthalylsulphathiazole on the incidence and severity of diarrhoea, mortality rate and body weight

Experimental Procedure

Three groups each of ten calves.

Group I Controls, given no treatment.
Group II Given 250 mgms. of chloromycetin twice daily by mouth.
Group III Given 2.5 gms. phthalylsulphathiazole twice daily by mouth.

Results

	Group I	Group II	Group III
Number of calves affected with diarrhoea	10	10	10
Number of calves that died	4	2	3
% expt. days surviving calves passed D1 faeces	15	10	16
% expt. days surviving calves passed D2 faeces	17	9	5
% expt. days surviving calves passed D3 faeces	44	11	5
% expt. days surviving calves passed diarrhoeic faeces	76	30	26
Mean value $\sin^{-1}\sqrt{p}$ for surviving calves	1.806	0.569	0.518
S.D. ±	0.229	0.197	0.222
Significance of difference between above mean and the control group mean P =		0.01	0.01
Mean value $\sin^{-1}\sqrt{p}$ for all calves (including dead)	1.278	0.761	0.832
S.D. ±	0.327	0.455	0.541
Significance of difference between above mean and the control group mean P =		0.02	0.05
Mean weight (lb.) change of surviving calves during expt.	-12	-4	-4
S.D. ±	6.5	2.8	3
Significance of difference between above mean and the control group mean P =		0.02	0.02

In Group I, four calves died after 5, 3, 3 and 4 days' diarrhoea and showed a loss of weight of 8, 11, 9 and 1 lb. respectively.

In Group II, two calves died after 2 and 1 day's diarrhoea and showed a loss of weight of 10 and 3 lb. respectively.

In Group III, three calves died after 4, 4 and 4 days' diarrhoea and showed a loss of weight of 9, 4 and 11 lb. respectively.

M

Experiment 10 Date: 7.6.60

The effect of oral chlortetracycline and milk and ostermilk feeding on the incidence and severity of diarrhoea, mortality rate and body weight

Experimental Procedure

Two groups each of ten calves and two groups each of five calves.

Group I	(10 calves)	Controls, given no treatment, fed ostermilk.
Group II	(10 calves)	Given no treatment, fed cow's milk.
Group III	(5 calves)	Given 250 mgms. chlortetracycline twice daily by mouth and fed ostermilk.
Group IV	(5 calves)	Given 250 mgms. chlortetracycline twice daily by mouth and fed cow's milk.

Results

	Group I	Group II	Group III	Group IV
Number of calves affected with diarrhoea	10	10	5	2
Number of calves that died	2	1	0	0
% expt. days surviving calves passed D1 faeces	17	21	16	4
% expt. days surviving calves passed D2 faeces	26	7	4	0
% expt. days surviving calves passed D3 faeces	11	3	5	0
% expt. days surviving calves passed diarrhoeic faeces	54	31	23	4
Mean value $\sin^{-1}\sqrt{p}$ for surviving calves	0.827	0.589	0.490	0.130
S.D. $\pm$	0.175	0.163	0.110	0.140
Significance of difference between above mean and the control groups mean $P =$		0.02*	0.01*	0.001[β]
Mean value $\sin^{-1}\sqrt{p}$ for all calves (including dead)	0.976	0.687	0.490	0.130
S.D. $\pm$	0.35	0.30	0.11	0.14
Significance of difference between above mean and the control groups mean $P =$		0.1*	0.02*	0.01[β]
Mean weight (lb.) change of surviving calves during expt.	-3	+2	+4	+7
S.D. $\pm$	4.5	2.7	4.4	4.4
Significance of difference between above mean and the control groups mean $P =$		0.02*	0.05*	0.1[β]

In Group I, two calves died after 3 and 9 days' diarrhoea and showed a loss of weight of 6 and 10 lb. respectively.

In Group II, one calf died after 2 days' diarrhoea and showed a loss of weight of 8 lb.

*Group I compared Groups II and III.

[β]Group II and Group IV means compared.

Discussion

The results obtained in the first part of this study can be considered to have both clarified and confused some aspects of the problem of diarrhoea in newborn calves.

As previously mentioned, the aetiology of diarrhoea in newborn calves has been associated with several factors which may act independantly or in conjunction. In this study diarrhoea appeared to be associated with three factors acting in conjunction. These three factors were, bacterial activity, diet and seasonal variations in the calves' susceptibility.

The significance of bacteria in the aetiology was evident in the way some oral antibiotics were effective in controlling diarrhoea and reducing mortality rate. E.coli was isolated from the faeces of healthy and diarrhoeic calves, from the tissues of dead calves and from the blood of some dying calves. The significance of these findings is discussed later. The results obtained in Experiment 7 with oral penicillin G (an antibiotic not supposed to be effective against E.coli) suggested that gram positive organisms may have been implicated in the aetiology of the diarrhoea. This observation concurs with that of Smith (1960). He was of the opinion that gram positive organisms such as Clostridia and Streptococci may be of unrecognised importance in the aetiology of diarrhoea in calves.

The significance of diet in the aetiology was evident from the results obtained in Experiment 10. In this experiment there was a marked difference in the incidence and severity of diarrhoea in calves fed cow's milk and those fed ostermilk. The latter which is prepared from cow's milk has a gross chemical composition similar to that of the natural product as shown in Table A (Page 35) There was presumably some factor either in the more detailed chemical composition or in the physical nature of the 'solution' of the reconstituted product implicated in the aetiology of the diarrhoea. This finding emphasises the importance of the composition of milk in relation to diarrhoea. It concurs with the

observations in the British Veterinary Association Handbook, The Husbandry and Diseases of Calves, and by Shanks (1950) and Cowie (1964).

Bacterial activity appeared to be more important than diet in the aetiology of the diarrhoea. If diet had been of primary importance it would have been expected that the diarrhoea would have occured soon after the animals were first fed ostermilk. Diarrhoea in fact usually occured after the 3rd or 4th day of the experiment. It would also have been expected that if diet had been of primary importance as the cause of the diarrhoea, those antibiotics which were effective, would not have completely prevented diarrhoea but only reduced its severity.

The third factor implicated in the aetiology of the diarrhoea was a seasonal variation in the calves susceptibility as indicated by the seasonal differences in mortality rate. Seasonal variations in susceptibility could have been due to either differences in the calves resistance or differences in the severity of the condition. Seasonal differences in the calves resistance could have been associated with differences in their vitamin or mineral reserves at different times of the year as has been previously discussed. Seasonal differences in the severity of the condition could possibly have occured due to differences in bacterial activity at different times of year or because of the stress associated with adverse weather conditions in the early spring months of the year. Since the calves were fed ostermilk at all times of year it was considered that seasonal differences in the severity of the condition were not likely to have been associated with differences in the composition of the diet.

As discussed in detail later, the clinical condition observed in this study simulated that described by previous workers and also that encountered under farm conditions. The fact that the present condition with its complex aetiology simulated clinically other outbreaks of diarrhoea with probably different aetiologies, emphasises that 'diarrhoea' in newborn calves is a clinical sign common to many different aetiologies. It illustrates the difficulty (or the

impossibility) of diagnosing the aetiology of diarrhoea from the clinical signs alone and thus emphasises that unless the aetiology has been defined, methods of treatment and prevention ideally should include attention to all possible aetiological factors.

Diarrhoea was the most characteristic clinical sign of the condition observed in the present study. It occured in all the calves which showed signs of ill health. However it appeared that diarrhoea was a clinical sign common to two recognised conditions. These were designated as the 'acute' and 'chronic' conditions. Clinically they simulated the diseases 'septicaemic colibacillosis' and 'enteric colibacillosis' respectively described by Blood and Henderson (1963). The conditions were also similar to those described in the British Veterinary Association Handbook, The Husbandry and Diseases of Calves. Blood and Henderson (1963) also described 'dietetic diarrhoea' in calves. The present 'chronic condition' simulated in some respects their description of this condition also. These authors stated that 'dietetic diarrhoea' of calves is frequently complicated by secondary bacterial activity, a situation comparable to that occuring in the present study.

Calves died from both the acute and the chronic conditions but the pathogenesis of these conditions appeared to differ. In the acute condition the calves appeared to die from the direct effects of bacteria. The precise cause of death was not defined but it was considered that an endotoxaemic-anaphylactic type of response may have occured in these calves comparable to those described in other species. This response was suspected as the calves were pyrexic and in the terminal stages appeared to be in circulatory failure. The circulatory failure in these calves was not considered to have resulted from same effects as that observed in calves affected with the chronic condition. Calves which died from the acute condition did not loose as much weight as those dying from the chronic condition and presumably they were not so depleted in body water or electrolytes. A terminal E.coli septicaemia was detected in some

calves dying from the acute condition. The significance of this finding is considered questionable as a terminal septicaemia was also observed in some calves dying from the chronic condition and as previously mentioned, Briggs (1951) recorded that a terminal septicaemia occured in most dying calves. It is considered an interesting problem as to whether the septicaemia observed in the acute condition in the present study and the septicaemic colibacillosis described by others, is either a 'normal' terminal event in a dying calf or if the septicaemia is a specific disease. In this respect it is of interest that Blood and Henderson (1963) described how in peracute endotoxaemic colibacillosis it may be impossible in some cases to isolate the bacterium from the blood as death occurs so rapidly from toxaemia that the intestinal bacterium do not have time to invade the tissues. Septicaemic colibacillosis could be merely a terminal event therefore of a protracted enteric endotoxaemia. However, septicaemia could foreseeably arise due to bacterial invasion by routes other than the intestine. As E.coli is ubiquitous in the environment of the calf, invasion could occur through the lungs, skin, umbilicus or urinary tract. The significance and origin of septicaemia in the acute condition is considered important as it could have a bearing on the administration of antibiotic therapy and prophylaxis. If septicaemia occured only as a natural sequence to an enteric endotoxaemia it would appear feasible to administer oral antibiotics to very young calves as a way of preventing death. However, if septicaemia occurred due to invasion by routes other than the intestine, it would appear advisable to administer antibiotics parenterally when treating or preventing the condition, since the majority of antibiotics given orally are not rapidly or effectively absorbed from the intestine.

The majority of calves which died were affected with the chronic condition. This condition also appeared to affect the other calves which had diarrhoea and survived. There was no indication that calves had the acute (septicaemic ?) condition and recovered, although it was impossible to state categorically this

did not occur as a complete bacteriological survey was not carried out.

Apart from diarrhoea the most outstanding clinical signs in calves affected with the chronic condition was emaciation and loss of body weight. Loss of weight could be due to either or both a reduction in total body solids or body water. That a loss in body solids probably occured was evident in the calves' emaciated appearance and the reduction in the muscle mass of the shoulders and hindquarters. Blaxter and Wood (1953) observed that diarrhoeic calves were in a negative nitrogen balance. This suggests that the loss in weight in diarrhoeic calves is partly due to catabolism of body solids. Loss of body water would be expected to occur in diarrhoeic calves in the same way as occurs in diarrhoeic infants. Previous studies by Blaxter and Wood (1953), McSherry and Grinyer (1954) and Roy et al (1959) indicated that diarrhoeic calves developed deficits of electrolytes. In view of the close association between the body's electrolyte content and its water, these results suggest that diarrhoeic calves loose body water.

As previously mentioned, some calves affected with the chronic condition were found to have an E.coli septicaemia shortly before death. It was considered however, that the cause of death was not due primarily to the effects of the septicaemia, but resulted from the long term effects of diarrhoea. In diarrhoeic infants the following effects of prolonged diarrhoea have been described and are possible causes of death; hypovolaemia, hyper and hypoelectrolytaemia, metabolic acidosis and starvation (Bland 1963). The results obtained by McSherry and Grinyer (1954), Blaxter and Wood (1953) and Roy et al (1959) suggest that similar effects occur in diarrhoeic calves.

In view of the above and also from other clinical signs observed in diarrhoeic calves (enopthalmus, changes in skin pliability, weak pulse, poor jugular filling, abnormal drinking and mental behaviour) it would appear that the effects of diarrhoea on the body fluids are of considerable importance. The effects of diarrhoea on the body fluids in infants has been investigated in

great detail. From these investigations there has developed an understanding of the importance of correcting these effects and thereby saving diarrhoeic infants' lives. In view of the known significance of the effects of diarrhoea on the body fluids in infants it was decided to investigate this aspect of diarrhoea in calves.

Before it was possible to define the effects of diarrhoea on the body fluids and then attempt to prescribe methods of correcting these effects, it was obviously necessary to know something about the volume and composition of the body fluids in health. As little was known of the volume and composition of the body fluids in healthy calves it was essential to define some of these parameters before investigating the effects of diarrhoea. These investigations form the basis of the subsequent studies described in Parts II and III of this thesis.

It is apparent that diarrhoea in newborn calves is a clinical sign common not only to conditions arising due to different aetiologies but also to several different clinical conditions which may cause the calf's death.

The term 'calf diarrhoea' has been often used as if to describe a specific disease, thus implying that "diarrhoea in neonatal calves" has a specific aetiology and pathogenesis. From the previous discussion it is apparent that this is not the case. Diarrhoea in newborn calves is but a clinical sign common to a neonatal syndrome. This syndrome has a multiple aetiology and pathogenesis and includes such recognised clinical conditions as coli-endotoxaemia, coli-septicaemia, enterocolibacillosis and dietetic diarrhoea. It is considered that there may be other clinical conditions which have not as yet been defined. It is apparent that before it is possible to interpret the efficacy of a prophylactic or therapeutic procedure, the aetiology and pathogenesis of the "diarrhoea" should be defined.

The interpretation of the efficacy of a prophylactic or therapeutic procedure can also depend apparently on the criterion used to assess its efficacy.

The criterion used by most previous workers and those used in this study were the efficacy of the procedure in (a) preventing diarrhoea, (b) reducing the severity of diarrhoea, and, (c) preventing deaths. The present results indicate that there is not always an absolute correlation between these criterion. This is not entirely unexpected in view of the complex aetiology and pathogenesis of diarrhoea but it is apparent that if there is not a correlation between these criterion, the interpretation of experimental results could be biased depending on the emphasis placed on each criterion.

It is considered that (a) the failure to appreciate that 'calf diarrhoea' is not a specific disease but a syndrome (b) failure to define the aetiology and pathogenesis of diarrhoea (c) the relative emphasis placed on the different criterion used to assess the efficacy of a therapeutic or prophylactic procedure, are the basis of some of the confusion and conflicting evidence apparent in previous studies on 'calf diarrhoea'.

The way in which a spurious interpretation of the efficacy of a prophylactic procedure could occur can be illustrated by the following hypothetical examples.

If an outbreak of 'calf diarrhoea' was due to a gross, but unrecognised error in diet, an oral antibiotic administered as a prophylactic would not be effective in preventing the diarrhoea. This would occur even though the antibiotic may have had an unrecognised effect in limiting the intestinal bacterial population.

The efficacy of the antibiotic in reducing the severity of the diarrhoea would depend on the relative 'contributions' to the severity by the diet and by bacterial activity. If the diet was the most outstanding factor, the antibiotic would have no apparent effect despite the fact that it may have limited the bacterial activity. If the bacterial activity made a substantial 'contribution' to the severity of the diarrhoea and the antibiotic was effective in controlling the intestinal bacteria then obviously the antibiotic would have an effect as assessed by this criteria.

The efficacy of the antibiotic on mortality rate would depend on what factors were causing death. If death was due solely to the effects of the severe diarrhoea which resulted primarily from the dietetic error, then the antibiotic would have no effect. If however the severity of the diarrhoea was partly due to bacterial activity and the severity of the diarrhoea was reduced by the antibiotic then fewer calves would die from the direct effects of the diarrhoea and the antibiotic would be effective in reducing the mortality rate.

In such an outbreak of 'calf diarrhoea' there is always the possibility that some calves may die from an enteric endotoxaemia. If the antibiotic was effective against the bacteria causing this condition then the apparent effects of the antibiotic in reducing mortality rate would depend on the relative number of calves which died from the direct effects of the diarrhoea resulting from the dietetic error and the number which died from the endotoxaemia.

Clinically the outbreak of 'calf diarrhoea' described above may simulate an outbreak in which bacterial activity was of primary importance. In this instance it is foreseeable that the prophylactic would be effective in preventing diarrhoea, reducing the severity of the diarrhoea and preventing deaths.

It is obviously impossible to completely relate the results obtained in an experiment to the overall problem of 'calf diarrhoea'. The results of an experiment can only be expected to be reproduced in another outbreak of 'calf diarrhoea' in which the aetiology and pathogenesis of the diarrhoea are identical.

The impossibility of relating experimental results to the overall problem of 'calf diarrhoea' is further illustrated by the fact that even in the present study in which the experimental conditions were standardised with regard to the type, feeding and management of the calves, it was found to be impossible to compare the results obtained in successive experiments.

Before attempting to draw conclusions from the present results it is important to consider the present experimental conditions. The calves originated from many farms and were in contact with numerous other calves in the

market. It would be expected therefore, that a wide range of bacterial strains would be represented in the calf population used in this study. As the antibiotics were administered daily throughout the experiments it would be expected that antibiotic resistant strains may have become established in the calf population. Thus the experimental conditions would be expected to favour the emmergence and the dissemination throughout the calf population of virulent, antibiotic resistant bacterial strains.

Although throughout this investigation the nature of the clinical condition was apparently consistent, the number of days on which surviving calves had diarrhoea, the severity of the diarrhoea and the mortality rate varied in each group of control calves. All the control calves became affected with diarrhoea so there was no obvious difference in the incidence of diarrhoea. The percentage of days on which the surviving calves in control groups had diarrhoea ranged from 33 to 76%. The percentage of days on which the surviving calves in control groups had the severest (D3) diarrhoea ranged from 16 to 59%. The mortality rate in the control groups of calves varied from 0 to 60%.

The reason for the variations in the severity of diarrhoea and mortality rate in the control groups is not known. It may have been due to differences in the virulence of bacteria in successive experiments and there was also an apparent seasonal variation in the calves susceptibility. As the severity of the diarrhoea was not constant in successive control groups of calves then presumably it was not constant in successive treated groups of calves either. It was therefore impossible to state categorically that a prophylactic used in one experiment was more or less effective than a prophylactic used in another experiment, since the two prophylactics were not exposed to the same 'challenge'. The only comparisons that can be made between prophylactics are when both were used in the same experiment.

The results obtained in the 10 experiments are summarised in Table This table shows the three criteria for assessing the effects of treatment,

Table N.

Summary of the effects of different methods of controlling diarrhoea and mortality rate

Experiment No.	Method	No. affected Treated/Control	No. died Treated/Control	% Diarrhoea days Treated/Control	'p' for surviving calves	'p' for all calves
1.	Oral Streptomycin	9/10	0/2	38/62	0.1	0.02
	Oral Streptomycin	10/10	1/2	38/62	0.1	0.1
	Par: Streptomycin	10/10	0/2	53/62	0.5	0.5
2.	Oral Streptomycin	10/10	0/1	39/37	1.0	0.8
	Par: Streptomycin	10/10	0/1	45/37	1.0	1.0
3.	Oral Neomycin	10/10	1/3	66/61	1.0	0.6
	Oral Oxytetine	7/10	0/3	17/61	0.001	0.001
4.	Oral Oxytetine	7/10	1/0	15/61	0.001	0.01
5.	Par: Oxytetine	10/10	2/2	56/63	0.6	0.8
6.	Oral Oxytetine	9/10	1/3	18/35	0.05	0.1
7.	Oral Penicillin	8/10	4/6	32/46	0.4	0.1
	Oral Chlortetine	10/10	3/6	20/46	0.2	0.3
8.	Fed T.I.D.	10/10	4/4	44/33	1.0	0.8
	Oral Chlortetine	8/10	0/4	18/33	0.02	0.01
9.	Oral Chloromycetin	10/10	2/4	30/76	0.01	0.02
	Oral Sulphs	10/10	3/4	26/76	0.01	0.05
10.	Cow's milk	10/10	1/2	31/54	0.02	0.1
	Cstk.+ Oral Chlortetine *	5	0	23	0.01	0.02
	Milk + Oral Chlortetine *	2	0	4	0.001	0.01

* These were groups each of five calves. Par: = Parenteral.

namely, (a) Preventing diarrhoea, (b) Reducing the severity of diarrhoea, and, (c) Preventing deaths. Also shown are the two P values for the statistical comparison of the mean values $\sin^{-1}\sqrt{p}$ of the treated and control groups. All the P values are shown, even though some are obviously of no biological significance. They do however provide a method of comparing results.

In Experiment 1, only oral streptomycin was effective in preventing diarrhoea. Oral streptomycin and parenteral streptomycin were effective in reducing the mortality rate. Oral streptomycin I was less effective. Oral streptomycin and oral streptomycin I reduced the percentage of days on which surviving calves had diarrhoea to a level which was slightly significant ($P = 0.1$). Parenteral streptomycin was not effective in this respect ($P = 0.5$). From the results shown in Table D it can be seen that all the treatments were effective in reducing the severity of the disease as indicated by the number of D3 days. When the statistical analysis included calves which died, the significance of treatment with oral and parenteral streptomycin was greater than when assessed on living calves only.

These experimental results illustrate the difficulty of interpreting the effectiveness of treatment. Obviously oral streptomycin was the most effective treatment in that it reduced the incidence, severity of diarrhoea and mortality rate. It is more difficult to decide whether the oral streptomycin I was a more effective treatment than parenteral streptomycin. Statistically the oral streptomycin I was more effective than the parenteral streptomycin in reducing the incidence of diarrhoea, but the latter was more effective in preventing death.

In Experiment 2, oral and parenteral streptomycin were effective in reducing the mortality rate. They were not effective however in either reducing the incidence of diarrhoea or the number of days on which surviving calves had diarrhoea. Neither, as shown in Table E were these drugs effective in reducing the severity of diarrhoea as indicated by the number of D3 days. Obviously the conclusion that can be drawn from this experiment

depends on whether the effect of the drugs in preventing death is the sole consideration. It is of particular interest that the results in this experiment are quite different from those of the previous experiment.

In Experiment 3, oral oxytetracycline was obviously effective in all respects and the statistical significance of the effectiveness of this treatment was very high. Neomycin was effective in reducing the mortality rate but not in reducing the incidence or the severity of diarrhoea as shown by the results in Table F Again the conclusions that can be drawn regarding the effectiveness of neomycin depend on which criterion ~~is~~ are used.

In Experiment 4, oral oxytetracycline had a highly significant effect on the incidence of diarrhoea in the surviving calves. The treatment also reduced the number of calves which became affected and the severity of diarrhoea. One of the treated calves however died and in this respect the result in the treated group was worse than in the control group.

In Experiment 5, parenteral oxytetracycline had no apparent effect. Experiment 4 and 5 commenced on the 3rd and 4th of June respectively and the calves were reared in close proximity. It is therefore possible to make some comparison between the results obtained in these two experiments. There was an obvious difference in the effectiveness of oxytetracycline when given orally and when given parenterally. A similar difference in the effectiveness of streptomycin administered orally and parenterally was apparent in Experiment 1. It is possible that these antibiotics were not effective when given parenterally as there was an insufficient concentration in the intestine to control the bacterial population.

In Experiment 6, oral oxytetracycline was effective in all respects though statistically it was not as effective as in Experiment 3.

In Experiment 7 an interesting result was obtained. Oral penicillin was effective in preventing diarrhoea and also reduced the mortality rate. It was not however effective in reducing the incidence of diarrhoea in the surviving

calves (P = 0.4). Neither was it effective in reducing the proportion of days on which the calves had D3 diarrhoea. When the statistical analysis was based on the results obtained in all calves the significance was higher than when based on surviving calves only (P = 0.1 and 0.4 respectively). Oral chlortetracycline did not prevent diarrhoea in any of the calves but reduced the mortality rate by half. The incidence of diarrhoea in the surviving calves was also considerably reduced (P = 0.2). This treatment did not obviously affect the proportion of days on which the calves passed D3 faeces. When the statistical analysis was based on the results obtained on all calves the significance was lower than when based on surviving calves only (P = 0.2 and 0.3 respectively). Obviously in this experiment it depends which criterion are used to define if oral penicillin was more effective than oral chlortetracycline.

In Experiment 8, oral chlortetracycline was obviously effective and feeding three times daily not effective.

In Experiment 9, oral chloromycetin was effective in reducing mortality rate and the incidence and severity of diarrhoea. Oral phthalysulphathiazole was slightly less effective.

The results obtained in Experiment 10 indicate that calves fed cow's milk were healthier than those fed ostermilk. Oral chlortetracycline was effective in preventing diarrhoea in calves fed cow's milk and also in reducing the death rate and the severity and incidence of diarrhoea. This treatment was also effective when administered to calves fed ostermilk but in this instance it did not prevent diarrhoea and neither did it have the same marked effect in controlling the incidence and severity of diarrhoea.

These results illustrate the problem of assessing the efficacy of prophylaxis from the three criterion when there is an obvious lack of correlation between the criteria. The lack of correlation between criteria was most evident in the results obtained in Experiments 1, 3, 4 and 7.

As described in the introduction, the antibiotics were administered as a

prophylactics as it was suspected that diarrhoeic calves died from the effects of bacteria (e.g. spoticaemia) or from the effects of diarrhoea (e.g. dehydration). It was considered that by administering the antibiotics as a prophylactic, the results obtained should provide an indication of their efficacy in controlling the bacterial infections associated with diarrhoea. The antibiotics were not expected to prevent death from the effects of diarrhoea such as dehydration nor could they reasonably be expected to prevent death from septicaemia since orally administered antibiotics are not effectively absorbed from the intestine. From the above it is apparent that in this investigation the important criterion for assessing the efficacy of the prophylactics were their ability to (a) prevent diarrhoea, and (b) to reduce the severity of the diarrhoea.

From this study it can be concluded that antibiotics administered as an oral prophylactic may sometimes be very effective in preventing diarrhoea and reducing its severity. They may also reduce the calf mortality rate. It is considered that it may be a feasible practice to administer oral antibiotics as a prophylactic under farm conditions to alleviate diarrhoea in newborn calves. It must be appreciated however that the efficacy of this practice can be expected to be extremely variable depending on the circumstances under which 'calf diarrhoea' occurs. Although the present results suggest that some antibiotics were not effective, e.g. neomycin and streptomycin, it should not be concluded that these preparations are of no value. Other workers have found differences in the efficacy of antibiotics from those in this study. This is not unexpected in view of the complexity of the aetiology and pathogenesis of "calf diarrhoea". For example Smith and Crabb (1960) found that neomycin was effective but chloromycetin was not effective in reducing the intestinal bacterial population and preventing diarrhoea. This finding was the converse of the one in this study. Smith and Crabb (1960) also found that furamazone was not effective and this result differs markedly from that of Henry and Blackburn (1957).

The variation in the efficacy of antibiotics to limit bacterial population

and control diarrhoea may be due to the emmergence of resistant strains of bacteria. Smith (1958) showed that antibiotic resistant strains of E.coli are widely distributed in the calf population. Smith and Crabb (1960) showed that the E.coli population in the calf's intestine can change from a predominantly antibiotic sensitive one to a resistant one within 24 - 48 hours of administering the antibiotic. The emmergence of resistant strains is an important consideration in both the treatment and prophylaxis of diarrhoea with antibiotics. It is obviously of value to determine the sensitivity of the intestinal flora of a diarrhoeic calf when using antibiotics for treatment or prophylaxis. In human medical practice the simultaneous administration of two or more antibiotics is advocated to avoid the emmergence of resistant bacterial strains, Jawetz, Melnick and Adelberg (1960). The principle being that the likelihood of the emmergence of a resistant strain is very much less if two antibiotics are used simultaneously than if used consecutively. This principle could probably be used to advantage in controlling the bacterial populations associated with calf diarrhoea.

Feeding calves three times daily instead of twice daily did not have any effect on the incidence or severity of disease. It is considered that this negative results should not be interpreted as an indication that the practice of multiple feeding is of no value. As previously described the aetiology of diarrhoea in the present study appeared to be related to bacterial and dietetic factors and seasonal variations in the calves susceptibility. It is therefore not unexpected that attention to only one possible facet of the aetiology need necessarily be effective.

It was found that some groups of calves given antibiotics were significantly heavier than their corresponding control groups at the end of the experiment. As all the calves consumed the same amount of ostermilk (apart from some in Experiment 10 fed cow's milk) the difference in the body weights of the treated and control groups cannot be attributed to differences in the amount consumed.

In Experiments 1 - 9 there were 15 groups of calves given treatment. Nine of these 15 groups had significant reductions in the incidence of diarrhoea when compared with their corresponding control groups. Of these nine groups of calves, five also had significant differences in body weight. Six of the 15 groups of treated calves had no significant reduction in the incidence of diarrhoea and neither did any of these groups have a significant difference in body weight. These results suggest that the effects of antibiotics on body weight were related to their effects in alleviating diarrhoea. Blaxter and Wood (1953) have shown that the digestibility of the diet is reduced in diarrhoea. It is possible that part of the difference in body weight of the calves in which treatment was effective against diarrhoea, was because these calves utilised their food better than the other calves. It was also shown that diarrhoeic calves lose weight, probably due to depletion of body solids and body water, this is believed was the primary factor causing the differences in body weight in those calves in which treatment was effective.

PART II

THE VOLUMES OF THE BODY FLUID COMPARTMENTS IN HEALTHY CALVES

Introduction

The body fluids consist of water as the solvent and many organic and inorganic substances as solutes. The maintenance of the volume and composition of the body fluids by the active movement of solute and solvents are essential functions in the living animal. In health the volume and composition of the body fluids remain constant within very narrow limits despite the dynamic activities constantly taking place within the body and its environment. In disease the normal mechanisms which regulate the body fluids may be impaired causing changes in their volume and composition. Such changes in the volume and composition of the body fluids may contribute either directly or indirectly to death.

The volume and composition of the body fluids in man have been extensively investigated in both health and disease. A few studies have also been made on animals including some on cattle. It is probable that in most mammals the volume and composition of the body fluids are generally comparable and that analogies can be made between man and other mammals on the main physiological and biochemical features of the body fluids. However, before it is possible to assess the extent and significance of changes in the volume and composition of the body fluids occurring in disease, it is essential to know the precise range of the normal values of the volume and composition of the body fluids in healthy animals.

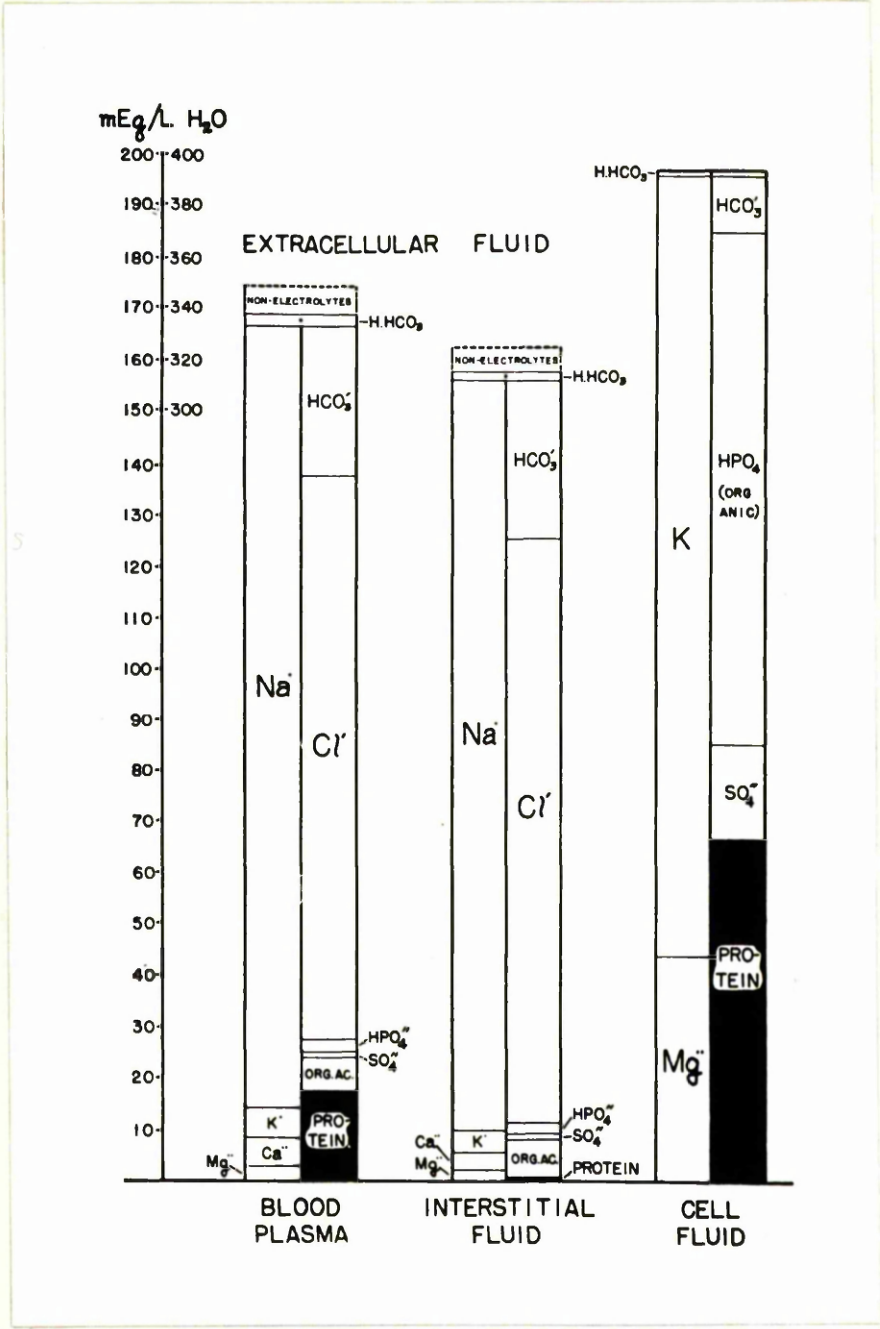
In the animal body as a whole the body fluids may be divided on a histological basis into two principal 'phases', the intracellular fluid and the extracellular fluid. The intracellular phase consists of water and solutes within the cells and is the site of the metabolic activities of the body. The

extracellular fluid bathes the cells and acts as the means of communication between the cells and the rest of the body and also with the environment. The extracellular fluid can be further subdivided on an anatomical basis into three compartments, the plasma, the interstitial fluid and the connective tissues. Within the body there are also special fluid depots in the gastro-intestinal tract, in serous and synovial cavities, in the lower urinary tract, in the gall bladder and bile ducts of the liver and in the central nervous system. These fluid depots have been termed the 'transcellular fluids', Moore (1948).

Water is freely diffusible from one part of the body to another, but the various solutes are more limited in movement, and their concentration in different parts of the body is strictly maintained. The distribution of water in the body is regulated principally by the concentration of the different solutes in the body fluid compartments. There are four basic mechanisms which lead to the movement of fluid within the body and also in relation to its environment, namely, diffusion, osmosis, active transport and hydrostatic pressure.

Diffusion along concentration gradients is the simplest type of movement of fluid and solutes and occurs whenever two solutions of different concentration or composition are in direct contact with each other. The transfer of fluid or solutes in osmosis is similar to that of diffusion apart from the limitations on the movement of some constituents of the solutions imposed by a semipermeable membrane. The active transport of fluid within the body is carried out by the cells moving solutes against concentration gradients, the changes in solute distribution being followed by the movement of the solvent. Fluid is also moved around in the body by the activity of the circulatory and lymphatic systems.

Figure 1. Comparative composition of extracellular and intracellular fluid - from Elkinton and Danowski (1956)



Since individual tissues have specific functions it is not surprising they differ in composition but, despite these differences, the composition of the intracellular and extracellular phases are similar enough in different tissues to justify consideration of the composition of these phases as a whole. This simplification becomes even more necessary when considering abnormalities that occur in disease.

The principal ionic solutes of the intracellular phase are potassium and magnesium as cations, and phosphate and protein as anions. In the extracellular fluid the major cation is sodium and the main anions are chloride and bicarbonate. Protein is present as an anion but is mainly restricted to the vascular system. The electrolytic composition of the extracellular and intracellular fluids are shown in Figure 1.

The differential distribution of ions, characterised by the location of sodium in the extracellular fluid and potassium in the intracellular fluid is a basic characteristic of the dynamics of the body fluids which is, as yet, not completely understood.

Conway (1947 and 1951) suggested that active transport of sodium occurs across the cell membrane, and that sodium ions which diffuse into the cell from the extracellular fluid are constantly being ejected. This active transport he hypothesises may involve an enzyme system in the cell wall which takes up an electron from the neutrally charged intracellular phase. The electron then attracts the sodium ion that has diffused into the cell to the cell wall. The electron and sodium ion are then liberated into the extracellular fluid where they dissociate and the liberated electron is returned to the intracellular phase to restore electrical neutrality.

Ling (1952) attempted to explain the ion distribution about the cell on a purely physico-chemical basis. He hypothesised that the separation of sodium

and potassium takes place throughout the whole cell and not just at the cell wall. He believed that this is due to the fact that the dielectric constant of the water between the protein anion and the electrolyte cation is lower as the diameter of the hydrated cation decreases. The diameter of potassium cation is smaller than that of sodium cation so that the former is attracted to the protein anion and the latter repelled.

Another important factor regulating the distribution of the solutes within the various body fluid compartments and thereby the amount of solvent is the Gibbs-Donnan Equilibrium. This is a phenomenon in which there may be an unequal distribution of ions on either side of a semipermeable membrane which is freely permeable to these ions but not to another ion present on one side of the membrane. The phenomenon may be explained as follows. Assume that a membrane which is freely permeable to the ions sodium and chloride is used to separate two solutions of different concentration of sodium chloride. The rate at which the sodium or the chloride ions diffuse from one side of the membrane to another depends on the concentration of the ions upon each side of the membrane, but the demand for electroneutrality of the solutions is such that it will not permit either ion to diffuse across the membrane without the other. However, if the sodium chloride on one side of the membrane were to be replaced by a solution of a sodium salt, the anion fraction of which was not freely diffusible through the membrane, a different set of conditions would prevail. Some of the chloride ions would diffuse into the compartment containing the non-diffusible anion because of the differences in the concentration gradient for this ion on either side of the membrane which is freely permeable to chloride. The non-diffusible anion is prevented from doing this because of the membrane. The chloride ions cannot diffuse without taking some sodium ions with them, otherwise the electroneutrality of the system would be disturbed.

Thus the total ion concentration rises on the side of the membrane which limits the movement of the non-diffusible ion. In the body the non-diffusible ion is generally protein which is an anion. The distribution of the ions in such a system gives rise to an osmotic pressure due to both the diffusible and the non-diffusible fractions and thus regulates the distribution of water between the two phases of the system.

The body fluid compartments are linked by the vascular system which permits the rapid and even distribution throughout the body of any disturbances in the equilibrium of the body fluids. The vascular system also acts as the pathway between the body fluids and the organs responsible for their exchanges with the environment. The four organs in which exchanges take place between the body fluids and the environment are the gastro-intestinal tract, the lungs, the skin and the kidneys.

The intestine is normally the only route of entry from the external environment of the fluid and solutes which constitute the body fluids. In order to function correctly the small intestine and the stomach secrete large volumes of fluid, similar in composition to the extracellular fluid and this secretion is later absorbed together with the ingested material in the lower parts of the alimentary tract. In health the process is so efficient that despite the large amounts of sodium and water which may be ingested and are secreted by the gastro-intestinal tract, the faeces usually contain little water and almost no sodium chloride.

The lungs are concerned primarily with the loss of water and regulation of the acid-base balance in the body by eliminating carbon dioxide. The water loss through the lungs is an obligatory function and continues with relatively little decrease in rate even when an animal is severely dehydrated (Danowski et al. 1944).

The skin is also the site of continuous vaporization and in man considerable amounts of fluid and electrolytes may be lost as sweat. The mechanism for heat regulation by sweating is not well developed in cattle but there is some loss of water and electrolytes through the skin, although not as extensively as in man.

The kidneys are mainly responsible for regulating the volume and composition of the body fluids. Their activity is closely related to the activity of other organs and in particular to the endocrine secretions of the pituitary and adrenal glands. The kidneys regulate the amount and proportion of the body water and electrolytes by a series of complicated reactions involving glomerular filtration, tubular secretion and tubular excretion. The precision of the kidneys and allied regulating mechanisms in controlling the volume and composition of the body fluids is quite spectacular. The overall fluid turnover in the kidney of man during a day is several times the volume of fluid in the body. About 180 litres of fluid are filtered, and of this, about 99% is reabsorbed. Also contained in the filtrate are electrolytes and the waste products of metabolism. These are carefully separated and the unwanted excreted and the required reabsorbed. Another important function of the kidneys is the regulation of the acid-base balance in the body by the selective absorption or excretion of ions.

About 60-80% of all the water, all the glucose, a large fraction of the urea and a proportion of the sodium cations are reabsorbed in the proximal tubules. Some secretory contributions to the urine are made here, too.

After the filtrate has traversed the proximal tubule there is left 20-30% of the original glomerular filtrate to make its way down the thin segment of Henle and through the distal tubules to the collecting tubules as the final

urine product. In the distal segment the tubular cells reabsorb variable amounts of sodium, potassium and chloride ions and other solutes. Creatinine and hydrogen ions are secreted by the tubular cells.

The rate of reabsorption of water in the tubules is controlled by the antidiuretic hormone secreted by the posterior lobe of the pituitary gland. Although it is well established that the antidiuretic hormone regulates the ability of the kidney to concentrate urine, the means by which this concentration is achieved is not well understood. Wesson and Anslow (1952) and Zak et al. (1954) have proposed some type of water pump which is sensitive to antidiuretic hormone. If the level of circulating antidiuretic hormone is increased the distal water pump will reabsorb more water and so increase the solute concentration of the urine.

Wirz et al. (1951) on the other hand, suggest that urine is concentrated by the passive diffusion of water out of the collecting ducts into the interstitial fluid of the renal pyramids. They propose that a solute pump located in the ascending limb transports solute from the urine to the interstitial fluid. It then diffuses from the interstitial fluid to the descending limb. As urine flows down the descending limb it becomes more concentrated, while as it moves up the ascending limb it becomes more dilute. Such a counter current multiplier system will establish an osmotic gradient along the length of the pyramids, the base of the pyramid having an osmotic pressure lower than that in the apex. In this scheme, antidiuretic hormone controls the pore size in the collecting ducts; the more hormone present the larger the pore size. Thus, in the presence of antidiuretic hormone, this osmotic gradient will draw water out of the collecting ducts and so concentrate the urine.

The tubular reabsorption and secretion of electrolytes is controlled by steroids secreted by the adrenal cortex. Many steroids have been isolated

from the adrenal cortex but to date the most important hormone discovered is aldosterone (Simpson et al. 1954). When injected into animals aldosterone causes salt retention and potassium excretion.

The kidneys have a remarkable ability in health to combat deficits or excesses of fluid or electrolytes by selective excretion and reabsorption. When diseased, however, the kidneys may be unable to maintain the normal equilibrium of the body fluids and the cardinal signs of renal insufficiency are associated with disturbances in body fluid and electrolyte balance.

The body fluids represent some 50-70% of the body weight. This range is conditioned largely by the degree to which the total body weight consists of adipose tissue which contains little fluid. The proportion of the body weight due to fluid also varies with age. In infants the plasma, blood, extracellular fluid and total body water volumes are greater than in adults (Edelman et al. 1952, Fellers et al. 1949, Wiggers 1949, and Flexner et al. 1947).

Total body water includes the transcellular water such as that in the gastro-intestinal tract. In man the gastro-intestinal tract contains about 1.5% of the total body water (Edelman et al. 1952). In adult ruminant animals the stomachs may contain a large volume of fluid. Several workers have shown that the amount of fluid in the stomachs may vary considerably and hence affect the volume of total body water. A more detailed account of their findings is described later in this section.

By definition the extracellular fluid is that portion of the body fluids which lie outside the cells. The extracellular phase can be sub-divided into at least three comparts; the plasma, the connective tissue fluid and the interstitial fluid. The plasma volume constitutes about 5% and the interstitial fluid about 15% of the total body weight in the adult animal. These two

compartments are not very different in composition except that the plasma contains protein, which apart from a very small fraction does not diffuse into the interstitial fluid. The electrolyte concentrations of plasma and interstitial fluid differ slightly as required by the Gibbs-Donnan effect previously described. The plasma proteins exert an osmotic pressure known as the "colloidal or oncotic pressure" which is normally equal to about 23 mm. of mercury and is opposed by the hydrostatic pressure in the circulation. At the arterial end of a capillary bed the hydrostatic pressure of blood is about 32 mm. of mercury and at the venous end about 12 mm. of mercury. Thus, at the arterial end of the capillary bed the hydrostatic pressure is greater than the colloidal osmotic pressure and fluid is forced out through the permeable walls into the extravascular compartments, while at the venous end of the capillary bed the colloidal osmotic pressure is greater than the hydrostatic pressure and fluid is attracted from the extravascular compartment back into the circulation. It is the balance between these two opposing forces aided by the action of the lymphatic system and the kidneys which keep the volume of plasma and other body fluids constant. Chambers and Zweifach (1944) have shown that the blood flow to tissues and hence the fluid transfer in them is partly controlled by the action of small muscular sphincters on the arterial end of the capillary bed.

Plasma and serum, apart from their protein contents, are almost identical in composition to the rest of the extracellular fluid. In Table 1 are shown the concentrations of the principal inorganic constituents of the serum and plasma of cattle determined by various investigators. As far as is known no studies have been carried out to determine the composition of intracellular fluid in cattle.

There are several methods available for estimating the volumes of the body

TABLE 1

The concentration in mEq/litre of the principal inorganic constituents of bovine serum or plasma

Reference		Cl	Na	K	Ca	Mg	Total Cations
Anderson et al. (1930) s					5.0 - 8.0		
Brown (1946) s		108					
Craige (1947) s		90 - 109			3.6 - 5.1		
Craige et al. (1949) p		89 - 108					
Dale et al. (1954) p			162	5.1	7.5	3.2	177.8
Dukes (1947) s					4.5 - 6.0		
Evans and Phillipson (1957) p			139 - 144	4.2 - 4.6			
Fisher (1960)	calves	100.3 ± 3.5	141.8 ± 3.5	5.1 ± 0.4	4.9 ± 0.2	1.14 ± 0.3	152.9
p	adults	103.3 ± 5.0	142.2 ± 2.0	4.4 ± 0.3	5.0 ± 0.6	1.46 ± 0.4	153
Godden and Allcroft (1932) s		85 - 98			4.0 - 5.8		
Lengemann et al. (1952) p		96.6 - 110					
McSherry and Grinyer (1954) s	calves	103 ± 2.5	142.0 ± 4.0	5.25 ± 0.54	5.08 ± 0.22		
s	adults	103.7 ± 3.5	142.0 ± 5.0	4.85 ± 0.47	5.42 ± 0.34		
Reibart (1939) s			151 - 165	6.1 - 6.4	5.5 - 5.8	1.5 - 1.8	
Sampson and Hayden (1935) s					4.6 - 6.2		
Sellers and Roepke (1951) p		103	139 - 146	3.9 - 4.4	4.9	1.8	154.7
Spector (1958) s		97 - 111	132 - 152	3.9 - 5.8	4.7 - 6.1	0.8 - 2.4	
Roy et al. (1959) s			135	5.9			
Ward et al. (1953) s		90 - 107			3.8 - 5.3		

s = serum : p = plasma

Unless otherwise stated values are for adult cattle.

fluid compartments and some of them have been used to determine the composition of cattle. The total body water of cattle cadavers was determined by dessication (Haigh et al. 1920, and Ellenberger et al. 1950).

Total body water can be calculated from the specific gravity of the body or from the percentage fat composition determined by dissection, as described by Behnke (1942). This method was used to determine the total body water in cattle cadavers by Kraybill et al. (1951 and 1952) and by Wellington et al. (1956).

In man the lean body mass can be determined by measuring the amount of oxygen consumed and creatinine excreted. Miller and Blyth (1952) have shown that the metabolism of oxygen and creatinine at rest are proportional to the lean body mass and the total body water.

The most widely used method for estimating the volumes of the body fluids is by determining the "apparent volume of distribution" or the "dilution" of a solute. This method depends on the relationship of the concentration of a solution to the amount of the solute in the solution and the volume of the solvent in which the solute is dissolved. Thus, if the amount of solute is A, and the concentration of the solution is C, then the volume V of the solution is equal to $V = A/C$.

The volume of distribution of a solute can be measured by either the "infusion method" or the "single injection method".

The volume of distribution of a solute is measured by the infusion method as follows. The solute is infused intravenously at a constant rate and during infusion the plasma or blood solute concentration is periodically determined. After the solute has been infused for some time it reaches equilibrium in concentration throughout the body fluid compartment and between the rate of infusion and of excretion. When the plasma or blood solute concentration is constant indicating that equilibrium has been established the infusion is stopped

and the amount of solute excreted from then on until the solute has completely disappeared from the body determined. The volume of distribution of the solute is calculated by dividing the amount of solute excreted from the time the infusion stopped by the plasma or blood solute concentration. The infusion method has been used to determine the extracellular fluid volume in man and dogs using inulin, mannitol and sucrose as solutes (Gaudino et al. 1948, Elkinton 1947, and Gamble et al. 1953). The estimation of the volumes of the body fluid compartments by the infusion method presents practical difficulties associated with infusion of the solute and collection of the excreted solute. Because of these difficulties and the time required to carry out a measurement, the infusion method has not been extensively used to investigate clinical problems in man nor, as far as is known, to determine the volumes of the body fluids in large domestic animals.

The "single injection method" is faster and simpler than the infusion method and has been used to study the volumes of the body fluids in many clinical conditions in man and in healthy large domestic animals. The theory and practical application of the method are as follows:

If a suitable solute is administered by rapid intravenous injection and then the plasma solute concentration determined at intervals, a series of changes in plasma solute concentration take place. Immediately after injection the plasma solute concentration rises and falls rapidly as the solute is mixed throughout the circulation. If the solute diffuses out of the circulation the concentration of the solute in the plasma will fall until the plasma concentration is in equilibrium with the concentration in the compartment into which the solute diffused. From the time the solute is injected it is being continuously excreted at an exponential rate proportional to its plasma concentration and after the initial mixing period the excretion rate is

proportional to the solute concentration in the fluid into which the solute diffused. If the rate at which the solute concentration falls after the initial mixing period is extrapolated back to the time of injection the theoretical concentration of the solute is obtained at the time when the solute was injected assuming instantaneous equilibration in the compartment and no excretion. The volume of distribution of the solute is determined by dividing the amount of solute injected by the theoretical concentration of the solute in the body fluid compartment at the time of injection. It is apparent that the type of solute required is one which rapidly equilibrates throughout the body fluid compartment after injection and is excreted at an exponential rate proportional to its concentration in the body fluid compartment. The time required for the excretion of the solute should be not less than 30 minutes or more than 24 hours. The primary error in this method is the assumption that during the initial period of mixing and equilibration, the rate of excretion is the same as that observed later, but provided a rapidly diffusible solute is used this error is of no great significance. In carrying out an estimate by this method usually not more than five blood samples are collected, the time and frequency of the collection after injection depending upon the solute used. The theoretical concentration at the time of injection is determined by plotting the solute concentrations on a semilogarithmic scale against the time of collection and extrapolation of the line drawn through these points to the time of injection. The semilogarithmic scale is used to simplify this determination as it converts the exponential curve which represents a geometrical progression into a linear trend. This is preferable to attempting to extrapolate the exponential curve when plotted on a linear scale.

A major problem in the dilution method is the selection of suitable solutes, for many diffuse out of the body fluid compartment they are intended to measure.

TABLE 7.

Solute spaces in adult man

Body Fluid Compartment	Mean volumes as % body weight determined by various workers				
Plasma					
T.1824 (Evans' Blue)	4.2	4.8	4.6	4.2	
I^{131} Albumin	4.0				
Extracellular Fluid					
Inulin	16	16	15	16	
Sucrose	20		21	18	
Mannitol	23	18	16	16	
Thiosulphate		17			
Sulphate	23				
Radiosulphate, S^{35}	17				
Bromide	27	23	29		
Radiochloride, Cl^{36}	27				
Radiochloride, Cl^{38}	18	27			
Radiosodium, Na^{24}	26	26	26	32	27
Thiocyanate	22	27	25	22	24
Total Body Water					
Deuterium oxide	72	63	53	62	60
Tritium oxide	65	52			
Antipyrine	55	52	56		
Desiccation	68	59	66		

Solute spaces in infants

Body Fluid Compartment	Mean volumes as % body weight determined by various workers	
Extracellular Fluid		
Thiocyanate	42	
Radiosodium, Na^{24}	44	43
Total Body Water		
Deuterium oxide	75	77
Desiccation	69	75

from Elkinton and Danowski (1956)

Figure 2. Solute spaces in man (from Elkington and Danowskii 1956)

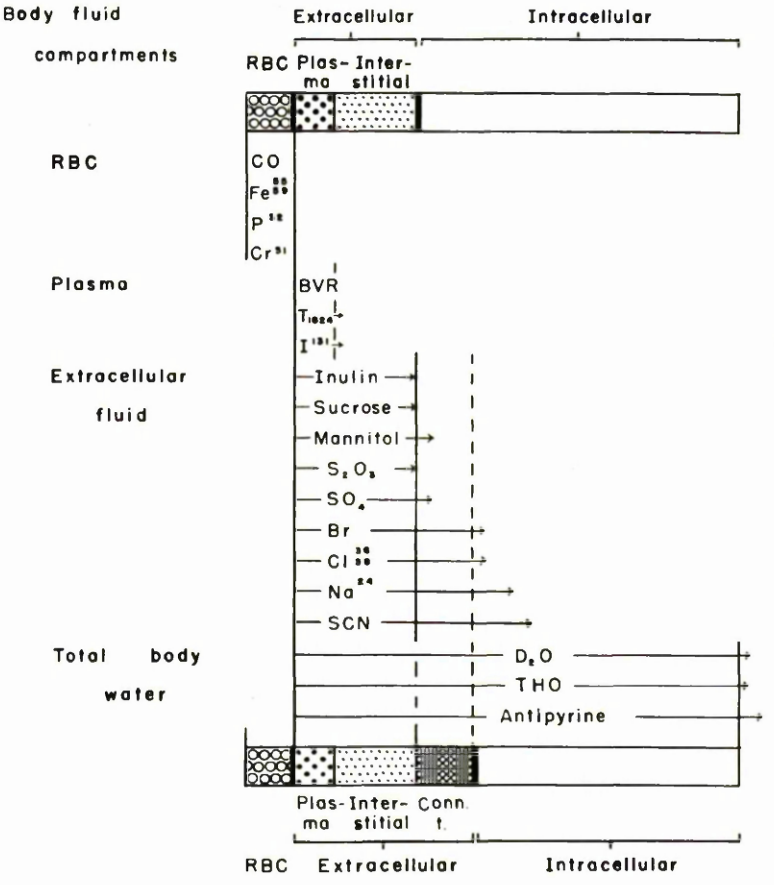


TABLE 3

Plasma and blood volumes in cattle

Reference	Method	Animals	Plasma % B. Wt.	Blood % B. Wt.
Turner and Herman 1931	Vital Red	54 Growing Dairy cows	3.5	5.8
		24 non-lactating cows	3.8	6.4
		41 lactating cows	4.9	8.1
Miller 1932	Vital Red	19 animals 81 determinations Weight range 400-1300 lbs.		6.0
Hansard et al. 1953	Erythrocytes tagged with Phosphorus 32	Hereford Cattle		
		2 2-6 weeks old		12.0
		1 3 weeks old		8.5
		3 2-3 months old		6.2
		5 6-8 months old		5.8
		3 14-15 months old		5.7
		4 8-12 years old		5.7
Reynolds 1953	T.1824	Guernsey Cattle		
		11 determinations on 1 heifer	3.7 ± 0.08	5.2 ± 0.14
		10 non-pregnant and non-lactating animals	3.9 ± 0.14	5.7 ± 0.21
Reynolds 1953 a	T.1824	Guernsey Cattle		
		20 pregnant animals	3.9 ± 0.18	5.9 ± 0.29
		7 lactating animals	4.4 ± 0.17	6.4 ± 0.27
Dale et al. 1956	T.1824	3 non-lactating Jerseys	3.7 ± 0.37	6.2 ± 0.50
		3 lactating Jerseys	4.7 ± 0.47	6.9 ± 0.70
		3 lactating Holsteins	5.5 ± 0.56	8.4 ± 0.98
Bianca 1957	T.1824	18 determinations on 6 Ayrshire cattle aged 4 months	4.8 ± 0.08	6.5 ± 0.11
Mixner and Robertson 1957	Bromosulphon-thalein	Holstein cattle		
		5 lactating cows	3.8 ± 0.65	5.7 ± 0.96
		9 bull calves Mean weight 48.7 kg.	6.8 ± 0.16	11.5 ± 3.4

TABLE 4

Extra-cellular fluid volume in cattle

Reference	Method	Animals	E.C.F. % B. Wt.
Inglis et al. 1955	Sodium 24	3 Cows 7 Stirks 8 Calves	27.5 ± 4.5 35.9 ± 2.5 41.3 ± 2.3
Hix et al. 1959	Sodium Thiocyanate	5 Cattle Av. Bdy. Wt. 165 ± 5 kilos	28.5 ± 0.9
Anderson and Mixner 1960	Inulin Single Injection	1 Cow Wt. 466 k. 1 Calf Wt. 44 k.	15.3 29.3

TABLE 5

Total body water in cattle

Reference	Method	Animals	T.B.W. % B. Wt.
Haigh et al. (1920)	Dessication of Cadaver	Newborn Jersey Calves	73.4
		Newborn Hereford Calves	72.4 - 73.5
Ellenberger et al. (1950)	Chemical analysis of tissues	7 Newborn Calves	74.2
		17 Calves 3 month old	71.7
		16 Calves 6 month old	69.1
		6 Adult Cattle	64.9
Kraybill et al. (1951)	Antipyrine	6 Crossbred Cattle	52.2 ± 5.3
	Antipyrine	24 Hereford Cattle	54.4 ± 5.3
	Specific Gravity	6 Crossbred Cattle	51.9 ± 4.8
	Specific Gravity	24 Hereford Cattle	54.1 ± 5.8
Kraybill et al. (1952)	Antipyrine	30 Beef Cattle	54.4
	Specific Gravity	30 Beef Cattle	54.3
Swanson and Neathery (1956)	Antipyrine	14 Adult Dairy Cattle	60.0
Wellington et al. (1956)	Antipyrine	20 Adult Cattle	68.0 ± 5.5
	Tissue Analysis	20 Adult Cattle	67.9 ± 5.6
McFadden and Richards (1956)	Antipyrine	5 Calves week old	72.0 - 74.0
		5 Calves month old	67.0 - 69.0
Hix et al. (1959)	Antipyrine	5 Cattle Av.Wt. 165 kgs.	63.1 ± 2.5
Garrett et al. (1959)	Antipyrine	30 Beef Cattle	56.5

Because of the considerable differences in the volumes of a body fluid compartment when determined with various solutes, it has become customary to refer to the volume of the compartment as a solute "space". In Table 2 and Figure 2 are shown the spaces of some solutes used to estimate the volumes of the body fluid compartments in adult man and infants.

The volumes of the principal body fluid compartments have been measured by dilution methods in adult cattle by many investigators but few studies have been made of the body fluid in calves. The results obtained and details of the methods used and animals studied by previous investigators are shown in Tables 3, 4 and 5, which show respectively the plasma and blood, extracellular fluid and total body water volumes expressed as a percentage of body weight. By comparing the results shown in these tables with those in Table 2 it can be seen that the body fluid volumes per unit body weight in cattle are generally comparable to the volumes in man.

From the results shown in Table 3 it can be seen that plasma and blood volumes have been previously determined in young calves by two groups of workers.

Hansard et al. (1953) measured blood volumes in two calves aged 2 - 6 days and one calf aged 3 weeks. The breed or sex of the calves was not recorded. Blood volumes were determined by the single injection dilution method, using phosphorus^{32} labelled erythrocytes.

These workers also measured blood volumes in older cattle and found that the blood volume per kilogram body weight decreased with age.

Mixner and Robertson (1957) determined plasma and blood volumes on nine Friesian bull calves. The average weight of these calves was 48.7 kilograms. Their ages were not recorded. Blood and plasma volumes were determined by the single injection dilution technique using bromosulphonthalein. They found the mean plasma volume of the calves was 6.8$\pm$0.16 per cent of body weight.

They also found that the mean blood volume of these calves was 11.5±3.4 per cent of body weight. Plasma and blood volumes were also measured in adult cattle and they found that the volumes of plasma and blood per kilogram body weight were greater in calves than in adult cattle.

From the results shown in Table 4 it can be seen that extracellular fluid volume has been previously measured in calves by two groups of workers.

Inglis et al. (1955) measured radio-sodium spaces in eight Ayrshire calves aged three to five weeks. They found the mean sodium space in these calves was equal to 41.3±2.3 per cent of body weight. They observed that the radio-sodium concentration in plasma did not fall at an exponential rate until two and a half hours after the injection of the solute. Theoretically it is not desirable that a solute should take so long to equilibrate throughout its space, if the space is being measured by the single injection dilution method. It has also been shown that thiocyanate and radio-sodium diffuse into cells and hence measure a volume greater than the true extra-cellular fluid (Winkler et al. 1943, Manery and Haege 1941).

Anderson and Mixner determined inulin spaces in one Friesian calf and one Guernsey cow. The age of the calf was not recorded. These workers determined the inulin spaces by the single injection dilution method. The validity of their result using this method is questionable. Elkinton and Danowski (1956) stated that owing to the slow rate of diffusion of inulin in the interstial fluid, inulin spaces cannot be successfully measured by the single injection dilution technique. The inulin space should ideally be measured by the continuous infusion dilution method.

It is interesting to note that despite the questionable validity of the results obtained by Inglis et al. and by Anderson and Mixner, that the radio-sodium and inulin spaces in calves were greater per kilogram body weight than in adult cattle.

From the results shown in Table 5 it can be seen that total body water has been previously determined in young calves by three groups of workers.

Haigh et al. (1920) determined the composition of an unspecified number of newborn Jersey and Hereford calves by dessication of the cadavers. Theoretically this should be the most accurate estimate of the body water volume in cattle.

Ellenberger et al. (1950) determined the composition of seven newborn calves and seventeen three month old calves by direct chemical analysis. They found the average body water in these calves was 74.2 and 71.7 per cent respectively of body weight. Their result compares closely with that of Haigh et al. who found the percentage body water in newborn calves ranged from 72.4 - 73.5 per cent. Ellenberger et al. found that newborn calves had little or no body fat. These workers also found that the proportion of body water to body weight decreased with increasing age.

McFadden and Richards (1956) determined antipyrine spaces in five Friesian bull calves at one and four weeks of age. They found that the antipyrine space in the calves at one week ranged from 72 to 74 per cent of body weight. In the same calves the antipyrine space at four weeks ranged from 67 to 69 per cent of body weight.

These workers also showed that the volume of the antipyrine space in older calves was affected by the amount the animals were fed before measurements were made. Garrett et al. (1959) made a similar observation when measuring antipyrine spaces in sheep. They found they could alter the volume of the antipyrine space by hydrating or dehydrating the animals. They concluded that the antipyrine dilution method gave values for the volume of total body water which were too

variable to be successfully used to resolve the composition of the adult ruminant animal.

Studies by Whiting et al. (1960) showed that the volume of the antipyrine space in cattle was larger than that of the n. acetyl. antipyrine space, since the former diffused more extensively into the rumen.

The difficulty in using the dilution technique in adult ruminant animals to determine total body water is apparently associated with the large and variable amount of fluid in the intestinal tract, and in particular in the rumen. Total body water includes the transcellular fluid in the alimentary tract. In man the amount of fluid in the intestine is normally fairly constant and equal to approximately 1.5% of the total body water (Edelman et al. 1952), and hence this problem does not arise to the same extent.

In the young calf the gastro-intestinal tract is anatomically and physiologically more like that of a non-ruminant animal. It is probable that the young calf's gastro-intestinal tract contains a fairly constant volume of fluid and hence the total body water is not subject to such marked variations in its volume as occurs in adult cattle.

In this study the plasma and blood volumes have been determined with the dye T.1824 (Evan's blue). The use of dyes to measure plasma and blood volumes was first described by Keith et al. (1915). They used the dye brilliant vital red, but this dye has a marked disadvantage because its absorption spectrum is very like that of haemolysed plasma. Gregersen et al. (1935) introduced the use of the dye T.1824 (Evan's blue) and this dye has since been extensively used to measure plasma and blood volumes in man and animals.

In this study extra-cellular fluid volumes have been determined with sodium thiosulphate. One of the major difficulties in measuring extra-cellular fluid volume in animals is the selection of a suitable solute. Elkinton and Danowski (1956) stated that as inulin has the smallest volume of distribution of the solutes used to measure extracellular fluid, it is really a true measure of this compartment. Unfortunately inulin diffuses slowly in the interstial fluid and hence its space should ideally be measured by the continuous infusion method. The use of this method in large domestic animals is technically difficult. Winkler et al. (1943) have shown that thiocyanate and radiosodium diffuse into cells and hence measure a space which is greater than the true extra-cellular fluid. Cotlove (1954) and Nichols et al. (1953) suggested that the concept of extracellular fluid has been over-simplified. They suggested that the extracellular phase should be subdivided into two phases; the interstial fluid which is an ultrafiltrate of plasma, and the connective tissue. They have shown that inulin and thiosulphate do not diffuse into the connective tissue, whereas radio-sodium does. Newman et al. (1946), Brun (1949) and Cardozo and Edelman (1952) have shown that thiosulphate has a similar volume of distribution to inulin in man and the dog.

In this study total body water was measured in calves using urea. Unlike most solutes used to measure total body water, urea is a natural component of the body and it is necessary to assume that the metabolism of endogenous urea remains constant during the time required for the determination. The urea dilution method has given values for the total body water similar to those obtained by other methods. Painter (1940) found that the urea space in dogs was similar to the volume determined by dessication. Eggleton (1951) found a similar result in cats. Pawan (1954) and Srikantia and Gopalan (1957) found

the antipyrine space in man was similar to the urea space. Bradbury (1961) showed that the volume of the urea space was about 5% less than that of the deuterium space in man.

Houpt (1959) investigated the utilization of urea by sheep and found that after intravenous injection urea diffused rapidly into the rumen where it was metabolized by the ruminal microflora. He measured the total body water eight times on two sheep using the method described by Painter and found the mean volume of the total body water was 43.8±2.3% of body weight. From these results he concluded that the urea space does not include the rumen water as the urea was metabolized as soon as it entered the rumen.

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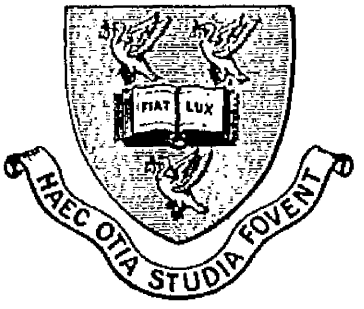

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R.

MATERIALS AND METHODS

The volumes of the principal body fluid compartments were measured in healthy calves by determining the T.1824 (Evan's Blue), sodium thiosulphate and urea spaces. The materials and methods used were as follows:

Determination of the plasma and blood volume

Plasma and blood volumes were determined from the T.1824 space and haematocrit by the method described by Gregersen (1951). The method was as follows:

A heparinised blood sample was collected from the jugular vein for determination of the haematocrit and the preparation of standards and blanks required for the photometric estimation of the dye concentration. A known amount of dye was then injected into the jugular vein. A venous blood sample was collected ten minutes later, or several samples were collected at intervals of up to an hour after injection. The dye concentration in the samples collected after injection was then determined photometrically by comparison with known standards. The volume of distribution of the dye was calculated by dividing the amount of dye injected, by the dye concentration ten minutes after injection, or by the theoretical dye concentration at the time of injection determined by extrapolation of the excretion curve. The blood volume was calculated from the plasma volume and the haematocrit using the equation

$$\text{Blood Volume} = \frac{\text{Plasma Volume}}{1 - (0.94 \times \text{Haematocrit } \%)}$$

Determination of the extracellular fluid volume

The extracellular fluid volume was determined from the thiosulphate space by the method described by Cardozo and Edelman (1952). The method used was as follows:

Before injection of the thiosulphate, a blood sample was collected from the jugular vein for the preparation of the plasma "blank" required in the analysis. The thiosulphate was then injected into the jugular vein as rapidly as possible. Venous blood samples were then collected at ten or fifteen minute intervals for the next hour. The thiosulphate concentration in the plasma was determined and plotted on a semilogarithmic scale against the time of collection. By extrapolation, the theoretical concentration of thiosulphate at injection time was determined. The thiosulphate space was calculated by dividing the amount injected by the theoretical concentration at the time of injection.

Determination of the total body water volume

The total body water volume was determined from the urea space by the method described by Painter (1940). The method used was as follows:

One or more venous blood samples were collected up to an hour before the injection of the urea, to determine the endogenous blood urea concentration and blood water content. The urea solution was then injected into the jugular vein and blood samples were collected thereafter at hourly intervals for up to four hours. The urea concentration in the samples was determined. The theoretical concentration of blood urea at the time of injection was determined by extrapolation of the excretion curve. The rise in blood urea concentration after injection was then calculated by subtracting the endogenous blood urea concentration from the theoretical concentration at the time of injection.

The effective rise in the urea concentration in the blood water was next calculated from the rise in the blood urea concentration and the blood water. The urea space was calculated from this figure and the amount of urea injected.

T.1824 used to determine plasma and blood volumes

The T.1824 used to determine plasma and blood volumes was purchased as a powder from British Drug Houses Limited[22]. The dye was prepared for injection by dissolving it in sterile physiological saline at a concentration of 0.05% to 0.2%. The volume of dye solution injected ranged from 2 - 10 ml., depending on the animal's size and the concentration of the dye used. No ill effects were observed following injection of T.1824, apart from one occasion when several calves died after injection of a solution contaminated with a pyrogenic bacterium.

Sodium thiosulphate used to determine extracellular fluid volume

The sodium thiosulphate used to determine the extracellular fluid volumes was "reagent grade chemical" purchased from British Drug Houses Limited and was prepared for injection by dissolving it in boiling distilled water. The amount of sodium thiosulphate injected ranged from 0.1 - 1.0 gm. per kg. body weight. In most cases a 10% solution was injected. Occasionally more concentrated solutions were injected to facilitate more rapid injection. Solutions of over 50% concentration were injected without apparent ill effects, and blood samples collected soon after injection showed no evidence of haemolysis.

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[22] British Drug Houses Limited, Poole, England.

Some calves were observed to "sniffle" immediately following injection and it was often observed that the urinary output was increased.

Urea used to determine total body water volumes

The urea used to determine total body water volumes was "reagent grade chemical" purchased from British Drug Houses Limited, and was prepared for injection as a 25% or 30% solution in sterile saline. Calves were injected with 50 ml. of the solution. No ill effects or haemolysis were observed following injection.

Determination of the spectoral absorption pattern of T.1824 in solution in bovine plasma and saline

Allen et al. (1953) showed that the spectoral absorption pattern of T.1824 varies when dissolved in the plasma of different animals. They and Reynolds (1953), found that the absorption peak of T.1824 and bovine plasma occurred at a wave length of about 620 mm.

An experiment was carried out to determine the absorption peak of T.1824 in bovine plasma. Solutions of different concentrations of T.1824 in bovine plasma and in saline were prepared. The percentage light absorption at different wave lengths of these solutions was measured in a Unicam S.P.600 Spectrophotometer[*]. Distilled water was used as a blank in the spectrophotometer when making these measurements.

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[*] Cambridge Instrument Company Limited, Cambridge, England

Determination of the relationship of the readings in a colorimeter and the concentration of T.1824 in bovine plasma

As an E.E.L. Photoelectric X.105 Colorimeter[++] was normally used to determine the T.1824 concentration in plasma when measuring plasma and blood volumes, an experiment was carried out to show that the colorimeter was suitable for this determination. The experiment was designed to show the relationship between the readings in the colorimeter and the concentration of T.1824 in bovine plasma.

Solutions of different concentrations of T.1824 in bovine plasma were prepared and their colorimeter readings determined. The colorimeter was zeroed on a plasma sample which did not contain T.1824. A No. 607 filter was used in the colorimeter. This filter has an optimum light absorption at a wave length similar to that found for a solution of T.1824 in bovine plasma.

Determination of the concentration of T.1824 in bovine plasma

The concentration of T.1824 in plasma was normally determined in an E.E.L. Photoelectric X.105 colorimeter with a No. 607 filter.

The results of the previous experiments showed that the colorimeter readings were proportional to the concentration of T.1824 in plasma. The T.1824 concentration in a sample was therefore determined by comparing its colorimeter reading with that of another sample which contained a known concentration of T.1824. The T.1824 concentration in the sample was calculated using the following equation:

$$\text{Unknown concentration} = \frac{S \times U}{R}$$

[++] Evans Electroselenium Limited, Harlow, England.

where S is the known concentration in a sample, and R is its reading in the colorimeter. U is the colorimeter reading of the sample whose concentration is unknown.

The method used to determine the T.1824 concentration in plasma was as follows; into two matched colorimeter tubes was pipetted a known volume (x) of plasma collected before the animal was injected with T.1824. To one of these tubes was added a known volume (y) of saline. This tube was used as a blank to set the colorimeter reading to zero. To the other tube was added the volume (y) of a saline solution containing a known concentration of T.1824. This tube was used as a standard.

Into a third matched colorimeter tube was pipetted the volume (x) of plasma collected after the animal had been injected with T.1824. To this tube was then added the volume (y) of saline. The colorimeter readings of the standard and the unknown sample were then determined. From these readings and the known concentration of T.1824 in the standard was calculated the concentration of T.1824 in the sample. The actual volumes of plasma (x) and saline (y) and the concentration of T.1824 in the standard solution, were varied in order to obtain the optimum readings in the colorimeter.

The concentration of T.1824 in blood was determined in a similar way to the T.1824 concentration in plasma. Into two centrifuge tubes were pipetted volumes (x) of blood collected before the animal was injected with T.1824. Volumes (y) of saline and the standard solution of T.1824 in saline were added to each of these tubes respectively. Into a third centrifuge tube was pipetted the volume (x) of blood collected after the animal had been injected with T.1824. To this tube was added the volume (y) of saline. The tubes were then centrifuged and the supernatant transferred to colorimeter tubes which were read in the colorimeter.

Determination of thiosulphate concentration in plasma

Thiosulphate concentrations in plasma were determined by two methods, the so-called "Indirect Iodometric Method", Newman et al. (1946), and the "Direct Method", Brun (1950). The first method was particularly suitable for micro-analysis but the second method was simpler and quicker. The principles and chemical reactions involved in these methods were as follows; in both methods plasma proteins are initially precipitated with sodium tongstate and sulphuric acid and the thiosulphate concentration is estimated on the supernatant obtained after centrifugation. In the direct method the thiosulphate in the supernatant is titrated directly with a dilute solution of iodine using starch solution as an indicator. The chemical reaction is shown in the following equation:

$$2Na_2S_2O_3 + I_2 = 2NaI + Na_2S_4O_6$$

The indirect method is a reverse titration which depends upon the liberation of iodine from potassium iodate by the thiosulphate in the test sample. The remaining iodine in the iodate is then titrated with sodium thiosulphate and from the amount of thiosulphate required to neutralize the remaining iodate can be calculated the amount of thiosulphate in the test sample. The chemical reactions in the process are shown in the following equations:

$$2KIO_3 + Na_2S_2O_3 + 2HCl = Na_2SO_4 + 2ICl + H_2O$$

$$2KI + 2ICl = 2I_2 + 2KCl$$

$$2KIO_3 + 10KI + 12HCl = 6I_2 + 12KCl + 6H_2O$$

$$2NA_2S_2O_3 + I_2 = Na_2S_4O_6 + 2NaI$$

The accuracy of the methods was determined by measuring the thiosulphate concentrations in solutions of known strengths.

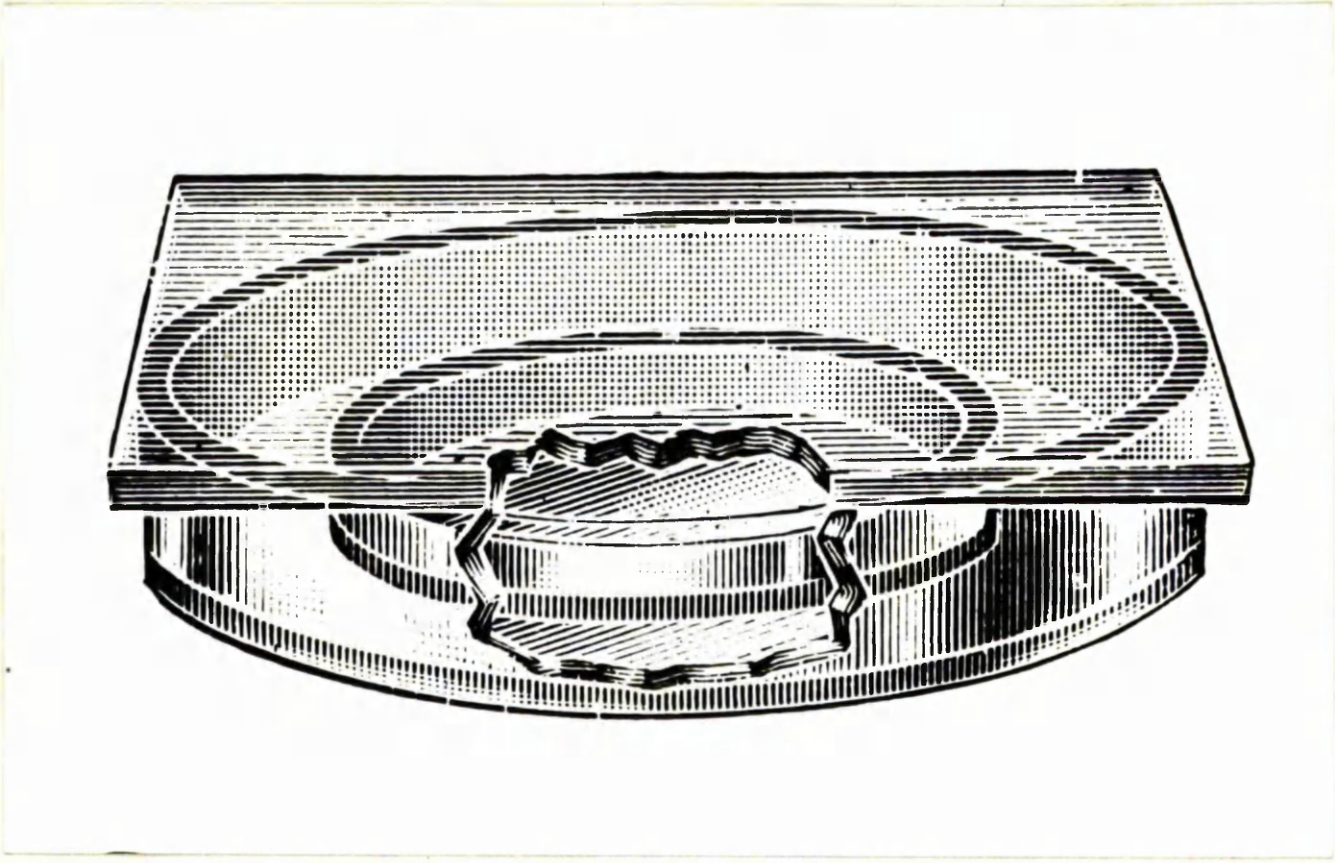
Determination of urea concentration in blood and urine

Blood urea concentrations were determined by the method described by Conway (1957) using the special unit illustrated in Figure 3. The chemical reactions involved can be summarised as follows. Blood urea is converted by the enzyme urease into ammonia and carbon dioxide; the liberated ammonia goes into solution in blood and is then displaced by addition of strong alkali; the ammonia is absorbed in a solution of boric acid, and this is finally titrated with hydrochloric acid.

The special unit consists of an inner and outer well separated from the atmosphere by a glass lid. The first two reactions, i.e. liberation of ammonia from the blood by urease and displacement by strong alkali take place in the outer well. The ammonia diffuses into the centre well where it is absorbed in boric acid and is there titrated with hydrochloric acid.

In this study blood urea concentration was determined on 1 ml. of blood instead of 0.2 ml. as described by Conway. Because a larger volume of blood was used it was necessary to increase the strength of the boric acid to 20 gm./litre instead of 10 gm./litre as suggested by Conway. Samples were incubated at room temperature with urease for six hours and with strong alkali for twelve hours.

Figure 3. A Conway Unit for Urea Determination



The accuracy of the method was determined by measuring the urea concentration in solutions of known strengths and by repeated determinations on a number of blood samples.

Determination of blood water

The water content of blood was determined by dessicating samples until the residue remained a constant weight. The samples were dessicated in porcelain dishes in an oven maintained at a temperature of 78° - 80°C. The time taken to dessicate samples was 3-4 days.

Determination of haematocrits

Haematocrits were determined by centrifugation of blood in Wintrobe haematocrit tubes at a centrifugal force of approximately 1,600g for at least two hours. Jennings et al. (1961) have shown that the haematocrit of bovine blood after centrifugation for two hours at a force of 1,600g contains approximately 6% trapped plasma.

The relation between centrifugation time and the haematocrit was demonstrated by the following experiment. Heparinised blood samples were collected from four calves and the haematocrits of these samples determined at half-hour intervals over a period of three hours. The samples were centrifuged in Wintrobe haematocrit tubes at a force of approximately 1,600g.

Collection of blood samples

Blood samples were collected from the external jugular vein. In healthy calves the jugular vein is not normally distended and to facilitate bleeding it was necessary to distend the vessel by compressing it at the base of the neck with a tourniquet or by digital pressure.

Venous blood samples were collected by venepuncture using a No. 16 or 18 British Wire Gauge 1 inch needle or through a thin nylon or polyethylene catheter. The nylon or polyethylene catheters used had an external diameter of 2 mm. Two methods were used to insert catheters. In the first method the jugular vein was punctured with a No. 9 British Wire Gauge 2 inch needle, and the catheter was threaded through the needle into the vein. The needle was then withdrawn, leaving the catheter in situ. The No. 9 British Wire Gauge needles were so big that they severely damaged the veins in calves, and the following technique using a smaller No. 12 British Wire Gauge 1½ inch needle was later adopted.

When the No. 12 needle was in the vein it was threaded with a stiff nylon rod of external diameter 1 mm. The needle was then withdrawn leaving the rod in the vein. The catheter was threaded over the rod into the vein and the rod then withdrawn, leaving the catheter in the vein. The catheter was inserted down the jugular vein so that the end was at the thoracic inlet. When the catheter was in situ a short piece of soft rubber tube was attached to the exposed portion to prevent it slipping into the vein and to facilitate the collection of blood samples or injection of solutions. When the catheter was not in use it was filled with heparinised saline to prevent blood clotting in the lumen, and closed with a Mohr's Clip. The blood pressure in the vein was usually sufficient to cause blood to flow spontaneously from the catheter, but, if not, a syringe was used to withdraw blood.

Preparatory to inserting the catheter the site of penetration was clipped free of most of the hair and the skin swabbed with a disinfectant solution. The site of penetration was then anesthetized by infiltration with a local anesthetic solution.

Blood samples were collected into clean, dry, heparinised centrifuge tubes or universal bottles. When plasma samples were required the blood was centrifuged as soon as possible after collection and the plasma separated.

Injection of solutes

T.1824 was injected by the following method. A volume of dye solution was drawn into a clean dry 2, 5 or 10 ml. capacity syringe. The syringe and a clean dry No. 18 British Wire Gauge 1 inch needle were then weighed. The needle was used to puncture the vein and when in situ the syringe was connected to it and the dye solution rapidly injected. Then, taking care not to suck blood into the syringe, the needle still attached to the syringe was withdrawn from the vein.

The syringe and needle were then carefully wiped free of any drops of blood and weighed. The difference in the weight of the syringe and needle before and after the injection of the dye, equalled the amount of dye injected.

Thiosulphate and urea solutions were injected from a pipette connected through an adaptor to a catheter inserted in the vein. The solution was blown out of the pipette. The adaptor and catheter were then flushed with saline to ensure that all the solute was injected.

Determination of body weight

Calves were weighed to the nearest pound on a spring or moving arm balance. Body weights were usually determined one to two hours after the calves had been given their morning feeds.

Experimental animals

The experimental animals used in this study were healthy male Ayrshire calves. They were purchased through a local market and nothing was known of their management or feeding before sale. The exact age of the calves was not known, but the majority of them would be about one week old when purchased. In the district from which the calves came, it is customary to sell the excess bull calves within the first week of life. It is the usual practice to feed such calves some colostrum during the first week of life. Care was taken when buying calves to choose animals which were obviously healthy, about one week old, and of a fairly uniform size.

The calves were fed twice daily on three pints of ostermilk*. Further details of the preparation, composition and method of feeding are described in Part II of the thesis. Some calves were given 500 mgm of chlortetracyclines per day to prevent diarrhoea. Body fluid compartments were measured within a week of the time that the calves were purchased.

* Ostermilk II, Glaxo Laboratories Ltd., Middlesex

Determination of the effects of altering the volume of the gastro-intestinal contents on the volume of the urea space

McFadden and Richards (1956) and Garrett et al (1959) have shown that the volume of the antipyrine space in adult cattle and sheep varies depending on the volume of the ingesta.

An experiment was carried out to determine if altering the volume of the gastro-intestinal contents affected the volume of the urea space in calves. Urea spaces and body weights were measured on two occasions on each of six calves. Three of the calves were fed on both occasions the measurements were made. The other three calves were fed on one occasion but starved on the next.

RESULTS

Spectoral absorption patterns of T.1824 in calf plasma and saline

Heparinised plasma samples were collected from two healthy calves. To 5 ml. of plasma from each animal was added a drop of a concentrated solution of T.1824 in saline. Different concentrations of these dye-stained plasma samples were prepared by diluting aliquots of them with more plasma in the proportions 1:4, 1:2 and 3:4. Solutions with similar concentrations of T.1824 in saline were also prepared.

The percentage light extinction of both plasma samples and solutions of T.1824 in plasma and saline were determined at different wave lengths in a Unicam S.P.600 Spectrophotometer++. Distilled water was used as a blank in the spectrophotometer when making these determinations.

++ Cambridge Instrument Company Limited, Cambridge, England

Figure 4 Percentage light extinction at different wave lengths of calf plasma and for T.1824 in plasma and saline

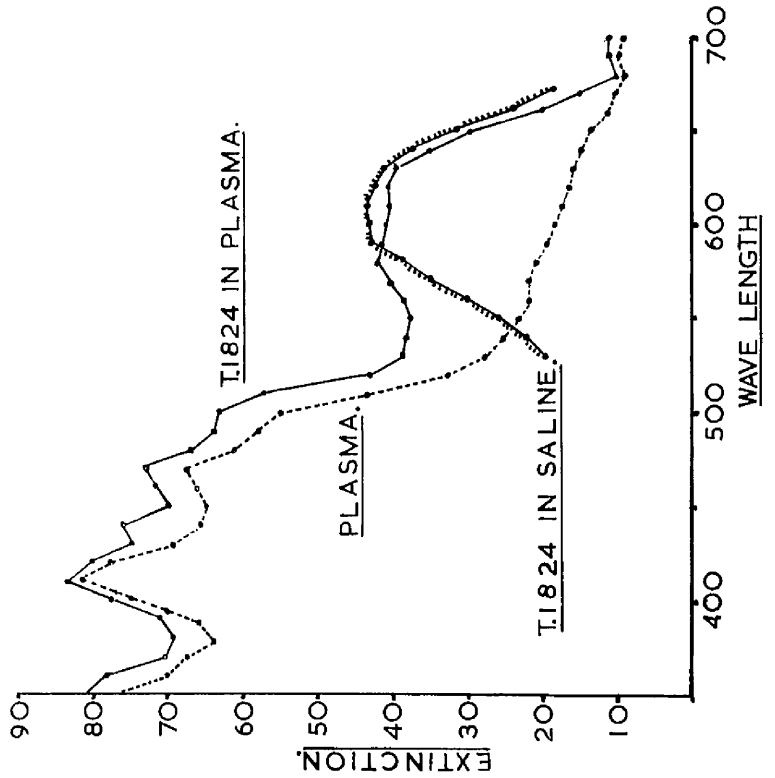
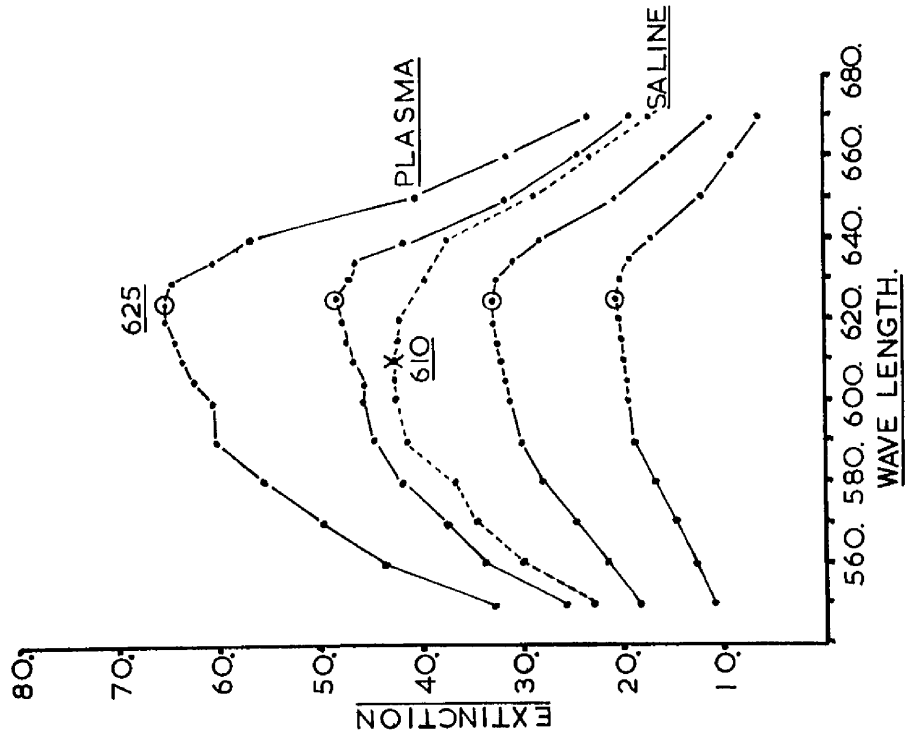


Figure 5 Percentage light extinction for different concentrations of T.1824 in plasma and saline



There was no difference in the absorption patterns of the plasma collected from the two calves. The absorption patterns of T.1824 in plasma from both calves were also identical.

In Figure 4 are shown the percentage extinctions at different wave lengths for plasma, for T.1824 in plasma, and for T.1824 in saline. From the results shown in Figure 4 it can be seen that both plasma and T.1824 in plasma had similar absorption patterns below a wavelength of 540 mu. Above the wave length of 540 mu the absorption patterns of plasma and T.1824 in plasma differed. The dye-stained plasma had an absorption peak at a wave length of about 620 mu which the unstained plasma did not have. The solution of T.1824 in saline had an absorption peak between wave lengths 550 to 650 mu.

In Figure 5 are shown in more detail the percentage extinctions between wave lengths 550 - 670 mu, for various concentrations of T.1824 in plasma and saline. From this figure it can be seen that the peak absorption for different concentrations of T.1824 in plasma always occurred at wave length 625 mu. The absorption peak for T.1824 in saline occurred at a wave length of 610 mu.

The results obtained in these experiments are similar to those obtained by Allen et al. (1953) and Reynolds (1953), who found the peak absorption for T.1824 in bovine plasma occurred at a wave length of about 620 mu.

Relationship of the readings in a colorimeter and concentration of T.1824 in bovine plasma

Plasma was collected from three calves, and the following experiment carried out using the plasma from each animal.

Into matched colorimeter tubes were pipetted aliquots of 3 ml. of plasma. To one of these tubes was added 4 ml of saline; this tube was used as a blank to zero the colorimeter. To the other tubes were added 4 ml. aliquots of standard solutions containing various concentrations of T.1824 in saline.

The tubes were read in the E.E.L. K.105 Colorimeter with a No.607 filter.

In Figure 6 are shown graphs representing the results obtained using plasma from the three calves. Each graph shows the colorimeter readings obtained when plasma was mixed with saline solutions containing different concentrations of T.1824.

From these graphs it can be seen that the colorimeter readings obtained were proportional to the concentration of T.1824.

Figure 6

Relation of T.1824 concentrations in plasma to colorimeter readings

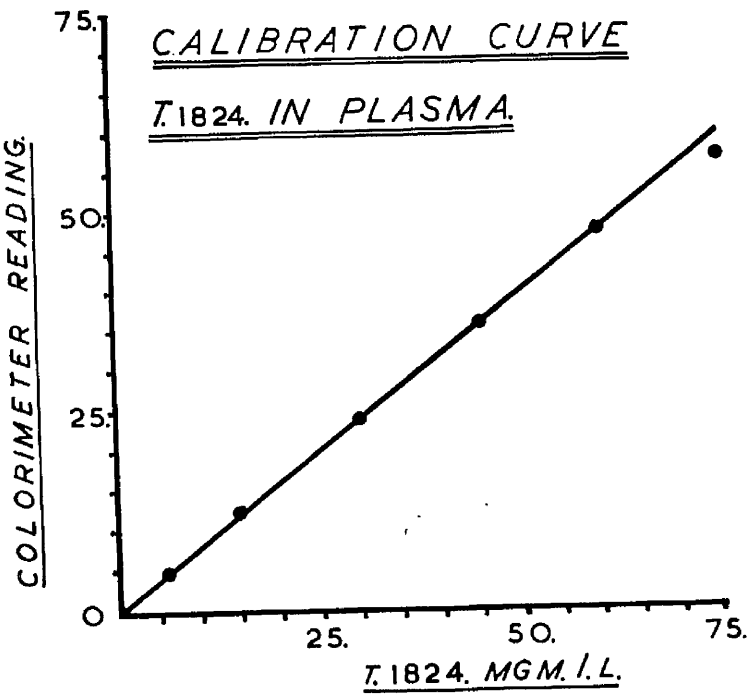
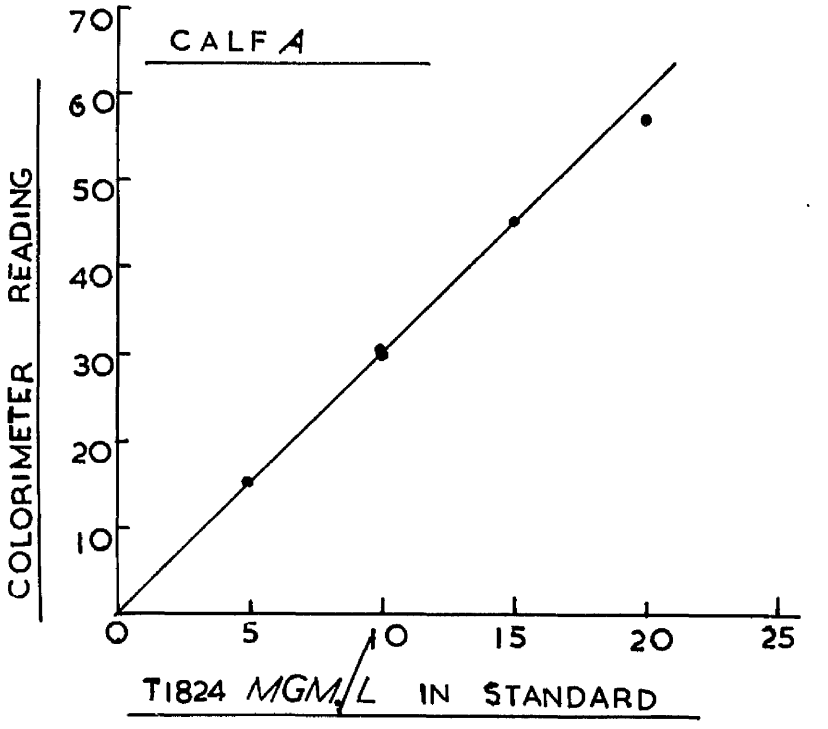
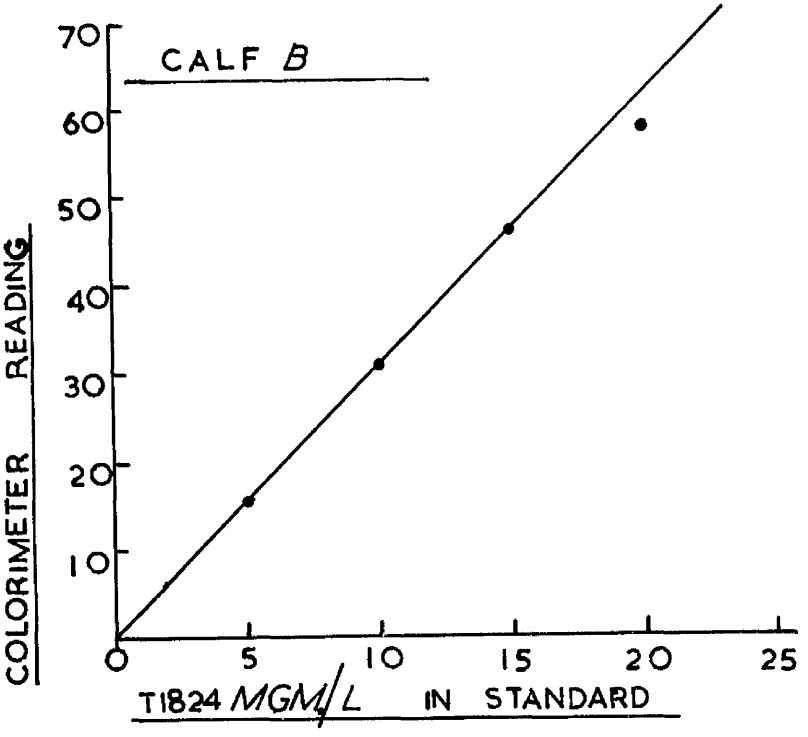


Figure 6 Relation of T.1824 concentrations in plasma to colorimeter readings





The accuracy of the Direct and Indirect Iodometric Methods for measuring sodium thiosulphate concentration in plasma

The accuracy of the two methods used to measure plasma thiosulphate concentrations was determined by measuring thiosulphate concentrations in plasma which contained a known amount of thiosulphate.

The concentration of thiosulphate was measured by the 'Direct Method' five times on each of two plasma samples. These two plasma samples contained 62 and 123 mgm. thiosulphate per 100 ml. of plasma respectively.

The concentration of thiosulphate was measured by the 'Indirect Method' twice on each of five plasma samples. These plasma samples contained 49, 62, 99, 123 and 246 mgm. thiosulphate per 100 ml. of plasma respectively.

The results obtained are shown in Table 6. This table shows the known and the measured concentrations of thiosulphate in the plasma samples.

TABLE 6

The accuracy of the methods used to measure thiosulphate concentration in plasma

Method	No. of measurements	Known conc. mgm./100 ml.	Measured conc. Mean and S.D. mgm./100 ml.
Direct	5	62	63 ± 2
	5	123	124 ± 2
Method	**No. of measurements**	**Known conc. mgm./100 ml.**	**2 Measured conc. mgm./100 ml.**
Indirect	2	49	49 and 49
	2	62	59 and 60
	2	99	100 and 100
	2	123	121 and 121
	2	246	249 and 249

From the results shown in Table 6 it can be seen that both methods used to measure plasma thiosulphate concentrations were accurate.

The accuracy of the Conway method for measuring urea concentration in blood

The accuracy of the Conway method was determined by measuring urea concentrations in solutions which contained known amounts of urea. Thirteen measurements were made of the urea concentration in a solution which contained 50 mgm./100 ml. and nine measurements of a solution which contained 100 mgm./100 ml. The results obtained are shown in Table 7. The table shows the known concentration of urea in the solutions, the number of measurements made, and the means and standard deviations for the measured urea concentrations.

TABLE 7

The accuracy of the Conway method for measuring urea concentration

No. of measurements	Known conc. mgm./100 ml.	Mean and S.D. of measured conc. mgm./100 ml.
13	50	49.7 ± 1.8
9	100	100 ± 0.8

Blood urea concentrations were also measured three times on blood samples collected from five calves. In Table 8 are shown the urea concentrations determined in triplicate on each of the five blood samples.

TABLE 8

Triplicate measurements of blood urea concentration

Calf	Blood Urea Conc. mgm./100 ml.		
	(1)	(2)	(3)
A	22	22	22
B	25	24	24
C	23	23	23
D	25	25	25
E	28	28	28

From the results shown in Tables 7 and 8, it can be seen that the Conway method was accurate for measuring urea concentrations and gave repeatable results on the same blood samples.

Blood water content

Blood water content was determined by dessication on thirty one blood samples collected from twenty five calves. The mean and standard deviation blood water content of the samples was 82.5 ± 1.69%. This value was similar to that found by Haigh et al. (1920) of 82.4%.

Normal variations in endogenous blood urea concentration

To determine if the normal variations in the endogenous blood urea concentration were likely to affect the determination of the urea space, five healthy calves were bled at hourly intervals for five hours and the blood urea concentration in the samples measured. The first blood samples were collected about two hours after the calves had been given their morning meal. The period of the day covered by this experiment corresponded to that used when measuring urea spaces in calves. The results obtained are shown in Table 9.

TABLE 9

Variations in the endogenous blood urea concentration

Calf	Blood urea mgm./100 ml.				
Hour	1	2	3	4	5
1	23	23	24	23	24
2	37	37	37	38	37
3	31	30	31	32	32
4	37	36	37	35	bottle broken
5	31	31	32	32	34

From the results shown in Table 9 it can be seen that the normal variations over a period of five hours in the endogenous blood urea concentrations, were unlikely to have any marked effect when determining urea spaces.

Relation between centrifugation time and haematocrit

Venous blood samples were collected from four calves and haematocrits determined at thirty minute intervals for three hours. The haematocrits were determined in Wintrobe haematocrit tubes which were centrifuged at a force of approximately 1600g. In Table 10 are shown the percentage haematocrits for each blood sample determined at thirty minute intervals for three hours.

TABLE 10

Relation of centrifugation time and haematocrit

	Minutes centrifugation					
	30	60	90	120	150	180
Calves	% Haematocrit					
1	41	37	36	35	35	34
2	39	34	33	33	32	32
3	34	31	29	28	28	27
4	28	25	24	23	22	22

From the results shown in Table 10 it can be seen that the haematocrits remained fairly constant after centrifugation for 120 minutes. The average difference in the haematocrit determined after 30 minutes and 120 minutes centrifugation was 6%. These results indicate the necessity for adequate centrifugation when determining the bovine haematocrit.

Figure 7 The change in plasma T.1824 concentration in a calf after the intravenous injection of 40 mgms of T.1824

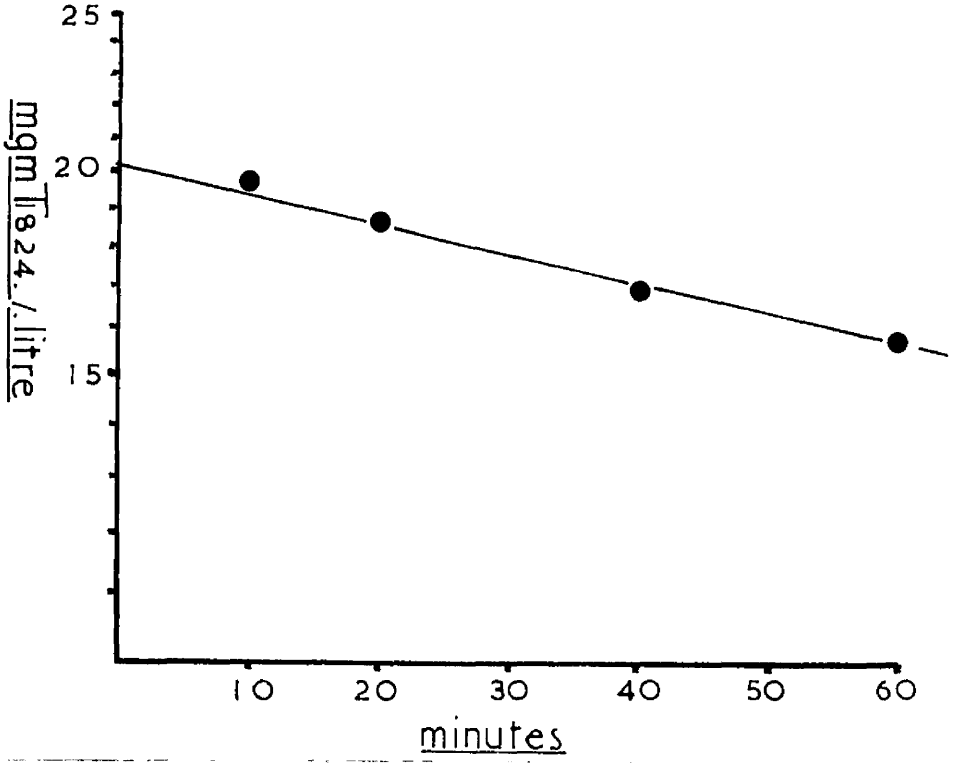
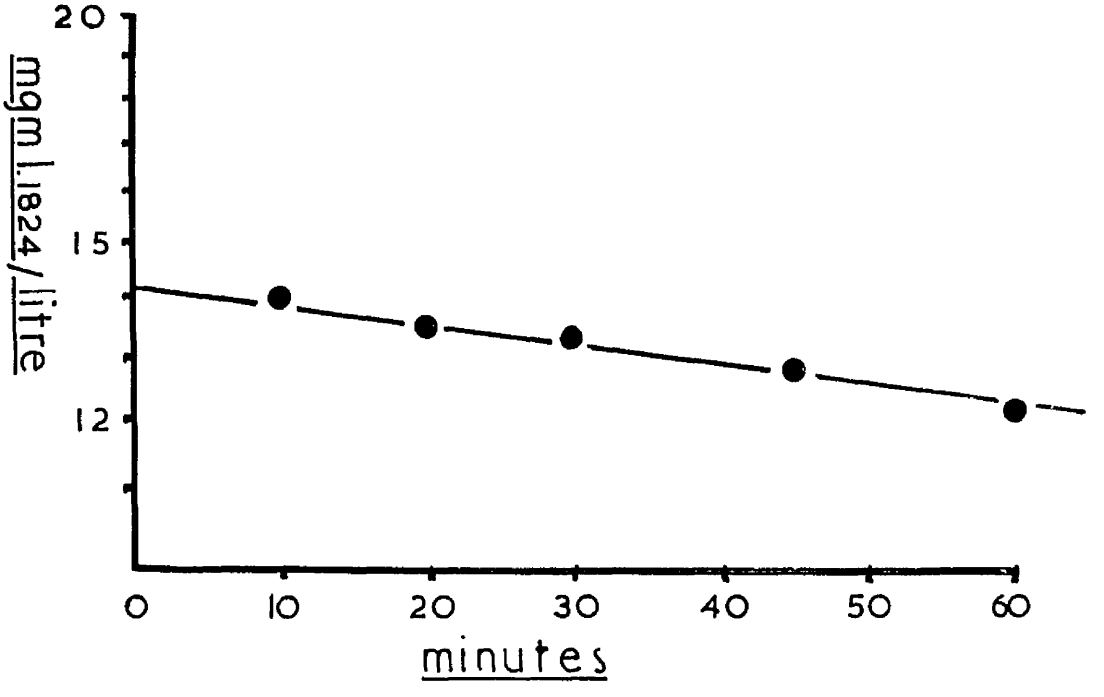


Figure 8 The change in plasma T.1824 concentration in a calf after the intravenous injection of 40 mgms of T.1824



Changes in plasma T.1824 concentration after intravenous injection of the dye

In Figures 7 and 8 are shown the changes in plasma T.1824 concentration following the intravenous injection of 40 mgms of dye into two calves. The excretion curves shown for these two calves were typical of those seen in other calves in this study.

From these figures it can be seen that the dye was excreted at an exponential rate. It can also be seen that the dye concentration ten minutes after injection was similar to the concentration at zero time determined by extrapolation of the excretion curve. The excretion curves for T.1824 in calves in this study were comparable to those described in adult cattle by Reynolds (1953).

Changes in plasma thiosulphate concentration after intravenous injection

In Figures 9 and 10 are shown the changes in plasma thiosulphate concentration following intravenous injection of 10 gms of thiosulphate into the calves. The excretion curves shown in these figures were typical of those seen in other calves in this study.

From these figures it can be seen that the thiosulphate concentration in plasma fell at an exponential rate. In most experiments it was found that when the plasma thiosulphate concentrations in samples collected at intervals after injection were plotted on a semilogarithmic scale, the line of fit drawn through these points usually passed through the point showing the plasma thiosulphate concentration ten minutes after injection. Occasionally it was found that the point showing the concentration ten minutes after injection was slightly above the line of fit drawn through the other points. This

Figure 9 The change in plasma thiosulphate concentration in a calf
The change in plasma thiosulphate concentration in a calf
after the intravenous injection of 10 gms of thiosulphate

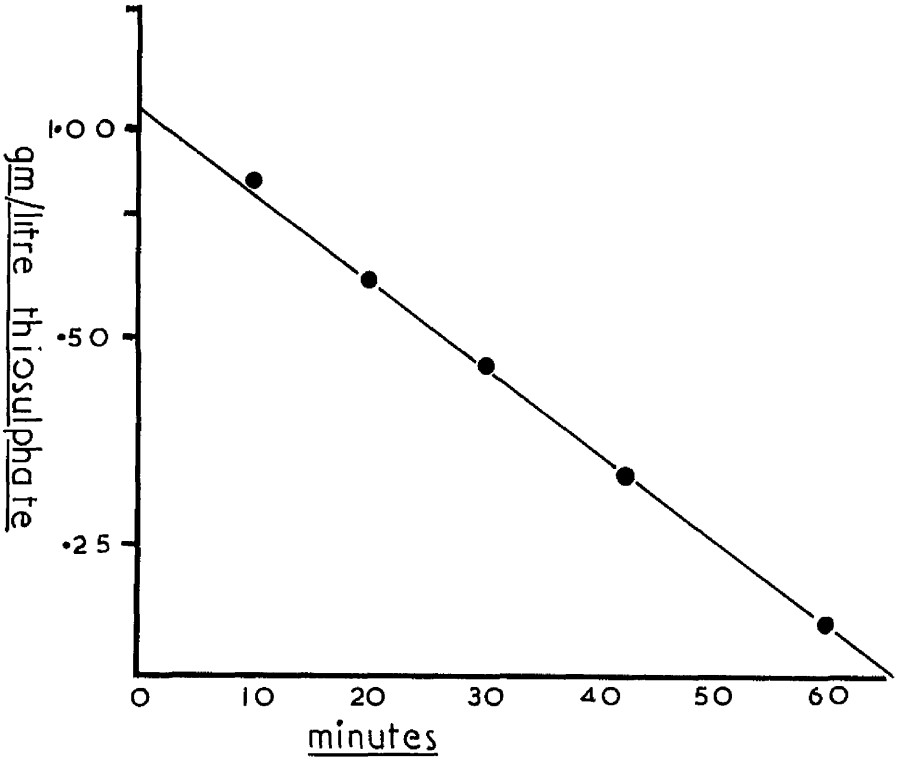


Figure 10 The change in plasma thiosulphate concentration in a calf after the intravenous injection of 10 gms of thiosulphate

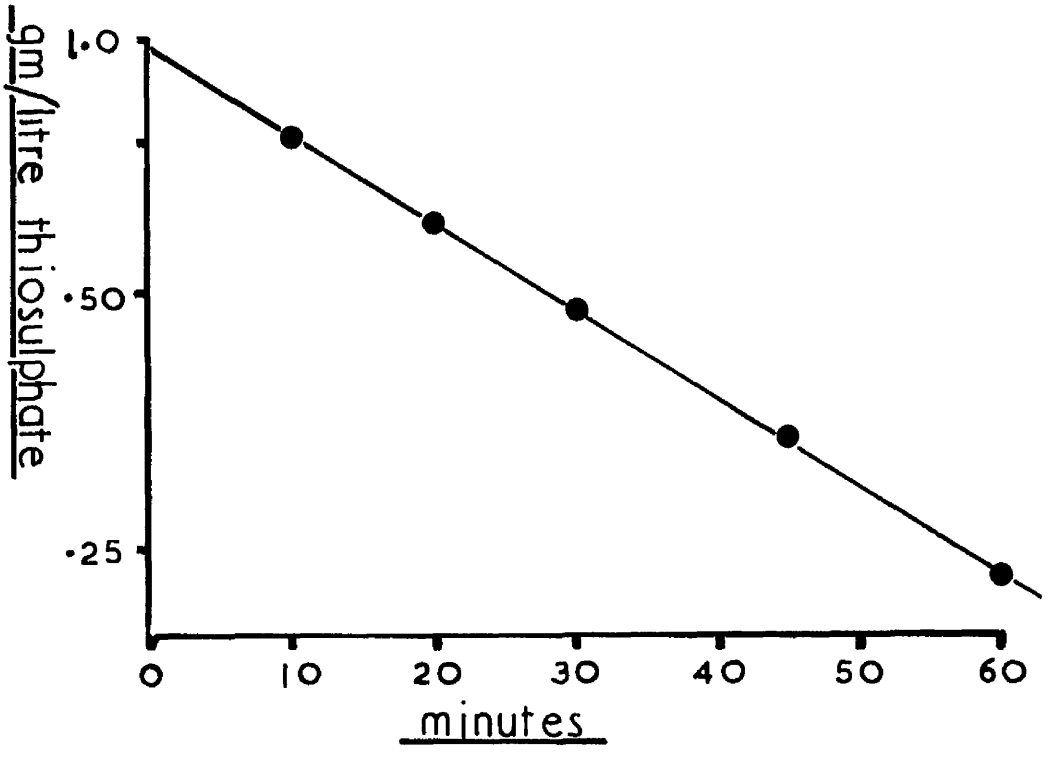


Figure 11 The change in blood urea concentration in a calf after the intravenous injection of 25 gms of urea

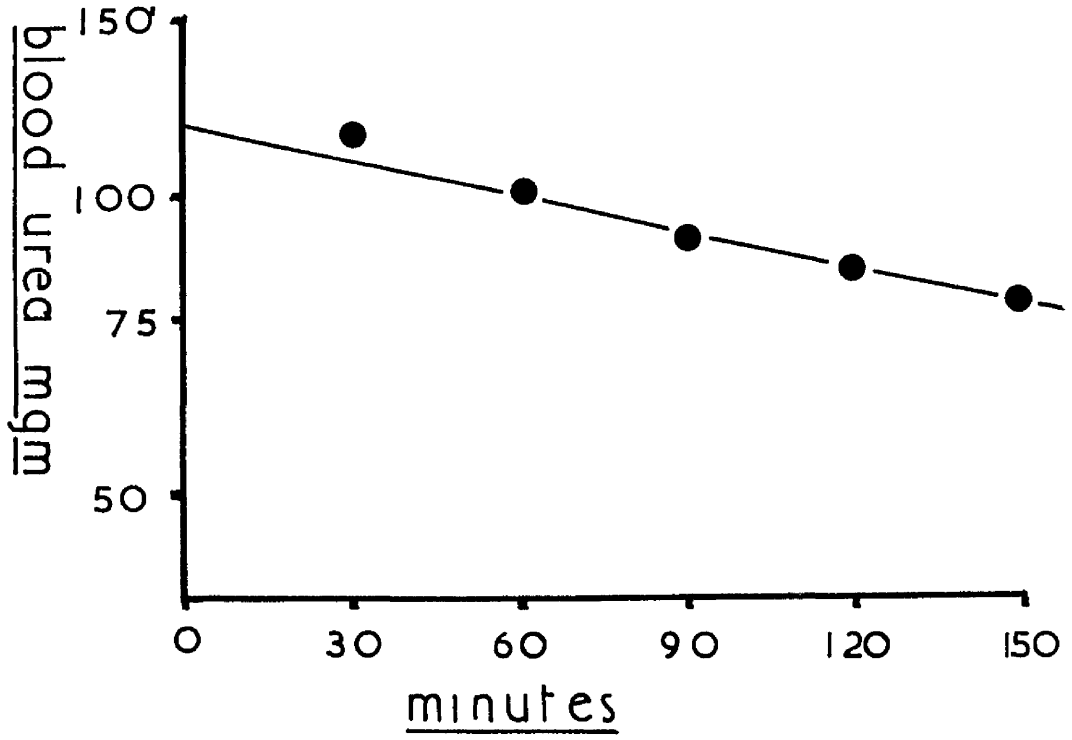
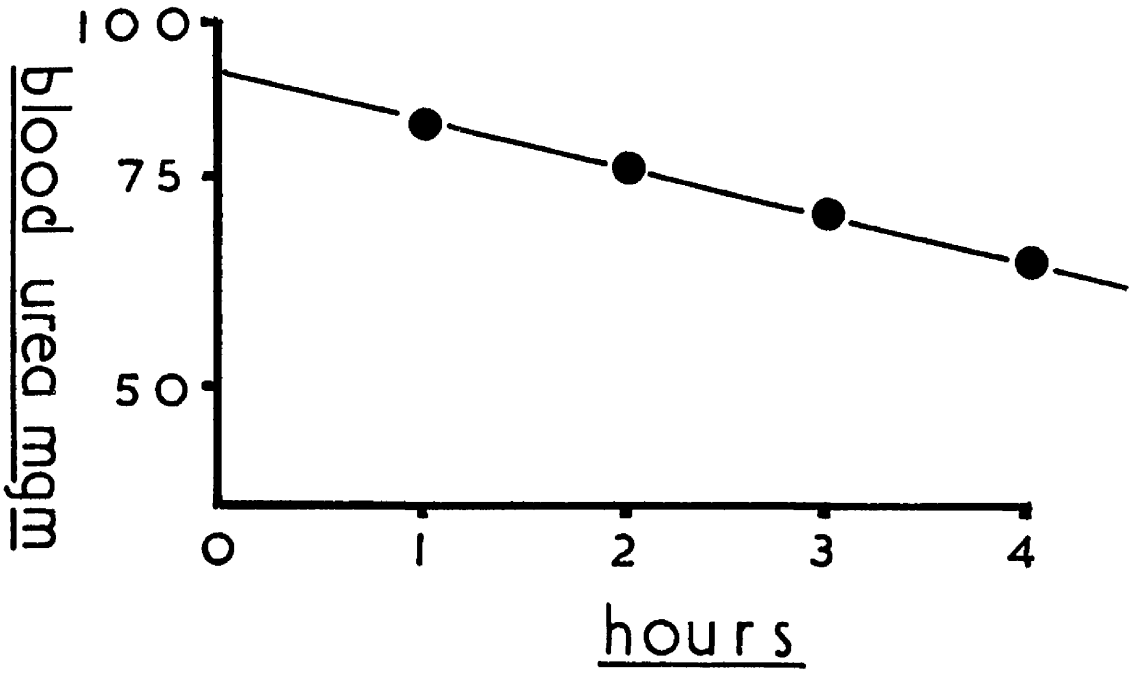


Figure 12 The change in blood urea concentration in a calf after the intravenous injection of 25 gms of urea



observation presumably indicates that the thiosulphate had usually established equilibrium in its concentration throughout the extracellular fluid within ten minutes of injection.

The type of excretion curve seen in calves in this study was similar to those described in humans by Cardozo and Edelman (1952)

Changes in blood urea concentration after intravenous injection

In Figures 11 and 12 are shown the changes in blood urea concentration in two calves which were injected intravenously with 25 gms of urea. The excretion curves seen in these two figures were typical of those seen in other calves in this study. In Figure 11 are shown the blood urea concentrations determined at half hour intervals after injection. From the figure it can be seen that the line of fit drawn through the points showing blood urea concentrations, passed below the point showing the concentration thirty minutes after injection. From this result it was assumed that the urea required more than thirty minutes after injection to equilibrate throughout body water. When blood samples were collected at hourly intervals after injection, as in Figure 12, it was found that the line of fit drawn through the points showing blood urea concentration, invariably passed through the point showing the blood urea concentration one hour after injection. This presumably indicates that the urea had established equilibrium in its concentration throughout the body water within an hour of the time of injection.

The type of urea excretion curves seen in calves in this study were similar to those described in dogs by Painter (1940).

Effects of altering the volume of the gastro-intestinal contents on the urea spaces in calves

The effects of altering the volume of the gastro-intestinal contents on the urea spaces were determined in an experiment on two groups each of three calves. Urea spaces were measured twice in each calf. The calves in the first group were fed one hour previously on both occasions urea spaces were measured. The calves in the second group were fed one hour previously on the first occasion and starved on the second.

In Table 11 are shown the urea spaces and body weights of the calves in the first group, which were fed on both occasions measurements were made. The table also shows the differences in the urea spaces and body weights of each calf when measured on two occasions. From the results shown in the table it can be seen that the body weights of none of the calves differed by more than 0.5 kg. and the urea spaces by more that 0.5 litre. The differences in the body weights of calves A and B were paralleled by similar differences in the urea spaces. In calf C there was a decrease in the body weight of - 0.5 kg. and an increase in the volume of the urea space of +0.2 litre.

In Table 12 are shown the body weights and urea spaces of the calves that were fed on one occasion and starved on the next. From the results shown in this table it can be seen that all three calves lost over 2 kg. in body weight when starved. The volume of the urea space decreased by 0.2 litre in one calf but increased in the other calves by 0.8 and 0.3 litre respectively.

From the results obtained it can be seen that the body weights and body waters did not vary by more that 0.5 kgms and 0.5 litres respectively, on repeated estimations, in calves which were fed on both occasions. When the calves were alternately fed and starved, it was found that they all lost more that 2 kgms in body weight but their body water did not vary by more than 0.8 litres.

TABLE 11

Body weights and urea spaces of calves fed on both occasions

Calf	1st Measurement		2nd Measurement		Difference	
	Body weight kg.	Urea space litres	Body weight kg.	Urea space litres	Body weight kg.	Urea space litres
A	34.3	27.4	34.0	27.1	-0.3	-0.3
B	29.5	24.5	30.0	25.0	+0.5	+0.5
C	27.5	20.2	27.0	20.4	-0.5	+0.2
H	29.1	22.0	29.5	23.2	+0.4	+1.2
Mean					+0.1	+0.4
S.D.					±0.507	±0.627

TABLE 12

Body weights and urea spaces of calves fed and starved

Calf	1st Measurement		2nd Measurement		Difference	
	Body weight kg.	Urea space litres	Body weight kg.	Urea space litres	Body weight kg.	Urea space litres
D	32.7	24.1	30.2	24.9	-2.5	+0.8
E	34.5	25.5	32.0	25.3	-2.5	-0.2
F	30.0	22.9	27.9	23.2	-2.1	+0.3
mean					-2.37	+ 0.3
S.D.					± 0.231	± 0.5

From these experiments it was concluded that varying the milk intake had a marked effect on the body weight of calves. Restricting the milk intake did not, however, have such a marked effect on the volume of the urea space. _see insert A.

Plasma and blood volume in calves

Plasma and blood volumes in calves were determined by the single injection dilution method using T.1824.

Plasma volumes were determined on sixty seven occasions on sixty five calves. The body weights of these calves ragned from 24 - 46 kilograms. The results obtained for individual calves are shown in Appendix I. The appendix shows the body weight of each calf and its plasma volume in litres and also expressed as a percentage of body weight. The mean and standard deviation of the plasma volumes of the calves was 6.6 ± 0.9 per cent of body weight.

Blood volumes were determined on forty occasions on thirty eight calves. The body weights of these calves ranged from 24 to 40 kilograms. The results obtained for individual calves are shown in Appendix I. The appendix shows the body weight of each calf and its blood volume in litres and also expressed as a percentage of body weight. The mean and standard deviation of the blood volumes of the calves was 11.0 ± 2.0 per cent of body weight.

Haematocrits of calves

Haematocrits were measured on blood samples collected from thirty seven calves whose body weight ranged from 24 to 40 kilograms. The results obtained for individual calves are shown in Appendix II. The mean and standard deviation of the haematocrits in the calves was 43 ± 8 per cent.

insert A

The differences were calculated between the body weights and urea spaces determined on the first and second measurements on each calf. The mean values of these differences were calculated for each group of calves and these means compared statistically using a 't' test. It was found that there was no significant difference in the extent by which the mean urea spaces of the two groups differed. There was however a significant difference in the extent by which the mean weights of the two groups differed. This difference was significant at the 0.1% level. It was concluded that starvation had no significant effect on the urea space but it did have a significant effect on body weight. The urea spaces measured in each calf were expressed as a % of body weight. The difference was then determined between this percentage when determined at the first and second measurements on each animal. The means of the differences were then calculated for the two groups of calves. The respective means were 2.6±2.1 in the calves fed on both occasions and 5.7±3.1 in the calves which were alternately fed and starved. These means were compared using a 't' test. They were not significantly different and it was concluded that starvation had no significant effect on the volume of the urea space when expressed as a percentage of body weight.

Extracellular fluid volumes in calves

Extracellular fluid volumes were determined in calves by the single injection dilution method using sodium thiosulphate.

Thiosulphate spaces were determined on ten calves. The body weights of these calves ranged from 27 to 45 kilograms. The results obtained for individual calves are shown in Appendix III. The appendix shows the body weight of each calf and its extracellular fluid volume in litres and also expressed as a percentage of body weight. The mean and standard deviation of the extracellular fluid in these calves was 24.2 ± 2.6 per cent of body weight.

Total body water in calves

Total body water volumes were determined in calves by the single injection dilution method using urea.

Urea spaces were determined on twenty five calves. The body weights of these calves ranged from 21 to 36 kilograms. The results obtained for individual calves are shown in Appendix IV. The appendix shows the body weight of each calf and its total body water in litres and also expressed as a percentage of body weight.

The mean and standard deviation of the total body water volume in the calves was 73.6 ± 6.4 per cent of body weight.

Discussion

From the results obtained in this study it was found that the average volumes of the body fluid compartments in Ayrshire bull calves aged between one and two weeks were as follows;

Plasma volume (T.1824 space) 6.6 ± 0.9 % of body weight,

blood volume (T.1824 space) 11.0 ± 2.0 % of body weight,

Extracellular fluid volume (Thiosulphate space) 24.2 ± 2.6 % of body weight,

Total body water (Urea space) 73.6 ± 6.4 % of body weight.

The values obtained for plasma and blood volumes in this study were comparable to those determined by previous workers in young calves. Hansard et al (1953) using radio-phosphorus found the average blood volume in two young Hereford calves was equal to 12 % of body weight. Mixner and Robertson (1957) determined plasma and blood volumes in nine calves with bromosulphonthalein. They found the mean plasma and blood volumes were equal to 6.8 ± 0.16 and 11.5 ± 3.4 % of body weight respectively. These workers both found that the plasma and blood volumes expressed as a per centage of body weight were greater in calves than in adult animals. Dalton and Fisher (1961) determined plasma and blood volumes in adult Ayrshire cattle and found that the mean plasma and blood volumes were equal to 5.0 ± 0.77 and 6.3 ± 0.83 % of body weight respectively. On statistical analysis it was found that the mean plasma and blood volumes in calves were significantly higher ($P < 0.1$ and $P < 0.1$ respectively) than the corresponding mean volumes in adult cattle.

From the results shown in Table 4 it can be seen that the mean volume of the thiosulphate space determined in calves was smaller than the volumes of the radio-sodium and inulin spaces determined by previous workers. The validity of the methods used by these workers has been previously discussed and it is difficult to compare the results obtained in this study with their

findings. From the results shown in Table 4 it can been seen that Inglis et al (1955) and Anderson and Mixner (1960) found that the volumes of the radio-sodium and inulin spaces were larger when expressed as a percentage of body weight in calves than in adult animals. Thiosulphate spaces were also determined in adult cattle, Dalton 1963, and it was found that the mean thiosulphate space in calves was significantly larger ($P < 0.1$ and $P < 0.5$) than the mean thiosulphate spaces in castrated male Ayrshire cattle aged 6-18 months and in Ayrshire cows respectively.

It is of interest to compare the ratio of the plasma volume to extracellular fluid in man and cattle. Elkinton and Danowski (1956) gave the range for the plasma volume (T.1824 space) in man as 4.2 - 4.8 per cent of body weight. This volume is equivalent to approximately one quarter of the mean volume of the thiosulphate space (17% of body weight). In this study it was found that the mean plasma volume in calves and adults were 6.6 and 5.0 % of body weight. These values are also approximately one quarter of the mean thiosulphate spaces determined in this study (calves 24.2 % and cows 21.0 % of body weight)

The values found for total body water in this study were similar to those determined in calves by previous workers using different methods. Haigh et al (1920) found the total body water in newborn calves by dessication ranged from 72.4 - 73.5 % of body weight. Ellenberger et al (1950) found the body water in seven newborn calves by chemical analysis was equal to 74.2 % of body weight. McFadden and Richards (1956) found the antipyrine space in week old calves was equal to 72 - 74 % of body weight.

It would therefore appear that the method used to determine body water in this study gave reliable values for resolving the composition of the young calf. It was also shown that varying the amount of milk fed to the calves had a marked effect on the body weight but did not substantially affect

the volume of the urea space.

The value found for the mean haematocrit in this study (43 ± 8 %) was similar to that determined by Greatorex (1954). He determined haematocrits in calves of four dairy breeds by centrifuging the samples for one hour. The centrifugal force applied was not recorded. He found the following mean values for haematocrits, six newborn calves 48.5 %; five week-old calves 42 %, and four calves aged two weeks 43 %. Holman (1956) measured haematocrits in Ayrshire calves by centrifugation of blood samples for one hour. The centrifugal force applied was not recorded. The mean values found by Holman were as follows; eleven newborn calves 42.5 %; twelve calves aged one week 36 %, and seven calves aged two weeks 37 %. Holman's values were lower than those previously determined by Greatorex and also those found in this study. Haematocrits were also determined in adult cattle as described in the enclosed publication. The mean haematocrit in adult cattle was 33.0 ± 3.8 % which was significantly lower ($P < 0.1$) than the mean haematocrit found in calves of 43.0±8.2 %.

PART III

THE COMPOSITION OF BLOOD AND PLASMA IN HEALTHY AND DIARRHOEIC CALVES

AND

THE EFFECTS OF DIARRHOEA ON HAEMATOCRIT, PLASMA VOLUME AND BODY WATER

Introduction

Diarrhoea is probably the commonest cause of disturbance in fluid and electrolyte metabolism in newborn animals. The biochemical and physiological changes associated with diarrhoea have been extensively investigated in human infants and a few studies also made upon diarrhoeic calves.

Blaxter and Wood (1953) found that in a healthy calf fed 8 kgms of milk per day, the daily faecal output over a period of ten days was; nil, 308gms, nil, 35gms, nil, nil, nil, 141gms, nil, 60gms. They also observed that other healthy calves often did not defaecate for several days. They found that the average faecal output of healthy calves over several days was approximately 50gms per day and that these faeces had a dry matter content of 25 - 35 per cent. In diarrhoeic calves they found that the faecal output was markedly increased and that the dry matter content of diarrhoeic faeces was sometimes less than 5 per cent. In one diarrhoeic calf fed 4 kgms of milk daily, the faecal output on successive days was; 118gms, 495gms, 1,165gms, 1,797gms, 935gms, 2,503gms, 1,063gms, 86gms, 186gms, 1,380gms, 900gms, and 548gms.

Blaxter and Wood analysed 160 faecal samples collected from 8 calves on 20 occasions. These calves were fed a constant amount (quantity not stated) of margarine homogenised into skim milk. The faeces were classified according to the total weight passed daily by each calf. A daily faecal output of 0 - 200 gms was considered normal, an output of 200 - 500 gms daily as 'loose' and an output of more than 500 gms daily as 'diarrhoeic'. The results obtained are shown in Table 33.

TABLE 33

Faecal constituents excreted by healthy and diarrhoeic calves

From Blaxter and Wood (1953)

Amount excreted per day	Type of faeces		
	Normal	Loose	Diarrhoeic
Water gms	51	280	927
Dry matter gms	12.5	42.5	93.5
Total fat gms	4.1	17.5	37.4
Protein gms	5.5	22.3	41.0
Ash gms	1.5	5.3	10.6
Calcium m.eq	21.6	31.2	98.8
Magnesium m.eq	11.4	16.0	24.0
Sodium m.eq	5.0	9.5	41.6
Potassium m.eq	2.2	3.0	39.9
Phosphorus m.eq	21.0	39.0	94.0

Normal stools..... daily output 0 - 200 gms / day

Loose stools..... daily output 200 - 500 gms / day

Diarrhoeic stools.. daily output over 500 gms / day

Their results showed that while the increase in faecal output in diarrhoea was mainly due to an increase in faecal water, there was also an increase in the amount of dry matter lost in the faeces. The dry matter content of the diarrhoeic faeces consisted mainly of undigested fat and protein. Blaxter and Wood calculated that the loss of fat and protein in the faeces during diarrhoea often reduced the digestibility of the diet to below 40%, compared with 97% digestibility in health.

From the results shown in Table 33 it can be seen that the amounts of electrolytes lost in diarrhoeic faeces were greater than the losses in normal faeces. Blaxter and Wood found that in some calves more sodium was lost in the faeces than potassium. In other calves, particularly those which were rapidly loosing weight, more potassium was lost than sodium. They stated that the losses of sodium and potassium in the faeces may exceed the dietary intake and that the excess lost in the faeces was due to failure to reabsorb the intestinal secretions. They also stated that the increased faecal loss of calcium, magnesium and phosphate in diarrhoea was due to a failure to absorb these electrolytes present in the milk diet.

Blaxter and Wood carried out balance studies on two calves fed the same diet. They did not state the type of diet fed. One calf produced normal faeces and the other was affected with diarrhoea. They found that despite a high faecal nitrogen loss in the diarrhoeic calf compared with the healthy calf, the urinary nitrogen loss was similar in the two animals. The amount of sodium and potassium excreted in the urine was also similar in the two animals despite a greater faecal loss of these electrolytes in the diarrhoeic animal. They quoted other experimental work on an unspecified number of calves, in which they found that healthy calves excreted an average of 124 m.eq. of sodium in the urine per day and 7 m.eq. per day in

the faeces. Diarrhoeic calves fed the same diet excreted on the average 85 m.eq. of sodium per day in the urine and 139 m.eq. per day in the faeces. They suggested that the high nitrogen and electrolyte loss in the urine of diarrhoeic calves, despite the high faecal losses, was probably due to catabolism of body tissue.

McSherry and Grinyer (1954) studied changes in acid-base balance in 18 diarrhoeic calves. The calves were field cases many of which had been treated by veterinarians and farmers before they were studied by McSherry and Grinyer. The fluid intake of the calves before and during study by these workers was not recorded. Some calves were given an electrolyte solution containing sodium, potassium, calcium, magnesium, chloride and bicarbonate.

McSherry and Grinyer found that in 8 of the 18 calves, the plasma pH fell below 7.3 pH units. This was below the normal value of 7.44 ± 0.14 pH units previously determined in healthy calves, McSherry and Grinyer (1954a) They found that in most calves the acidosis was compensated until serum bicarbonate concentrations fell below 20 m.eq. / litre. Serum sodium, potassium and chloride concentrations above and below normal were observed in diarrhoeic calves. The maximum and minimum serum sodium concentrations found before the administration of electrolyte solution were 173 and 130 m.eq. / litre compared with the normal value of 142 ± 4 m.eq. / litre. Similarly, the maximum and minimum potassium concentrations found were 11.0 and 3.7 m.eq. / litre compared with the normal 5.2 ± 0.5 m.eq. / litre; the maximum and minimum chloride concentrations found were 124 and 84 m.eq. / litre compared with the normal 103 ± 2.5 m.eq. / litre. They also found that serum calcium concentrations were frequently in the lower range of normal and that in three of the 18 calves concentrations were below normal. Hypoglycaemia was observed in some calves and in two, blood glucose levels had fallen below 10 mgms per 100 ml blood.

McSherry and Grinyer measured haematocrits on the calves but the results obtained were variable. In 4 calves the haematocrits were observed to decrease when the calves recovered from diarrhoea. They suggested that this was associated with expansion in plasma volume as the animals became rehydrated.

Roy et al (1959) studied changes in serum sodium and potassium concentration in 152 experimental calves. Forty calves were deprived colostrum, 102 were given 400 ml of colostrum and 10 were given 3,410 ml of colostrum. When healthy, the calves were fed 1 lb. of milk / 10 lbs body weight but when the calves were affected with diarrhoea, their milk intake was reduced. Forty three of the 102 calves died. Twelve calves died of an E.coli septicaemia and 28 calves died of what was described as a 'localised intestinal infection'. All the calves which died of septicaemia, except one, had been deprived of colostrum. In these animals the mean serum sodium concentration at death was 127 m.eq. / litre which was below normal. The mean serum potassium concentration nine hours before death was 6.7 m.eq. / litre which was slightly above normal. Roy et al stated that the average serum sodium and potassium concentrations in calves were 135 and 5.9 m. eq. / litre respectively. They did not give the standard deviations about these means. The 28 calves which died from localised intestinal infections had low serum sodium concentrations and some had high potassium concentrations shortly before death. In three calves plasma potassium concentrations over 9.5 m.eq. / litre were observed shortly before death. Roy et al stated however that these results may not be strictly valid because of the way in which serum samples were collected.

Roy et al arranged the 109 calves which survived into five groups as follows; 20 calves which did not have diarrhoea or had diarrhoea on one

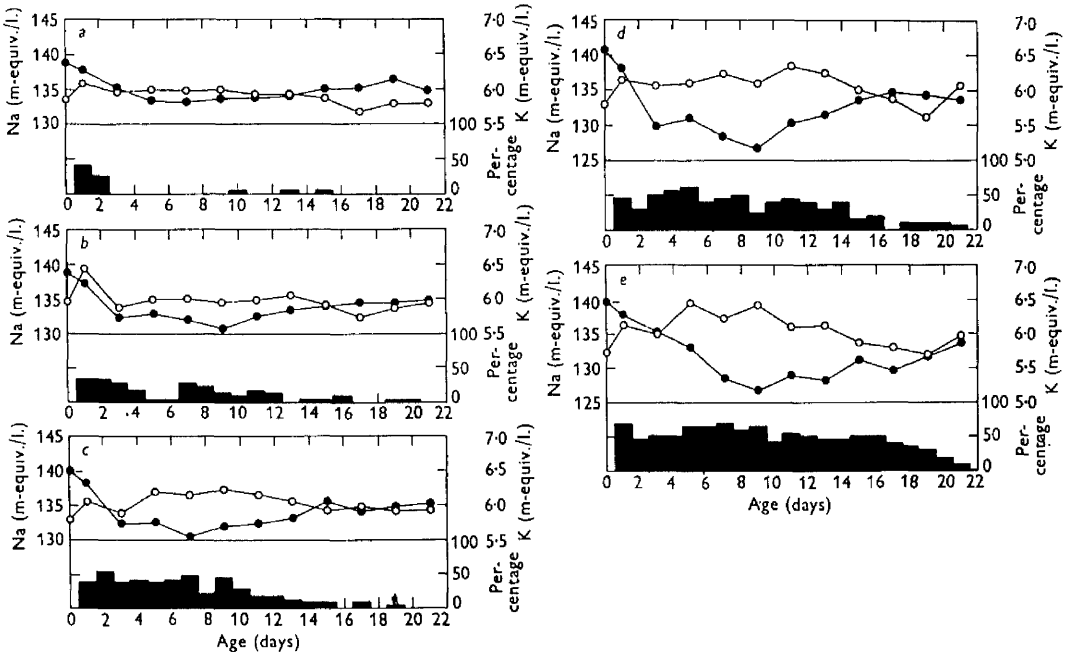
day only, 22 calves which had diarrhoea on 2 or 3 days, 25 calves which had diarrhoea on 4 or 5 days, 20 calves which had diarrhoea on 6 or 7 days and 22 calves which had diarrhoea on 7 days or more. The results obtained by Roy et al are shown in Figure 18, reproduced from their publication. From the results shown in Figure 18 it can be seen that as the incidence of diarrhoea increased a greater fall in serum sodium concentration occured. When diarrhoea occured on 6 days or more the mean serum sodium concentrations fell to 127 m.eq. / litre. The mean serum potassium concentrations of the different groups of calves did not vary extensively but the mean potassium concentration of the most severly affected group of calves was slightly above that of the least affected group.

Roy et al suggested that the hyperkalaemia observed by them, and also by McSherry and Grinyer, may be associated with death in some diarrhoeic calves. They cited the work of Bergman and Sellers (1953 and 54) which showed that experimentally induced hyperkalaemia in calves affected cardiac and respiratory function. Sellers and Bergman showed that severe cardiac signs developed in calves when the serum potassium concentration was raised to 8 m.eq. / litre and cardiac arrest occured when the concentration reached 12.7 m.eq. / litre.

The following part of this thesis describes an investigation of the changes in plasma electrolytes associated with diarrhoea in calves. A study was also made of the changes in blood urea concentration and haematocrit in diarrhoeic calves. In these experiments the amount fed to the calves was kept constant, irrespective of whether the calves were healthy or affected with diarrhoea. This was done in order to eliminate any variations in electrolyte and fluid balance which could arise due to differences in intake.

Figure 18 Changes in serum sodium and potassium concentrations in calves affected with diarrhoea

From Roy et al. (1959)



Mean values for serum sodium (●——●) and potassium (○——○) of calves that survived, and their relationship with incidence of scouring and age of calf. *a*, calves that did not scour or scoured on 1 day only; *b*, calves that scoured on 2 or 3 days; *c*, calves that scoured on 4 or 5 days; *d*, calves that scoured on 6 or 7 days; *e*, calves that scoured on 8 days or more. The shaded areas show the percentage of calves that scoured on any one day.

Materials and Methods

The effects of diarrhoea on the concentration of plasma electrolytes and blood urea, body weights and haematocrits were studied in the sixty calves used in the last two experiments described in Part II of the thesis.

These calves were male Ayrshires aged about one week at the start of the experiment. During the fourteen days that they were studied many of them became affected with diarrhoea and some died. The calves were bought through a local market and nothing was known of their management before they were sold. It is the usual practice however to feed such calves some colostrum during the first week of life.

The thirty calves that were studied in Experiment 9 in Part II of this thesis, were all fed three pints of ostermilk+ twice daily. Details of the preparation and composition of the ostermilk are described in Part II of the thesis. Fifteen of the thirty calves used in Experiment 10 in Part II of this thesis were fed twice daily on three pints of ostermilk. The other fifteen calves in this experiment were fed twice daily on three pints of cow's milk. Calves which refused to consume the full ration at each meal were fed by stomach tube.

Of the thirty calves studied in Experiment 9 ten were given no treatment, ten were given 5 gms of phthalylsulphathiazole daily by mouth and ten were given 500 mgms of chloromycetin daily by mouth. Of the thirty calves studied in Experiment 10, five of which ostermilk and five which were fed cow's milk were also given 500 mgms of chlortetracycline daily by mouth.

A daily record was kept of the type of faeces passed by each calf in the experiment and body weights were measured on alternate days. The type of faeces passed by the calves was classified as described in Part II of this thesis.

+ Glaxo Laboratories Ltd., Middlesex, England.

Blood samples were collected at intervals during the experiment. Blood was collected from the calves studied in Experiment 9 on the 1st, 4th, 7th, 10th and 13th days of the experiment. Blood samples were collected from the calves studied in Experiment 10 on the 1st, 7th, 9th, 11th and 14th days. Blood samples were collected from the jugular vein into heparinised bottles. The blood samples were collected two to three hours after the calves had been given their morning feed. Plasma was separated from the blood samples by centrifugation. The plasma was always separated from the blood within an hour of the time when the blood samples were collected.

Plasma sodium and potassium concentrations were determined with an E.E.L. model A flame photometer+.

Plasma sodium concentrations were found by comparison with a series of standard solutions of sodium chloride, each of which contained 5 m.eq. / litre of potassium in order to minimise the possible effects of the potassium emission spectra on the sodium flame. Plasma potassium concentrations were found by comparison with a series of standard solutions of potassium chloride each of which contained 130 m.eq. of sodium chloride / litre in order to minimise the possible effects of the sodium emission spectra on the potassium flame.

Plasma chloride concentrations were determined by the mercuric nitrate method of Schales and Schales (1941). In this method chloride is estimated in a protein free filtrate of plasma by titration with mercuric nitrate using diphenyl-carbazone as an indicator.

Blood urea concentrations were determined by the Urease Nesslerization method described by Varley (1958). This method depends on the conversion of urea into ammonia by the enzyme urease. The ammonia then

+ Evans Electroselenium Ltd., Halstead, Essex.

reacts with Nessler's Reagent causing a change in colour which is measured in a photoelectric colorimeter.

Haematocrits were determined by centrifugation of blood samples at a force of 1,600 g for two hours.

Plasma electrolyte concentrations were determined in the samples collected from the sixty calves. Blood urea concentrations were determined in samples collected from the thirty calves in Experiment 10. Blood urea concentrations were also determined in samples collected from the fourth day onwards from some of the calves in Experiment 9. Haematocrits were determined in blood samples collected from the calves in Experiment 9.

Results

In Appendix V are shown tables of results for each of the sixty calves studied in this experiment. Each table shows the days on which the calf was affected with diarrhoea and its body weight on alternate days during the experiment. The table also shows the plasma sodium, potassium and chloride concentrations determined at intervals during the experiment. Haematocrits and blood urea concentrations were determined on some calves and these results are also shown in the tables.

The effects of diarrhoea on plasma electrolyte and blood urea concentrations.

It was difficult to draw general conclusions about the effects of diarrhoea on the plasma electrolyte and blood urea concentrations from the tables of results for sixty individual calves. In order to draw such conclusions, it was necessary to describe the effects of diarrhoea on plasma electrolyte and blood urea concentrations in groups of similarly affected animals. This was done by dividing the calves into five groups depending on the number of consecutive days on which the calf had been affected with diarrhoea prior to the determination of plasma electrolyte and blood urea concentrations. By dividing the calves into groups it was possible to compare similarly affected calves, irrespective of the time during the experiment when they developed diarrhoea. Calves which were affected with diarrhoea on many consecutive days automatically appeared in more than one group. Of the sixty calves studied in this experiment, forty were selected into groups for analysis. The other twenty calves were unsuitable for selection into groups either because of early deaths, absence of diarrhoea or because they did not have diarrhoea on consecutive days.

The forty calves were selected into five groups as follows;

(a) Thirty one calves affected with diarrhoea on two or three consecutive days

before plasma electrolyte and blood urea concentrations were determined.

(b) Eighteen calves affected with diarrhoea on four or five consecutive days before plasma electrolyte and blood urea concentrations were determined.

(c) Twelve calves affected with diarrhoea on six or seven consecutive days before plasma electrolyte and blood urea concentrations were determined.

(d) Seven calves affected with diarrhoea on eight or nine consecutive days before plasma electrolyte and blood urea concentrations were determined.

(e) Four calves affected with diarrhoea on ten or more consecutive days before plasma electrolyte and blood urea concentrations were determined.

In order to assess if significant changes had occured in plasma electrolyte and blood urea concentrations in the diarrhoeic calves it was necessary to define the 'normal' values for plasma electrolyte and blood urea concentrations in 'normal' healthy calves. As none of the calves was affected with diarrhoea on the first day of the experiment their plasma electrolyte and blood urea concentrations were considered to be 'normal'. These 'normal' values were used in preference to the values found by other authors in order to eliminate any constant inherrent errors in the methods of analysis. The mean plasma electrolyte and blood urea concentrations together with the 'normal' ranges of the sixty calves on the first day of the experiment are shown in Table 34.

The concentrations of plasma electrolytes and blood urea in calves on the first day of the experiment.

	Mean & S.D.	'Normal' Range.
Plasma sodium m.eq./litre	140 ± 5	130 - 150
Plasma potassium m.eq./litre	4.9 ± 0.4	4.1 - 5.7
Plasma chloride m.eq./litre	98 ± 3	92 - 104
Blood urea mgm./100 ml.	16 ± 8	0 - 32

To determine if there were any significant difference in the mean plasma electrolyte and blood urea concentrations of the 'normal' calves and the mean concentrations of the groups of affected calves, 't' tests were carried out.

The 'normal' ranges for plasma electrolyte and blood urea concentrations were also calculated in order to determine if an individual calf affected with diarrhoea had a concentration which was outside the 'normal' range. The 'normal' range that has been accepted in this study, was that defined by two standard deviations above and below the arithmetical mean. In a normal population distribution, 95 per cent of the population lies within the limits of two standard deviations about the mean (Snedicar 1956). There would therefore be a 95 per cent chance that a sample which had a concentration outside this 'normal' range would not be a 'normal' concentration in a 'normal' animal.

In Table 35 are shown the plasma electrolyte and blood urea concentrations of the thirty one calves which had been affected with diarrhoea for two or three consecutive days prior to the determination of these concentrations. A 't' test was carried out to compare the mean plasma electrolyte and blood urea concentrations of the group of affected calves with the corresponding 'normal' mean values in healthy calves. It was found that the mean plasma sodium concentration in the affected calves was significantly lower ($P < 0.001$) than the 'normal' mean value. Similarly it was found that the mean blood urea concentration in the affected calves was significantly higher ($P < 0.001$) than the 'normal' mean value. There were no significant differences in the mean plasma chloride and potassium concentrations in the affected calves from the mean values in 'normal' calves.

From the results shown in Table 35 it can be seen that nine of the

Table 35

Plasma electrolyte and blood urea concentrations in calves affected with diarrhoea for two or three consecutive days

Calf No.	Sodium m.eq.	Potassium m.eq.	Chloride m.eq.	Urea mgm.
4	133	4.2	102	21
8	131	4.6	95	22
9	135	4.8	99	
12	129 low	4.9	100	
17	141	4.8	103	52 high
18	138	4.6	97	24
19	131	4.4	102	66 high
20	129 low	4.8	96	22
22	138	4.3	96	43 high
23	140	4.0 low	98	17
25	135	4.6	98	
26	122 low	4.3		
27	131	4.5	99	27
28	141	4.3	94	23
30	131	4.5	100	24
31	148	5.2	95	15
34	128 low	5.1	92	18
35	145	5.5	99	25
37	120 low	4.4	93	42 high
38	133	3.9 low	95	28
40	136	4.7	98	26
43	128 low	5.4	92	53 high
44	128 low	4.8	99	18
45	136	4.9	95	25
47	145	7.6 high	103	100 high
49	128 low	4.6	94	35 high
51	140	4.8	100	29
53	136	4.6	95	19
57	128 low	4.2	104	15
58	150	5.4	97	39 high
59	133	4.8	94	33 high
Mean and S.D.	134±7	4.8±0.6	98±3	32±18

Samples with concentrations higher or lower than the 'normal' range are marked in the table.

thirty one calves (28 %) had plasma sodium concentrations below the 'normal' range. Nine of the twenty seven calves (33 %) in which blood urea concentrations were determined had blood urea concentrations above the 'normal' range. Two of the thirty one calves (6 %) had plasma potassium concentrations below the 'normal' range and one calf (3 %) had a concentration above the 'normal' range. The plasma chloride concentrations of all the affected calves were within the 'normal' range.

In Table 36 are shown the plasma electrolyte and blood urea concentrations of the eighteen calves which were affected with diarrhoea for four or five consecutive days. The 't' tests were carried out as previously described and it was found that the mean plasma sodium and mean plasma potassium concentrations were significantly lower ($P < 0.001$ and $P < 0.02$ respectively) than the corresponding 'normal' mean concentrations. The mean blood urea concentration was significantly higher ($P < 0.001$) than the 'normal' mean blood urea concentration. The mean plasma chloride concentrations of the affected calves was not significantly different from the 'normal' mean concentration.

From the results shown in Table 36 it can be seen that of the eighteen affected calves, nine (50 %) had plasma sodium concentrations below the 'normal' range. Six of the fourteen calves (43 %) in which blood urea concentrations were determined had blood urea concentrations above the 'normal' range. One (5 %) of the eighteen calves had a plasma potassium concentration above and another a concentration below the 'normal' range. One calf (5 %) had a plasma chloride concentration below the 'normal' range.

In Table 37 are shown the plasma electrolyte and blood urea concentrations of the twelve calves affected with diarrhoea on six or seven consecutive days before concentrations were determined.

Table 36

Plasma electrolyte and blood urea concentrations in calves affected with diarrhoea on four or five consecutive days

Calf No.	Sodium m.eq.	Potassium m.eq.	Chloride m.eq.	Urea mgm.
3	137	6.0 high	103	71 high
6	135	4.4	104	24
9	127 low	4.9	97	
10	129 low	4.9	103	
13	134	4.9	102	76 high
15	136	4.9	94	
18	129 low	4.6	99	27
27	129 low	4.4	98	22
28	140	5.1	104	29
35	133	4.1	99	
38	128 low	3.8 low	95	36 high
40	128 low	4.1	99	28
43	120 low	4.6	88 low	44 high
44	125 low	4.4	96	24
48	128 low	4.8	95	47 high
51	130	4.1	100	28
56	140	4.6	93	31
58	136	4.2	98	39 high
Mean and S.D.	131±5	4.6±0.5	98±4	38±17

Samples with concentrations higher or lower than the 'normal' range are marked in the table.

Table 37

Plasma electrolyte and blood urea concentrations in calves affected with diarrhoea on six or seven consecutive days

Calf No.	Sodium m.eq.	Potassium m.eq.	Chloride m.eq.	Urea mgm.
4	129 low	4.5	97	26
6	136	4.6	96	26
12	127 low	4.6	89 low	
15	129 low	4.7	93	
17	129 low	4.0 low	101	31
25	131	5.1	99	
38	128 low	3.8 low	94	22
40	133	4.4	100	31
46	136	3.8 low	99	28
48	136	4.0 low	99	28
50	133	4.1	94	28
58	136	5.0	96	36 high
Mean and S.D.	132±2	4.4±0.4	96±3	28±4

Samples with concentrations higher or lower than the 'normal' range are marked in the table.

On statistical analysis it was found that the mean plasma electrolyte and blood urea concentrations of this group of calves were all significantly different from the corresponding 'normal' mean values. The mean plasma sodium, mean plasma potassium and mean plasma chloride concentrations were all significantly lower ($P < 0.001$, $P < 0.001$ and $P < 0.05$ respectively) than the corresponding 'normal' mean values. The mean blood urea concentration of the affected calves was significantly higher ($P < 0.001$) than the 'normal' mean.

Five of the twelve (42 %) affected calves had plasma sodium concentrations below the 'normal' range. Four calves (33 %) had plasma potassium concentrations below the 'normal' range but the plasma chloride concentrations of all the affected calves were within the 'normal' range. One of the nine calves (11 %) in which blood urea concentrations were determined had a urea concentration above the 'normal' range.

In Table 38 are shown the plasma electrolyte and blood urea concentrations of seven calves which were affected with diarrhoea for eight or nine consecutive days. The mean plasma sodium concentration of the group was significantly lower ($P < 0.001$) than the 'normal' mean. Similarly the mean plasma chloride concentration was significantly lower ($P < 0.001$) and the mean blood urea concentration significantly higher ($P < 0.001$) than the 'normal' means. There was no significant difference in the mean plasma potassium concentration from the 'normal' mean. All the affected calves had plasma sodium concentrations below the 'normal' range and three calves (42 %) had plasma chloride concentrations below the normal range. One calf (14 %) had a plasma potassium concentration below and one calf had a concentration above the 'normal' range. Two of the four calves (50 %) on which blood urea concentrations were determined had concentrations

Table 38

Plasma electrolyte and blood urea concentrations in calves affected with diarrhoea on eight or nine consecutive days

Calf No.	Sodium m.eq.	Potassium m.eq.	Chloride m.eq.	Urea mgm.
9	113 low	5.4	85 low	
12	120 low	5.5	93	
25	116 low	5.6	84 low	
28	129 low	4.3	98	31
44	120 low	4.4	95	39 high
48	125 low	3.2 low	96	26
50	128 low	7.2 high	88 low	44 high
Mean and S.D.	122±6	5.1±0.4	91±5	35±8

Samples with concentrations higher or lower than the 'normal' range are marked in the table.

Table 39

Plasma electrolyte and blood urea concentration in calves affected with diarrhoea on ten or more consecutive days

Calf No.	Sodium m.eq.	Potassium m.eq.	Chloride m.eq.	Urea mgm.
9	125 low	5.0	86 low	
12	135	4.7	93	
25	113 low	5.5	80 low	
48	125 low	3.9 low	106 high	22
Mean and S.D.	125±9	4.8±0.7	91±11	

Samples with concentrations higher or lower than the 'normal' range are marked in the table.

above the 'normal' range.

In Table 39 are shown the plasma electrolyte and blood urea concentrations of the four calves which were affected with diarrhoea for ten or more consecutive days. The mean plasma sodium and chloride concentrations of this group were significantly lower ($P < 0.001$ and $P < 0.01$ respectively) than the 'normal' mean values. There was no significant difference in the mean plasma potassium concentration of the affected calves. Three of the four calves (75 %) had plasma sodium concentrations below the 'normal' range. Two calves (50 %) had plasma chloride concentrations below the 'normal' range. One calf (25 %) had a plasma chloride concentration above the 'normal' range and one calf a plasma potassium concentration below the 'normal' range. Blood urea concentration was determined on only one calf and in this animal the concentration was within the 'normal' range.

Table 40 summarises the results shown in Tables 35, 36, 37, 38 and 39 and the statistical analyses previously described. From the results shown in this table it can be seen that the commonest abnormality found in diarrhoeic calves was a lowered plasma sodium concentration. The five groups of calves all had mean plasma sodium concentrations which were significantly lower than the 'normal' mean value. Of the seventy two plasma samples collected from the forty affected calves, thirty three (46 %) of the samples had plasma sodium concentrations below the 'normal' range. From the results shown in Table 40 it can be seen that the percentage of calves with low plasma sodium concentrations was higher in the groups of calves which were affected with diarrhoea for more prolonged periods. The mean plasma sodium concentrations of the groups of affected calves were also lower in those groups which were affected for the longer periods.

Table 40

Mean plasma electrolyte and blood urea concentrations in groups of calves affected with diarrhoea from two to three up to ten or more consecutive days

Group	Sodium m.eq.	Potassium m.eq.	Chloride m.eq.	Urea mgm.
31 calves affected for 2-3 consecutive days	134±7 (31 samples) P<0.001 28% hyponatraemic	4.8±0.6 (31 samples) not significant 6% hyperkalaemic 3% hypokalaemic	98±3 (30 samples) not significant	32±18 (27 samples) P<0.001 33% uraemic
18 calves affected for 4-5 consecutive days	131±5 (18 samples) P<0.001 50% hyponatraemic	4.6±0.5 (18 samples) P<0.02 5% hyperkalaemic 5% hypokalaemic	98±4 (18 samples) not significant 5% hypochloraemic	38±17 (14 samples) P<0.001 43% uraemic
12 calves affected for 6-7 consecutive days	132±5 (12 samples) P<0.001 42% hyponatraemic	4.4±0.4 (12 samples) P<0.001 33% hypokalaemic	96±3 (12 samples) P<0.05	28±4 (9 samples) P<0.001 11% uraemic
7 calves affected for 8-9 consecutive days	122±6 (7 samples) P<0.001 100% Hyponatraemic	5.1±0.4 (7 samples) not significant 14% hypokalaemic 14% hyperkalaemic	91±5 (7 samples) P<0.001 42% hypochloraemic	35±8 (4 samples) P<0.001 50% uraemic
4 calves affected for 10 or more consecutive days	125±9 (4 samples) P<0.001 75% hyponatraemic	4.8±0.7 (4 samples) not significant 25% hypokalaemic	91±11 (4 samples) P<0.01 50% hypochloraemic 25% hyperchloraemic	1 sample only which was within 'normal' range.
'Normal' mean concentration	140± 5	4.9±0.4	98± 3	16± 8
'Normal' range of concentrations	130-150	4.1-5.7	92-104	0-32

The second most common biochemical abnormality seen in the diarrhoeic calves, was an elevation in the blood urea concentration. Blood urea concentrations were determined in fifty five samples and eighteen (33 %) samples had concentrations above the 'normal' range. There were also statistically significant differences in the mean blood urea concentrations of four of the groups of calves when compared with the 'normal' mean. In one group of calves insufficient observations were made to carry out a 't' test.

Plasma potassium concentrations below the 'normal' range were found in nine (12 %) of the seventy two plasma samples collected. Three (4 %) samples had plasma potassium concentrations above the 'normal' range. Statistically significant differences in plasma potassium concentration were found in the groups of calves affected for four or five and six or seven consecutive days.

Plasma chloride concentrations below the 'normal' range were found in six (9 %) of the seventy one samples analysed. Significant differences occured in the mean plasma chloride concentrations of the groups of calves which were affected with diarrhoea for the longer periods.

The effects of diarrhoea on body weights and haematocrits

In order to assess if diarrhoea caused significant changes in body weights and haematocrits it was necessary to determine the extent to which these measurements 'normally' varied from day to day in healthy calves.

The extent to which body weights 'normally' varied was determined in calves which had not previously been affected with diarrhoea over periods of two to three, four to five, six to seven, eight to nine and ten to more consecutive days. These results are shown in Tables, 41, 42, 43, 44 and 45 and are summarised in Table 46.

Table 41

Differences in haematocrits and body weights of calves not previously affected with diarrhoea on two or three consecutive days

Calf No.	Difference in Haematocrit %	Difference in Body weight lbs.
1	+1	+3
2	-1	+1
4	+6	0
7	-2	+4
8	-1	+5
14	-3	+6
17	-4	+5
19	+2	0
20	0	+4
23	+2	0
26	+1	+4
29	+1	+2
31		0
32		+4
33		+1
34		+5
35		+4
37		0
39		+5
40		+3
41		+1
42		0
43		+4
44		+2
45		+4
47		0
49		+4
51		+3
52		0
53		+5
55		+2
57		+4
58		+3
59		+7
Mean and S.D.	0±3	3±2

Table 42

Differences in body weights of calves not previously affected with diarrhoea on four or five consecutive days

Calf No.	Difference in Body weight lbs.
32	+5
33	0
34	+2
37	-1
39	+3
41	-1
44	+1
51	+2
55	-3
	+1±2

Table 43

Differences in body weights of calves not previously affected with diarrhoea on six or seven consecutive days

Calf No.	Difference in Body weight lbs.
32	+5
33	-1
39	+4
41	-5
55	+5
Mean and S.D.	+2±4

Table 44

Differences in body weights of calves not previously affected with diarrhoea on eight or nine consecutive days

Calf No.	Difference in Body weight lbs.
32	+5
33	-1
39	+2
41	-2
55	+3
Mean and S.D.	+1±3

Table 45

Difference in body weights of calves not previously affected with diarrhoea on ten or more consecutive days

Calf No.	Difference in Body weight lbs.
32	+8
33	0
39	+7
41	-1
55	+7
Mean and S.D.	+4±4

Table 46

Mean differences in haematocrits and body weights of groups of calves not affected with diarrhoea

Group of calves	Difference in Haematocrit %	Difference in Body weight lbs.
Not affected on 2 or 3 consecutive days	0±3 (12 samples)	+3±2 (34 samples)
Not affected on 4 or 5 consecutive days		+1±2 (9 samples)
Not affected on 6 or 7 consecutive days		+2±4 (5 samples)
Not affected on 8 or 9 consecutive days		+1±3 (5 samples)
Not affected on 10 or more consecutive days		+4±4 (5 samples)

Similarly the extent to which haematocrits 'normally' varied was determined in calves which were not affected with diarrhoea for periods of two to three consecutive days. It was not possible to extend these observations for longer periods since none of the calves in which haematocrits were measured were unaffected with diarrhoea for more than two to three days. These results are shown in Table 41 and are summarised in Table 46.

In Tables 47, 48, 49, 50 and 51 are shown the changes in the body weights of the five groups of calves which were affected with diarrhoea for periods of two to three up to ten or more consecutive days. The results shown in these tables are summarised in Table 52.

From the results shown in Table 52 it can be seen that the mean body weights of the affected calves all fell during the periods of observation. It can also be seen that the mean losses in body weight increased as the duration of the diarrhoea increased. The 't' tests were carried out to determine if there were significant changes in the mean body weights of the groups of affected calves compared with the changes in the mean body weights of the groups of calves which were not affected with diarrhoea over similar periods. It was found that the mean body weights of the five groups of affected calves were all significantly lower than the mean body weights of the corresponding groups of calves which were not affected with diarrhoea. The statistical significance of the differences in the mean body weights of the different groups of calves are shown in Table 52.

In Tables 47, 48, 49, 50 and 51 are shown the changes in the haematocrits of the five groups of calves affected with diarrhoea. The results shown in these tables are summarised in Table 52. The 't' tests were carried out to compare the changes in the mean haematocrits of the groups

Table 47

Changes on haematocrit and body weight of calves previously affected with diarrhoea on two or three consecutive days

Calf No.	Difference in Haematocrit %	Difference in Body weight lbs.
4	-4	-1
8	+1	-6
9	-1	-7
12	-4	-2
17	+3	-3
18	+1	-3
19	+11*	-4
20	-1	0
22	+1	-2
23	-5	-2
25	-5	-4
26	+10*	-8
27	0	+1
28	-3	+3
30	+4	-3
31		-3
34		-1
35		-1
37		-4
38		-2
40		0
43		-1
44		-3
45		-2
47		-8
49		-3
51		-3
53		-2
57		0
58		-3
59		-1
Mean	0±5	-2±2

* above 'normal' range

Table 48

Changes in haematocrit and body weight of calves previously affected with diarrhoea on four or five consecutive days

Calf No.	Difference in Haematocrit %	Difference in Body weight lbs.
3	+6	0
6	-1	+2
9	+7*	-7
10	+1	-5
13	+11*	-1
15	-1	-3
18	-2	0
27	+2	-8
28	-1	-1
35		-4
38		-7
40		0
43		-4
44		-3
48		-4
51		0
56		-1
58		-3
Mean and S.D.	+2±4	+3±3

* above 'normal' range

Table 49

Changes in haematocrits and body weights of calves previously affected with diarrhoea on six or seven consecutive days

Calf No.	Difference in Haematocrit %	Difference in Body weight lbs.
4	-3	-5
6	-1	-6
12	-3	-8
15	0	-5
17	+3	-6
25	-2	-6
38		-7
40		-1
46		-7
48		-4
50		-3
58		-9
Mean and S.D.	-1±2	-6±2

Table 50

Changes in haematocrit and body weight of calves previously affected with diarrhoea on eight or nine consecutive days

Calf No.	Difference in Haematocrit %	Difference in Body weight lbs.
9	+2	-11
12	-6	-11
25	-3	-13
28	-3	-5
44		-5
48		-8
50		-4
Mean and S.D.	-2±3	-8±4

Table 51

Changes in haematocrits and body weights of calves previously affected with diarrhoea on ten or more consecutive days

Calf No.	Difference in Haematocrit %	Difference in Body weight lbs.
9	+6	-13
12	-3	-14
25	0	-17
48		-5
Mean and S.D.	+1±4	-12±5

Table 52

Mean difference in haematocrits and body weights of groups of calves affected with diarrhoea

Group of calves	Difference in Haematocrit %	Difference in Body weight lbs.
Affected on 2 or 3 consecutive days	0±5 (n.s.) (15 samples)	-2±2 (P<0.001) (31 samples)
Affected on 4 or 5 consecutive days	+2±4 (n.s.) (9 samples)	-3±3 (P<0.01) (18 samples)
Affected on 6 or 7 consecutive days	-1±2 (n.s.) (6 samples)	-6±2 (P<0.001) (12 samples)
Affected on 8 or 9 consecutive days	-2±3 (n.s.) (4 samples)	-8±4 (P<0.01) (7 samples)
Affected on 10 or more consecutive days	+4±4 (n.s.) (3 samples)	-12±5 (P<0.01) (4 samples)

n.s. = not a significant statistical difference

The results shown in this table were compared statistically with those shown in Table 46.

of affected calves with the change in animals not affected with diarrhoea. It was found that there was no biologically significant difference in the changes of the mean haematocrits of the affected groups when compared with the mean of the non-affected group.

From the results shown in Table 45 it can be seen that there was no difference in the average haematocrit of the group of calves which were not affected with diarrhoea over a period of two to three days. While there was no difference in the mean haematocrit over this period there was however a standard deviation of 3 % about the mean. Adopting a similar criterion to that previously described, it was assumed that an animal which had a change in its haematocrit of twice the standard deviation (i.e. of 6%) would have a 95 % chance that this variation was outside the range of 'normal' variation in 'normal' healthy calves.

From the results shown in Tables 47, 48, 49, 50 and 51 it can be seen that four of the thirty seven (9 %) blood samples collected had an increase in their haematocrit values of more than 6 %. There is thus some evidence that calves affected with diarrhoea may occasionally have increases in their haematocrit concentrations.

The effect of diarrhoea on the urea space

Urea spaces were measured in three calves. Initial measurements were made when the calves were healthy and subsequent measurements when the calves had been affected with diarrhoea and had lost weight. The calves were affected with diarrhoea from 3 to 5 days. The time between the initial and subsequent measurements did not exceed 8 days. The calves consumed the same ration of 6 pints of skimmilk each day over the period of the experiment. All urea space measurements and weighings were made at approximately the same time after feeding.

In Table 53 are shown, for each calf, the urea space expressed as a percentage of body weight and the differences between the volume of the urea space and body weight measured on successive occasions.

It can be seen that all the calves lost weight when affected with diarrhoea and that the volumes of their urea spaces also decreased. In calf X, the decrease in urea space was equivalent to approximately 90% of the loss in weight. Similarly, in calf Y it was equivalent to 50% and in calf Z to 70%.

The mean values were calculated of the differences between body weight and urea space determined in successive measurements on these diarrhoeic calves. The mean differences in body weight and urea space had been previously determined in 4 healthy calves on which duplicate measurements had been made when these animals were fed on both occasions. These mean values of healthy and diarrhoeic calves were compared with a 't' test. It was found that the mean differences in body weight and urea space of diarrhoeic calves were significantly different ($P = 0.02$ for both) from the means of the healthy calves. It was concluded that the decrease in urea space and body weight in these diarrhoeic animals was a significant effect of diarrhoea.

When the urea spaces in these calves were expressed as a percentage of body weight, an interesting result was obtained. In 2 calves, the percentage

Table 53

The effect of diarrhoea on urea space and body weight

Calf No. and Condition	Urea Space as % Body Weight	Difference between measurements when healthy and when affected with diarrhoea	
		Body Weight (Kg)	Urea Space (litres)
X - Healthy	78.3		
Diarrhoeic	76.4	-4.1	-3.7
Y - Healthy	70.5		
Diarrhoeic	72	-1.8	-0.9
Z - Healthy	74.5		
Diarrhoeic	75.0	-2.3	-1.6
Diarrhoeic	76.0	-5.5	-3.8
Mean		-3.4	-2.5
S.D.		+1.7	±1.47

increased and in one calf it decreased when they were affected with diarrhoea. The reasons for this variation was presumably associated with the relative amounts of water and tissue the calves had lost when affected with diarrhoea. The effect of losing different proportions of tissue and body water can be readily appreciated by considering the following hypothetical example.

Assume a calf, when healthy, weighs 100 lb. and has a body water volume equivalent to 75% of its weight. It becomes affected with diarrhoea and loses 10 lb. in weight. This loss in weight is due to loss of body water and to catabolism of boddy tissue in varying proportions.

If the 10 lb. loss in weight is due to a loss of 9 lb. of body water and 1 lb. of tissue, the percentage body water in the animal is now:

$$\frac{\text{Body water } (75 - 9)}{\text{Body weight } (100 - 10)} \times 100 = 73.3\%$$

If the 10 lb. loss in weight is due to a loss of 7.5 lb. of body water and 2.5 lb. of tissue, the percentage body water is now:

$$\frac{\text{Body water } (75 - 7.5)}{\text{Body weight } (100 - 2.5)} \times 100 = 75\%$$

If the 10 lb. loss in weight is due to a loss of 5 lb. body water and 5 lb. of tissue, the percentage body water is now:

$$\frac{\text{Body water } (75 - 5)}{\text{Body weight } (100 - 10)} \times 100 = 77.7\%$$

The results obtained in the present study and the hypothetical example considered above suggest that a single measurement of urea space in a diarrhoeic calf would be of no value in determining whether the animal had a deficit of body water.

Although it has been shown that, in healthy calves, the urea space is apparently similar in volume to the total body water determined by other methods, it is questionable how accurate this space is as a measure of total body water in diarrhoeic calves. It has been shown that a significant number of calves

affected with diarrhoea developed uraemia. It is possible that, in such animals, the injected urea may not measure the same space as in healthy ones. In the severely dehydrated animal, the equilibration time after injection may be greater than in a healthy animal and, if this was excessive, it could foreseeably affect the measurement. The fact that the urea excretion rate would probably be slower in the dehydrated, uraemic animal should not theoretically affect the measurement of the space provided the rate of excretion was exponential or linear.

In the present study, the 3 diarrhoeic calves were not uraemic. The equilibration times and excretion curves of injected urea in these calves were apparently similar to those in healthy calves.

N.B. Expressing the plasma volume as a % of body weight in diarrhoeic calves poses the same problems as expressing Total Body Water as a % of body weight. Ideally another parameter is required to relate the volumes of the body fluids to than body weight. This problem has been overcome in man by relating various physiological measurments to surface area. As far as it is known the surface area of cattle of different ages has not been determined.

The effect of diarrhoea on plasma volume

Plasma volumes were measured by the T.1824 dilution method in calves on the 1st, 4th, 7th, 10th, 13th and 16th days of Experiment 9. The results obtained are shown in Appendix V. Some results are marked with an asterisk. These are considerably higher than others determined in successive measurements on the same calf. This is believed to be because a small amount of T.1824 solution was either spilt or injected subcutaneously when measuring plasma volumes.

To determine if diarrhoea had an effect on plasma volume, it was necessary to know the extent plasma volume varied between successive measurements in 'healthy' calves. Since all the calves became affected with diarrhoea, this observation was made on 7 calves affected with diarrhoea for 3 days or less that did not have marked changes in body weight. The results obtained on these calves are shown in Table 54. It can be seen that there was some variation between the plasma volumes of these calves when measured on successive occasions during the experiment. It is not known how much these variations were due to normal physiological changes in plasma volume or to the experimental error in measurement.

In Table 55 are shown the plasma volumes of 10 calves. These animals were affected with diarrhoea for many days and some animals lost a considerable amount of weight. It can be seen, by comparing the results shown in Tables 54 and 55 and , that the variations in plasma volume of the 7 'healthy' calves were similar to those of the 10 severely affected calves. There was no marked indication that the plasma volumes of the 10 severely affected calves decreased.

Three calves had a decrease in plasma volume which appeared to be significant. The plasma volumes of these calves are shown in Table 56. These 3 calves died and had lost a considerable amount of weight.

Since an increase in haematocrit could be associated with a decrease in plasma volume, it is of particular interest to compare these measurements.

Table 54

Plasma volumes of seven calves which were affected with diarrhoea on three or less days

Calf No.	Day of Experiment 1	4	7	10	13	16
1	2.5	2.7	3.6*	2.8	2.6	2.9
2	2.3	1.9	2.3	2.0	1.9	1.9
7	2.3	3.1	2.3	2.1	2.0	-
14	2.8	2.1	2.6	2.0	2.0	2.1
23	2.8	2.5	2.8	2.4	2.1	2.6
24	2.3	2.1	2.2	1.7	1.9	1.9
29	2.8	2.9	-	2.5	2.5	-

*Experimental error due to faulty injection.

Table 55

Plasma volumes of ten calves severely affected with diarrhoea

Calf No.	Day of Experiment 1	4	7	10	13	16	**
	Plasma volume in litres						
4	2.4	-	2.7	-	1.9	2.2	7
6	2.2	2.0	2.5	2.7	1.9	2.1	6
9	2.2	2.3	2.2	2.2	2.1	-	12
12	1.7	1.7	2.0	-	1.7	-	13
15	2.4	2.7	2.6	2.8	2.3	3.3	9
17	2.2	2.0	-	2.2	2.4	2.2	8
18	3.0	2.6	3.0	2.9	2.8	-	6
25	1.8	-	1.9	1.7	1.5	-	13
27	2.5	2.2	2.6	-	2.2	2.1	8
28	2.2	1.6	2.0	2.1	2.3	-	12

** Number of days diarrhoea [redacted]

Table 56

Plasma volumes of three calves that died

Calf No.	Day of Experiment 1	4	7	10	13	16	**
	Plasma volume in litres						
13	1.7	1.9	1.0				4
16	2.7	1.6					3
26	2.7	2.4	1.6				3

** Number of days diarrhoea before death

Four calves, Nos. 9, 13, 16 and 26, had had an increase of more than 6% in haematocrit value, which was considered to be significant.

As shown in Table 56, Calf 13 had a decreased plasma volume and, on the 7th day of the experiment, the haematocrit had increased by 10%. Calf 26 had a decreased plasma volume and, on the 7th day, the haematocrit had increased by 11%. The other calf, No.16, that had a decreased plasma volume, had an increase in its haematocrit of 5%. This increase in haematocrit was slightly below the level that was considered to be significant.

The other calf, No. 9, that had an increase in its haematocrit, 7% and 6% on the 7th and 13th days respectively, had no obvious decrease in its plasma volume as shown in Table 55.

From these results, it would appear that, although all the calves became affected with diarrhoea, only a small minority had marked changes in plasma volume and haematocrit. ~~Three~~ Two of the 30 calves had both an increase in haematocrit and a decrease in plasma volume. One calf had a decrease in plasma volume and a slight increase in haematocrit. One calf had an increase in haematocrit but no obvious decrease in plasma volume. From this small number of calves, which showed changes in haematocrit and plasma volume, it was impossible to draw an absolute conclusion. However, it would appear that there was possibly some association between an increased haematocrit and a decreased plasma volume in diarrhoeic calves.

In this experiment, 9 calves died, 4 of these were animals which had an increased haematocrit and 3 were animals that had a decreased plasma volume. This suggested that an increased haematocrit in a diarrhoeic calf is a grave prognostic sign and also that some diarrhoeic calves may succumb due to circulatory failure associated with a decreased plasma volume.

Discussion

The mean plasma electrolyte concentrations determined on the first day of the experiment which were considered as 'normal' concentrations of healthy calves, were similar to the values determined in healthy calves by previous workers. Their values for plasma electrolyte concentrations in healthy calves are shown in Table 1. The plasma electrolyte concentrations of calves are similar to those of human infants, Weisberg (1962). The results obtained in Part II of this study also showed that the volumes of the principle body fluid compartments expressed as a % body weight were similar in calves and infants.

The results obtained by Blaxter and Wood (1953), McSherry and Grinyer (1954), Roy et al (1959) and those of the present study, indicate that diarrhoea causes changes in the blood chemistry and volumes of the body fluids in calves similar to those in infants, Bland (1956), Elkinton and Danowski (1956) Welt (1959) and Brusilow and Cooke (1959).

In human infants affected with diarrhoea, three types of dehydration namely, hypertonic, isotonic and hypotonic have been recognised Bland (1956). A deficit of water in greater than isotonic proportion to a deficit of electrolyte is known as hypertonic dehydration. A deficit of water and electrolyte in isotonic proportions is isotonic dehydration. A deficit of water in less than isotonic proportions to a deficit of electrolyte is known as hypotonic dehydration. The type and extent of dehydration which occurs in a diarrhoeic infant depends on the relative amounts of water and electrolyte lost through the faeces, lungs, skin and urine; and the relative amounts gained by ingestion or parenteral therapy. It will be shown later that similar forms of dehydration apparently occur in diarrhoeic calves.

In the present study a significant incidence of hypoelectrolytaemia was observed in diarrhoeic calves. Elkinton and Danowski (1956) state however, that the plasma electrolyte concentration is a measure only of the relative proportion of water to electrolyte in plasma and is not necessarily an indication

of an overall excess or deficit of either electrolyte or water. Typically in hypertonic dehydration the plasma electrolyte concentration is raised, in isotonic dehydration it is normal and in hypotonic dehydration it is below normal. High, low and normal plasma electrolyte concentrations occur however if the amounts of body water and electrolytes are increased in varying proportions. For example in congestive cardiac failure the plasma sodium concentration may be low or normal despite retention of sodium and oedema.

In 46% of the plasma samples collected, the sodium concentrations were below the normal range. The hyponatraemia could theoretically be due to either hypotonic dehydration or an increase in body water. Since the calves lost weight when affected with diarrhoea and as part of the loss in body weight was shown to be due to loss of body water, it is obvious that the calves were hypotonically dehydrated and not overhydrated. The other 54% of the plasma samples from diarrhoeic calves had plasma sodium concentrations within the normal range, since these calves had also lost weight and body water, the overall loss of sodium and water was in isotonic proportions and the calves were therefore isotonically dehydrated.

It was found that in some diarrhoeic calves the plasma sodium concentration varied from being within to being below the normal range in successive determinations. This indicates that the type of dehydration may change during the course of the disease. It was found that the incidence of hyponatramia and therefore hypotonic dehydration, was greatest in calves affected with diarrhoea for the longer periods.

While changes in plasma sodium concentration were the most obvious effect of diarrhoea, changes also occurred in plasma potassium and chloride concentrations. These confirm that the diarrhoeic calves were either isotonically or hypotonically dehydrated.

The deficit of electrolytes and water in diarrhoeic calves presumably

occurs in the same way as in diarrhoeic infants due to failure to absorbe the ingesta and gastro-intestinal secretions. The composition of intestinal secretions of the calf is not known but is probably similar to that of infants. Intestinal secretions in infants have the following composition, sodium 135 m.eq./litre, potassium 5 m.eq./litre, chloride 110 m.eq./litre and bicarbonate 30 m.eq./litre, Welt (1959). It is apparent that the loss of such secretions could produce the greatest effect on the concentration of plasma sodium and less effect on the concentrations of the other plasma electrolytes.

Hypernatraemia was not observed in the present study. In 3 of the 72 samples collected, the potassium concentrations were above the normal range (7.6, 7.2 and 6.0 m.eq./litre respectively). The plasma chloride concentration was above the normal range in one sample (106 m.eq./litre). The plasma sodium concentrations in these samples were however within or below the normal range (145, 128, 137 and 125 m.eq./litre respectively). It would therefore appear that hypertonic dehydration did not occur in the calves kept under the experimental conditions of the present study.

Although the results obtained in the present study indicate that the major electrolyte deficit in diarrhoeic calves was sodium, Blaxter and Wood (1953) and Darrow (1946) have shown that diarrhoea causes considerable deficits of body potassium due to faecal losses of electrolyte. Elkinton and Danowski (1956) describe how large deficits of potassium invariably occur in diarrhoeic infants even though the plasma potassium concentration may be normal or even raised. Potassium is the principle electrolyte in intra-cellular fluid. It is released from the cell into extra-cellular fluid as a cellular response to dehydration and also when cells are catabolised.

In addition to the extensive losses of potassium in diarrhoeic faeces, Blaxter and Wood (1953) observed that calves lost large amounts of potassium in the urine. They suggested this resulted from catabolism of body protein as

the calves were in negative nitrogen balance. The liberation of intracellular potassium due to cell catabolism and in response to dehydration may account for the hyperkalaemia observed in the present study and by McSherry and Grinyer (1954 and Roy et al (1959).

An excessive urinary excretion of potassium by diarrhoeic calves may occur in the same way as in man. Elkinton and Danowski (1956) described how in man there may be excessive urinary excretion of potassium despite an overall body deficit of potassium. They suggested this reflects an inability of the kidney to conserve potassium during periods of potassium deprivation which may be associated with changes in adreno-cortical function. In this respect it is interesting that Hawkins et al (1957) and Ingram (1959) observed an increase in the size of the adrenal glands of diarrhoeic calves. They suggested this may be associated with abnormal fluid and electrolyte balance in the diarrhoeic calf.

The hyperkalaemia observed in the present study was not so marked as that described by McSherry and Grinyer (1954) and Roy et al (1959). Sellers and Bergman (1953 and 1954) showed that in calves, experimentally induced hyperkalaemia caused cardiac signs when the potassium concentration reached 8.0 m.eq./litre and cardiac arrest occured when the concentration reached 12.7 m.eq./litre. The plasma potassium concentrations observed in the present study were therefore probably too low to have had obvious effects of hyperkalaemia on cardiac function.

In their studies of diarrhoeic calves, McSherry and Grinyer (1954) observed both hyper and hypoelectrolytaemia and Roy et al (1959) observed hyponatraemia and sometimes marked hyperkalaemia. These results would indicate that some calves were hypertonically dehydrated. The reason for the differences in their results from those of the present study may be associated with the feeding of calves.

McSherry and Grinyer (1954) studied calves brought directly from farms and it questionable if such calves had had an adequate fluid intake before they were examined. Roy et al (1959) reduced the amount of milk fed to their calves when

affected with diarrhoea. Reducing the milk intake of diarrhoeic calves would predispose to hypertonic dehydration in the same way as occurs in diarrhoeic infants, Bland (1956). In the present study the milk intake was maintained when the calves were affected with diarrhoea. Because they always had a large fluid intake they became either isotonically or hypotonically and not hypertonically dehydrated. The advantages and disadvantages of limiting or maintaining the milk intake of diarrhoeic calves is discussed later.

Major deficits of salt or water have different physiological effects in man and dog, Nadal et al (1941), Elkinton and Danowski (1956).

Dehydration resulting from a primary water deficit (as in hypertonic dehydration) is characterised in man and dog by thirst and oliguria. It does not lead to marked impairment of the circulatory system but affects the central nervous system. Sensory and motor functions are impaired and eventually the respiratory centre is depressed and death is often due to respiratory failure. (Winkler et al 1944).

In man and dog a major deficit of sodium chloride (as in hypotonic dehydration), leads to a reduction in the extracellular fluid volume. This causes a decrease in blood volume. Death is usually due to circulatory failure. In primary sodium chloride deficit the central nervous system is not affected to the same degree as in primary water deficit. Other clinical signs of sodium chloride deficit include wrinkling of the skin and loss of tissue turgor. However there is no marked thirst but a craving for salt, Elkinton and Danowski (1956).

It is of interest to compare the clinical signs in man and dog of primary water and salt deficits with the clinical signs in diarrhoeic calves.

In calves affected with the acute coli-septicaemia it would be expected that the major deficit is water. Such calves are typically febrile, anorexic and have only slight diarrhoea. Little water and electrolytes would be lost through the faeces but water deficit probably occurs due to failure to ingest milk and because

of the excessive water losses through the lungs and skin of a febrile animal. Calves affected with coli-septicaemia often show nervous signs such as incoordination, paddling movements of the limbs and nystagmus. They also appear to have impaired motor and sensory fadulties. Although these signs may be the effects of bacterial endotoxin, it is possible that in some instances they may result from a primary water deficit.

Although thirst is sign of a primary water deficit, it is of interest that some calves in the present study were apparently so obtunded they did not attempt to drink unless assisted. It is possible that the adipsia in calves with coli-septicaemia may often be an apparent rather than a real lack of thirst.

The periodic apnoea and gasping respiratory movements of some calves dying of septicaemia could possibly be an effect too of a primary water deficit on the respiratory centre.

In the present study diarrhoeic calves were observed that had a reduced or no thirst for milk. Typically these calves had been affected with diarrhoea for long periods, had lost weight (and therefore body water) and often had enopthalmus and changes in skin pliability which may have been an additional signs of dehydration. Calves which had been affected with diarrhoea for long periods were in addition frequently hyponatraemic. It is possible that the lack of thirst in such calves could be a manifestation of a sodium chloride deficit.

Four diarrhoeic calves had increased haematocrits and three of them also had an apparent decrease in plasma volume. Three of these four calves were also hyponatraemic. However, many other calves were hyponatraemic without either increased haematocrits or decreased plasma volumes. It was therefore impossible to conclude conclusively, that a sodium chloride deficit in diarrhoeic calves caused hypovolaemia.

Potassium depletion causes muscular weakness and paralysis in man. Cardiac function is also impaired and there are characteristic changes in the

electrocardiograph, Bland (1963). The effect of potassium depletion in calves is not known. It is possible that the muscular weakness, physical incoordination and bradycardia observed in calves affected with diarrhoea for long periods may have been related to potassium deficiency.

A significant incidence of uraemia occured in diarrhoeic calves. The uraemia in these animals was believed to be due to several factors. It has been shown that in dehydrated humans, uraemia occurs due to back diffusion of urea from the concentrated urine in the renal tubules. In advanced cases there is a lower glomerular filtration rate and renal blood flow due to circulatory failure. It is possible that similar effects occur in the diarrhoeic calf.

Blaxter and Wood (1953) have shown that diarrhoeic calves are in a negative nitrogen balance and the results of the present study showed that diarrhoeic calves loose body tissue. It is expected that an excess of urea would be therefore produced as protein is catabolised and this could lead to uraemia.

McCance and Widdowson (1957) have shown that newborn infants and some newborn mammals cannot produce concentrated urine. It is not known if the calf is able to produce concentrated urine. If it is similar to the newborn of other mammals, it would be expected that uraemia would occur in the dehydrated calf producing a small volume of dilute urine as it would be unable to excrete endogenous urea.

Only a small proportion of diarrhoeic calves had changes in haematocrit or plasma volume which indicated they might be hypovolaemic. It is possible that the practice of maintaining the fluid intake of the calves when affected with diarrhoea may have reduced the incidence of hypovolaemia. However McSherry and Grinyer (1954) also observed that very few diarrhoeic calves had increased haematocrits.

It is not known by how much the calf's blood volume can be reduced before clinical signs are produced. In man, whose normal blood volume is 70 - 80 ml / kg body weight, a reduction in blood volume due to haemorrhage of 40 - 45 ml / kg is

fatal or will lead to irreversible changes unless promptly replaced. A decrease in blood volume of 10% causes no effect but clinical symptoms and signs appear with a 20% loss in blood volume, Bland (1963). It is impossible to draw an absolute comparison between the effects of acute haemorrhage in man and diarrhoea in calves. In the former there is a loss of erythrocytes and plasma proteins whereas in the latter there is no evidence of a loss of erythrocytes or serum proteins, (except that the latter may be catabolised in the calf in negative nitrogen balance). Brusilow and Cooke (1959) calculated that losses in body water greater than 160 ml / kg in diarrhoeic infants result in death due to circulatory failure. It was shown that diarrhoeic calves which died lost on the average 8 - 6lbs in weight. It was also shown that 50 - 90% of the loss in weight of diarrhoeic calves was due to a decrease in the urea space, and presumably in body water. From these figures it was calculated that the worst affected diarrhoeic calves had a deficit in body water ranging from 135 to 240 ml / kg.

As the volumes of the principle body fluid compartments and the plasma electrolyte composition are similar in calves and infants and as diarrhoea has apparently similar effects on plasma composition, body water in both species, it is probable that some diarrhoeic calves die of circulatory failure due to hypovolaemia.

The clinical signs in diarrhoeic calves of a weak and terminally slow pulse, poor jugular filling and coldness of the skin and extremities, suggest that circulation is impaired. While these effects may be due to hypovolaemia, they could also result from shock due to endotoxaemia or heart failure due to acid-base and electrolyte disturbances.

The present results and those of previous workers indicate that diarrhoea has similar effects in calves and human infants. The correction of these effects is a vital aspect of the therapy of infantile diarrhoea and has been most significant in reducing the mortality rate, Welt (1959). In the past the therapy of calf diarrhoea has been primarily directed at limiting bacterial activity and little attention has been paid to correcting the effects of diarrhoea per se.

In view of the similarity of the effects of diarrhoea in calves and infants and the spectacular results obtained with fluid and electrolyte replacement therapy in infantile diarrhoea, it would appear foolish to continue to ignore this aspect of therapy in calf diarrhoea. The general principles of replacement therapy are presumably applicable to both species.

Therapy of diarrhoea necessitates attention to the following; (a) correcting fluid and electrolyte disturbances already present in the diarrhoeic subject, (b) maintaining a balance with the excessive faecal losses and, (c) meeting the normal physiological or obligatory requirements of the subject. In addition measures to combat the cause of the diarrhoea and careful nursing have to be considered..

Brusilow and Cooke (1959) calculated the deficits of water and electrolytes present in diarrhoeic infants with moderately severe dehydration. Their results are shown in Table 57 In view of the similarity of the body fluids in healthy infants and calves and also the effects of diarrhoea in the two species, it is considered that the results shown in this table are equally applicable in diarrhoeic calves. On this assumption it was calculated that the fluid and electrolyte deficit in a moderately severely dehydrated calf weighing 33 kgs was as shown in Table 58 By further comparison with Brusilow and Cookés calculations, it is estimated that a very severely dehydrated calf has a deficit of as third as much again as that shown in Table 58 A mildly dehydrated calf has a deficit of one third less. Hypertonically dehydrated calves would have a greater overall deficit of water and hypotonically dehydrated calves a greater overall deficit of electrolyte in ratios comparable to those calculated by Brusilow and Cooke (1959).

Having assessed the present deficits in the diarrhoeic calf the next object is to assess the water and electrolytes loss in faeces. On the basis of the results obtained by Blaxter and Wood (1953), a calf with moderately

severe diarrhoea has an estimated faecal loss of one litre of water and 40 m.eqs each of sodium and potassium per day.

The obligatory requirements of the healthy calf are not known. In an infant weighing 33 kgs they are approximately 1.75 litres of water and 30 m.eqs each of sodium, potassium and chloride. In addition the infant requires 1,300 Calories per day, Welt (1959).

The estimated deficits and requirements of a 33 kg calf affected with moderately severe isotonic dehydration are summarised in Table 58

Having estimated the electrolyte, water and calorific requirements of the diarrhoeic calf, the problems of treatment in both a well equipped Veterinary Hospital and under average farm conditions will be considered.

Hospitalisation of the majority of calves encountered in veterinary practice is impracticable for economic reasons. Hospitalisation would be a consideration principally in treating a very valuable animal.

Under hospital conditions it would seem feasible to adopt the same principles for diagnosis and treatment of the effects of diarrhoea in a calf as those used by the physician dealing with a child affected with diarrhoea.

When treating a calf in a Veterinary Hospital it would be necessary to carry out frequent biochemical analyses and to weigh the animal regularly to determine both its requirements and the effect of therapy. It may also be desirable to administer a proportion of the therapy by continuous intravenous infusion as is done in treating diarrhoeic infants.

Diagnosis and treatment under farm conditions imposes several economic and practical limitations and it is necessary to effect some compromises from the principles used in a hospital. For economic reasons it would be impracticable for the veterinarian to visit a calf on a farm more than once or twice. In addition, the time that can be devoted to the animal at each visit is limited.

Under farm conditions it is unlikely that facilities would be available to

determine the requirements of the calf or follow the effects of therapy. Therefore the type of therapy has to be standardised as far as possible. The amount of therapy that can be administered by continuous intravenous injection is limited. Most of the therapy has to be administered by the farmer who can be expected to administer it only by mouth or subcutaneous injection.

It is common practice in treating diarrhoeic infants, to withold food for 24 - 48 hours and then to gradually reintroduce feeding as the child's condition improves. Food is restricted to allow the alimentary tract to 'rest', to allow any inflammation to subside and to limit the intestinal bacterial population by attaining a high antibiotic concentration in the 'empty' intestine. The same benefits would presumably apply in treating diarrhoeic calves and the practice is advocated, provided adequate fluid, electrolytes and calories are given during the period of restriction. The British Veterinary Association Handbook, The Husbandry and Diseases of Calves, advises that diarrhoeic calves should not be fed milk for a day. Instead they should be given three meals each of one pint water plus one tablespoonful of sugar. Milk should then be gradually introduced over the next three or four days. It also suggests that lime water may be substituted for the sugar and water and in addition 500 - 1,000 ml of saline may be given subcutaneously. It is considered that the amount of water prescribed is inadequate. The value of giving sugar to the calf is also questioned as the calf is unable to utilise sucrose, Okamoto et al (1959). The validity of administering saline is also questionable and is discussed later.

In the present study the milk intake of the diarrhoeic calves was maintained at six pints daily. This practice is believed to have had advantages and disadvantages. Its advantages were possibly that it provided a large volume of water and also small amounts of electrolytes to the dehydrated animal. It may have alleviated the effects of starvation which are considered to be important in the diarrhoeic calf. The practice may have prevented

hypovolaemia and also hypertonic dehydration with its attendant risk of hyperkalaemia. Its disadvantages were that it may have exacerbated the diarrhoea, given no opportunity for the intestine to 'rest', diluted the intestinal concentration of orally administered therapeutics and caused an excessive loss of electrolytes in a large volume of faeces. Whether the advantages of this practice outweigh the disadvantages is debatable. The practice is considered to be less valuable however than the oral administration of a solution of electrolytes and glucose.

One of the first considerations in the treatment of a diarrhoeic infant is the maintenance of circulating blood volume. Brusilow and Cooke (1959) suggested that treatment of a diarrhoeic infant should be started with an intravenous infusion of Ringer's Lactate Solution followed by a whole blood or plasma transfusion. Ringer's Lactate Solution has the following composition:

Sodium	130 m.eq./ litre	Chloride	111 m.eq./ litre
Potassium	4 m.eq./ litre	Lactate	27 m.eq./ litre
Calcium	4 m.eq./ litre		

Ringer's Lactate Solution rapidly diffuses out of the circulation into the intracellular and interstial compartments. Hence it does not maintain the circulating blood volume unless given continuously. For this reason it is advocated that it should be followed by a whole blood or plasma transfusion.

As transfusions reactions are not a problem in cattle and the practice of taking blood from a donor cow and administering it to a calf is relatively simple, it is probably easier when treating diarrhoeic calves to forgo the use of Ringer's Lactate solution and to initiate treatment with a blood transfusion.
The blood volume of the average calf is approximately 2.5 litres and as signs of circulatory failure are known to occur in other species when blood volume is reduced by 20 - 50% it can be calculated that a transfusion of 500 - 1,000 ml of blood is required.

Many solutions have been formulated to treat the effects of diarrhoea in infants. Welt (1959) and other authors on the use of fluid therapy in man, emphasise that fixed standards of the amount and composition of replacement fluids cannot and should not be prescribed for the treatment of patients. They emphasise that while certain general rules can be made, each patient must be treated as an individual. Therapy must be adjusted to conform with the patient's clinical condition and with information gained by frequent biochemical estimations. The same principles regarding the prescription of fluid therapy should ideally apply when treating a calf under hospital conditions.

For the treatment of calves under hospital conditions, therapy may be based on the use of a few standard solutions as described by Welt (1959). The solutions of particular value for formulating therapy of the diarrhoeic patient are;

(a) 0.9% Sodium chloride, an isotonic solution containing 154 m.eq./litre of sodium and chloride ions.

(b) 5.0% Glucose solution, an isotonic solution containing the equivalent of 200 Calories per litre.

(c) 14.9% Potassium chloride, contains 2 m.eq./ml of potassium and chloride ions

(d) 11.2% Sodium lactate solution, contains 1 m.eq./ml of sodium and lactate ions.

(e) 7.5% Sodium bicarbonate solution, contains 0.9 m.eq./litre of Na and HCO_3 ions.

From these solutions it is possible to prepare a wide range of replacement fluids of varying composition and tonicity. For example if it was estimated that a solution was required with the following composition:

Sodium	145 m.eq./ litre
Potassium	10 m.eq./ litre
Bicarbonate	45 m.eq./ litre
Chloride	110 m.eq./ litre

It can be prepared from the standard solution as follows:

50 ml of 7.5% $NaHCO_3$ solution provides 45 m.eq. each of sodium and bicarbonate.
5 ml of 14.9% KCl solution provides 10 m.eq. each of potassium and chloride.
650 ml of 0.9% NaCl solution provides 100 m.eq. each of sodium and chloride.
Add 300 ml of distilled water to make up to a total volume of one litre.

Important details regarding the administration of fluid therapy are:

(a) Solutions given intravenously should be isotonic and given slowly.

(b) Solutions containing a high potassium content are dangerous when given intravenously and if given by this route must be administered very slowly.

(c) Solutions with a high potassium content are irritant if given subcutaneously. The maximum concentration given to man by this route is 40 m.eq./litre.

(d) Hypertonic solutions are best avoided. If they have to be given they should be given slowly by intravenous injection and not subcutaneously.

(e) Physiological saline is acid. If a large volume has to be administered, lactate or bicarbonate must be included to avoid an iatrogenic acidosis.

(f) Water alone should not be administered intravenously or subcutaneously. To provide a rapidly available supply of water administer 5% glucose solution intravenously. This solution should not be given subcutaneously.

Treatment under farm conditions necessitates compromises and one is to prescribe if possible a routine course and type of therapy. The therapy must be such that it caters for all the predicted effects of diarrhoea and yet be safe if given in excess. Where no facilities are available for determining the calf's blood chemistry it is considered best to assume the animal is isotonically dehydrated, has a metabolic acidosis and is in a state of subnutrition. The replacement therapy should be such, that provided the calf has adequate renal function and access to water, it would be able to correct any reasonable iatrogenic excesses of either water, electrolytes or base. A flexible attitude must obviously be adopted regarding treatment under farm conditions. If an animal recovered more quickly than predicted it would be foolish to continue therapy

which may lead to hypervolaemia and oedema. Conversely if recovery was not as rapid as predicted it may be necessary to increase or change the therapy.

It is suggested that the regimen of treatment of a diarrhoeic calf under farm conditions would be as follows. The veterinarian would administer a plasma or blood transfusion of 500 - 1,000 ml. if he suspected hypovolaemia. A litre of replacement fluid would also be administered by intra-peritoneal injection. The farmer would be instructed to deprive the calf of milk for 24 hours. During this period he should administer 2 litres of replacement fluid by subcutaneous injection and two litres by mouth. He should give this fluid in repeated small doses over the 24 hour period, rather than in a few massive doses. If the condition of the calf improved by the second day, the farmer should inject a further two litres subcutaneously and give three litres by mouth. If the condition of the calf continued to improve, parenteral administration should be stopped and the amount of fluid given orally gradually reduced and replaced by milk. In the event of the calf's condition not improving the farmer should continue parenteral administration of replacement fluid and seek professional advice.

Of the many replacement fluids formulated for treating diarrhoeic infants, that described by Talbot et al (1953) is considered most suitable on theoretical grounds for treating diarrhoeic calves under farm conditions.

Talbot's solution has the following composition.

Sodium	40 m.eq./litre	Lactate	20 m.eq./litre
Potassium	36 m.eq./litre	Phosphate	15.5 m.eq./litre
Chloride	40 m.eq./litre	Glucose	50 gms/litre

The electrolytes in this solution have an osmolality of 163 m.osms./litre. The solution is therefore half isotonic so far as electrolytes. Since the solution contains 5% glucose the solution is in fact hypertonic. Glucose rapidly diffuses throughout the body after administration and the final effect of this

solution is half isotonic. The excess of water provided by the administration of a half isotonic solution is considered an advantage. With such a solution there is less risk of an iatrogenic hyperelectrolytaemia in a hypertonically dehydrated calf. In the isotonically and hypotonically dehydrated calf the excess of water would be of potential value in the excretion of any retained end-products of catabolism. Hypotonic solutions also appear to be tolerated better when given orally and as this route of administration is important in the proposed regimen of therapy it is a significant consideration.

The main disadvantage of this solution is that it does not contain as much electrolyte as an isotonic solution. It can be seen from the estimated deficits of a diarrhoeic calf, shown in Table 58, that the administration of 5 litres of Talbots solution per day would almost meet the estimated water requirements of the animal but only half the electrolyte requirements. At least two days would therefore be required to correct the electrolyte deficits in the diarrhoeic calf.

Talbot's solution contains phosphate and lactate and these would help to correct any negative phosphate balance or metabolic acidosis. The solution also contains glucose. Five litres of solution would theoretically provide a 1,000 Calories which would almost meet the predicted energy requirements of the calf. Goodwin (1957) has shown that the newborn calf is not so susceptible to starvation as the newborn piglet and can survive for over a week without food. It is considered desirable however to include glucose therapy since McSherry and Grinyer (1954) have shown that hypoglycaemia occurs in diarrhoeic calves. In addition starvation leads to an undesirable catabolism of tissues with the consequent liberation of intra-cellular potassium and urea. Other end products of catabolism are of an acid nature and if they were not excreted by the dehydrated calf could lead to metabolic acidosis. It is also likely that the starving calf's resistance to adverse environmental conditions and to infection is reduced.

A small number of calves have been treated with Talbot's solution but so

far it has been impossible to make an accurate assessment of the effects of therapy. To assess the effects of therapy requires a large number of experimental calves with a high mortality rate so that an adequate number of treated and untreated calves can be compared. Since the complete therapy of the diarrhoeic calf should include antibiotics, it is apparent that an absolute assessment requires a number of calves given antibiotics and Talbot's solution and others given antibiotics alone.

The results obtained on a few diarrhoeic calves given Talbot's solution and antibiotics suggest treatment saved some calves which prior to treatment were considered to have a grave prognosis.

It has been found that calves will readily drink Talbot's solution. When allowed to drink this solution in lieu of milk for 24 - 48 hours, it was observed that the faecal output was considerably reduced and in some calves no faeces were produced. Talbot's solution has been administered subcutaneously and intraperitoneally without producing any obvious adverse reaction. It was found that 500 ml of the solution could be injected at one site under the skin and this solution was almost completely absorbed within three hours.

McSherry and Grinyer (1954) described the treatment of a few calves with a solution of the following composition.

Sodium	140 m.eq./litre	Potassium	10 m.eq./litre
Chloride	103 m.eq./litre	Calcium	5 m.eq./litre
Magnesium	3 m.eq./litre		

This solution is isotonic and as much is considered to have some disadvantages. It is foreseeable that the administration of isotonic solution to a newborn isotonically or hypertonically dehydrated calf, which may be unable to produce a concentrated urine, may not provide sufficient water for the excretion of iatrogenic excesses of electrolyte or base. This may be of particular importance if a hypertonically dehydrated calf which is slightly

hyperkalaemic is given an isotonic solution containing a high potassium concentration. An isotonic replacement solution would also provide less water for the calf to utilise to excrete end products of catabolism which may have accumulated in the body prior to treatment. The oral or subcutaneous administration of a large volume of isotonic solution to a hypotonically dehydrated calf could foreseeably lead to movement of body water into the solution. This could cause a serious hypovolaemia and death from circulatory failure. Isotonic solutions do not appear to be so palatable as hypotonic solutions to calves. This is an important consideration as it is desirable that the calf should drink the replacement fluid.

The solution prescribed by McSherry and Grinyer (1954) does not contain sufficient potassium to restore the calf's predicted deficit unless given in large volumes. This is an obvious disadvantage. The solution does not contain glucose and this too is a disadvantage.

It is difficult to assess the effects of this solution from McSherry and Grinyer's study as only a small number of calves were treated. A solution of similar composition is available commercially for the treatment of calf diarrhoea but there is no known evidence of its value.

McSherry and Grinyer (1964) included calcium and magnesium in their solution as they observed that some diarrhoeic calves were hypocalcaemic and hypomagnesaemic. Blaxter and Wood (1953) also found that diarrhoeic calves were in negative calcium and magnesium balance.

Solutions similar in composition to Talbot's solution but also containing calcium and magnesium have been formulated. However it is probably easier when treating diarrhoeic calves under farm conditions to administer 50 - 100 ml of one of the proprietary solutions* used to treat post-parturient metabolic disorders of cattle. These solutions contain approximately 20% calcium

* Myrilos P.M. Burroughs Welcome M.F.C. May & Baker.

Table 57

Probable deficits of water and electrolytes in infants with moderately severe dehydration due to diarrhoea.. from Brusilow and Cooke (1959).

Type of Dehydration.	Deficit per kilogram body weight			
	Water litres	Sodium m.eqs.	Potassium m.eqs	Chloride m.eqs.
Isotonic	100 - 120	8 - 10	8 - 10	8 - 10
Hypertonic	100 - 120	2 - 4	0 - 4	-2 to -6*
Hypotonic	100 - 120	10 - 12	8 - 10	10 - 12

* Patients with hypertonic dehydration have an excess rather than a deficit of chloride.

Table 58

Calculated deficits and requirements of a 33 kg calf affected with a moderately severe isotonic dehydration due to diarrhoea.

	Water litres	Na m.eq.	K m.eq.	Cl m.eq.	Calories
Initial Deficit	3.6	300	300	300	
Obligatory Requirements	1.75	30	30	30	1300
Continued loss in faeces	1.0	40	40	*	

* Principle loss of anions in faeces is bicarbonate.

borogluconate and 3% magnesium hydroxide.

The British Veterinary Association Handbook, The Husbandry and Disease of Calves, suggests that diarrhoeic calves should be given 500 - 1,000 ml of physiological saline subcutaneously. This practice has a value in providing sodium chloride and water but is not considered ideal. The amount administered is believed to be too little. Saline is acid and hence its administration would be undesirable to an animal with a metabolic acidosis.

The administration of vitamins and antibiotics to the diarrhoeic calf has been previously discussed in Part I. These would naturally be included in a course of therapy under either farm or hospital conditions.

In addition to the actual treatment of the diarrhoeic calf the nursing of the animal must be considered. Ideally the calf should be isolated from its neighbours and kept in a clean, dry, draught free and warm pen. If the calf is recumbant it should be periodically raised to its feet and if possible made to stand and walk. It has been observed that some recumbant calves will stand and eventually walk if a serious attempt is made to get them to do so. Calves which have been recumbant for several days appear to loose the use of their limbs and will only stand and walk when really forced to do so. If the animal does not stand it should not be allowed to lie on one side continually as there is a risk of haemostasis and possibly ischaemia of the limbs and pulmonary oedema.

Conclusions

The results obtained in this study have posed many interesting problems about diarrhoea in newborn calves.

It is apparent that there are many aspects of the aetiology and pathogenesis of diarrhoea in newborn calves which require elucidation. There is also an obvious need to relate these to the clinical signs of the syndrome of neonatal diarrhoea. Until these problems have been solved it is almost inevitable that present therapeutic and prophylactic procedures will be variable in their efficacy if indiscriminately applied to all outbreaks of "calf diarrhoea".

It would be of particular interest to determine if the pathogenesis of E.coli infections in calves is explicable in terms of the recent concepts of the endotoxin-anaphylactic reaction. If this reaction does occur in calves there may be a need in the therapy, for cortisone and antihistamindo preparations to alleviate the reaction. Other aspects of the aetiology and pathogenesis of "calf diarrhoea" which warrant further investigation are the role of viruses, the composition of the diet and of feeding methods and the factors which influence the calf's susceptibility.

While the present studies have elucidated some of the basic anatomy and composition of the body fluids in newborn calves there is obviously a need to extend these studies and also to learn about the dynamism of these fluids. Of special interest are the normal energy, fluid and electrolyte requirements of the calf, the processes of digestion and absorbtion, the homeostatic mechanisms of the calf and renal function. A knowledge of these subjects is an obvious prerequisite to an understanding of the pathology of the body fluids and ultimately of therapy.

The effects of diarrhoea and milk deprivation on the calf warrant further investigation. Previous studies have been made on experimental animals kept under varying conditions and it is considered desirable to extend these studies to an investigation of diarrhoeic calves maintained under farm conditions. It would be of special interest to define the effects of various disorders of the body fluids and their clinical effects. This definition would be of value in the diagnosis and consequently of the therapy of the effects of diarrhoea.

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APPENDIX I

Plasma and blood volumes in cattle determined from the T.1824 space and the haematocrit

Plasma and blood volumes in calves

Calf	Weight kg.	Plasma litres	Plasma % B.Wt.	Blood litres	Blood % B.Wt.
14880	24.1	2.1	8.6	3.2	13.4
14755	25.4	1.5	5.8	2.2	8.5
z	25.4	1.7	6.8	2.8	10.9
14756	25.9	1.3	5.1	1.9	7.5
14796	26.0	1.5	5.8	2.2	8.6
14766	26.4	1.9	7.2	2.9	11.3
y	27.3	1.9	7.0	3.0	11.1
14881	29.1	2.3	7.9	3.6	12.3
14881	29.5	2.4	8.1	3.7	12.4
94	30.0	1.7	5.7	3.2	10.7
82	30.4	1.7	8.7	2.6	8.7
93	30.4	1.7	5.7	3.1	10.2
02	30.9	2.1	6.7	3.1	10.1
86	30.9	2.1	6.7	3.5	11.4
84	31.8	1.9	6.0	3.8	11.9
00	31.8	1.9	6.1	3.7	11.5
87	31.8	2.2	6.8	3.8	11.8
89	33.6	2.4	7.2	3.5	10.4
83	34.0	2.2	6.6	3.3	9.6
08	34.0	2.5	7.4	4.1	12.0
03	34.0	2.2	6.7	4.1	12.0
98	34.5	2.3	6.6	3.9	11.4
88	34.5	2.3	6.8	3.2	9.2

Plasma and blood volumes in calves (Cont'd.)

Calf	Weight kg.	Plasma litres	Plasma % B.Wt.	Blood litres	Blood % B.Wt.
92	35.0	1.9	5.7	4.2	12.0
09	35.0	1.9	5.7	4.2	12.0
06	35.0	1.8	5.1	3.1	8.9
91	35.0	2.8	8.0	4.7	13.5
OF	35.0	2.4	6.8	2.6	7.5
11	35.5	1.9	5.6	3.4	9.5
99	35.9	2.3	6.4	3.9	10.9
97	36.8	2.1	5.7	4.2	11.3
07	37.3	2.7	7.2	7.0	18.9
05	37.3	2.3	6.0	5.1	13.5
04	38.2	2.3	6.1	3.5	9.1
95	38.2	2.8	7.4	5.2	13.7
90	38.2	2.2	5.7	3.6	9.3
01	39.1	2.7	6.8	5.0	12.8
85	39.5	2.4	6.2	4.1	10.4
96	40.0	2.4	6.0	4.7	11.7
10	40.5	2.8	7.0	4.2	10.3
27	27.2	2.5	9.0		
07	27.3	1.9	7.0		
06	28.2	1.6	5.7		
05	28.2	1.9	6.8		
02	28.6	2.5	8.9		
14	30.4	2.0	6.4		

Plasma and blood volumes in calves (Cont'd.)

Calf	Weight kg.	Plasma litres	Plasma % B.Wt.	Blood litres	Blood % B.Wt.
11	30.4	2.1	7.0		
17	31.3	2.2	7.0		
08	31.8	2.0	6.3		
97	31.8	2.1	6.7		
21	32.2	2.1	6.6		
06	32.7	2.6	8.1		
07	33.6	2.6	7.7		
24	34.0	2.6	7.7		
15	35.0	2.2	6.3		
01	35.0	2.1	5.9		
01	35.0	2.3	6.5		
FF	35.0	2.8	7.9		
25	35.4	2.5	6.9		
06	35.9	2.3	6.3		
23	37.2	2.3	6.2		
03	37.9	2.1	5.6		
30	38.0	2.4	6.4		
32	38.2	2.0	5.3		
35	38.5	2.8	7.4		
29	42.8	2.4	5.7		
28	45.6	3.1	6.7		
Mean			6.6		11.0
S.D.			±0.9		±2.0

APPENDIX II

Extracellular fluid volumes in cattle determined from the thiosulphate space

Extracellular fluid volumes in calves

Calf	Weight kg.	E.C.F. litres	E.C.F. % B.Wt.
y	27.3	6.2	22.9
6	28.2	8.2	28.9
F	35.0	9.5	27.0
1	35.0	8.4	23.9
3	37.9	9.0	23.7
30	38.0	9.0	23.6
32	38.2	8.0	21.0
35	38.5	10.0	25.9
29	42.8	8.6	20.1
28	45.6	11.3	24.8
Mean			24.2
S.D.			±2.6

APPENDIX III

Total body water in cattle determined

from the urea space

Total body water in calves

Calf	Weight kg.	T.B.W. litres	T.B.W. % B.Wt.
14596	21.4	14.6	68.2
14880	24.1	17.7	73.4
14755	25.4	17.9	70.5
13368	25.9	20.5	79.1
13369	25.9	16.0	61.8
14370	25.9	19.3	74.5
14796	26.0	17.3	66.6
14756	26.4	20.5	77.6
14289	27.5	20.2	73.4
14289	27.0	20.4	75.6
y	27.3	19.2	70.3
14371	27.7	19.1	68.9
14881	29.1	22.0	75.6
14881	29.5	23.2	78.6
4	29.5	21.1	71.5
6	29.5	24.5	83.0
6	30.0	25.0	83.0
3	30.0	22.9	76.3
X	31.4	21.6	68.8
T86	31.4	22.0	70.0
1	32.7	24.1	74.6
14598	33.6	22.4	66.7

Total body water in calves (Cont'd.)

Calf	Weight kg.	T.B.W. litres	T.B.W. % B.Wt.
5	34.0	27.1	79.7
5	34.3	27.4	84.8
2	34.5	25.5	77.3
T.11.S	34.5	25.7	74.5
T.88.S	34.5	27.2	78.9
F.C.	35.0	22.6	64.5
T.7.C	35.9	23.8	66.3
Mean			73.6
S.D.			±6.4

APPENDIX IV

The haematocrit in cattle

Haematocrits in Calves

Calf Number	Haematocrit %
98	45
83	33
2	36
4	35
95	49
99	44
85	43
92	56
82	39
89	33
9	47
84	53
97	53
7	42
96	52
93	47
86	44
6	46
90	41
94	50
0	50
3	47
11	44
87	45
88	28
10	34
1	50
91	42
5	59
80	38
55	34
56	34
56	39
96	35
81	38
81	37
F	36
Mean	43
S.D.	8

APPENDIX V

Body weights, haematocrits and plasma electrolyte and blood urea concentrations in calves.

Calf 1

	Day of Experiment															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Diarrhoea						D1		D1				D2				
Body weight lbs.		65		68		67		68		65		66		65		66
Plasma sodium m.eq	144			145			137			137			134			135
Plasma potassium m.eq	4.6			5.0			4.6			4.6			5.0			4.2
Plasma chloride m.eq	98			101			103			99			98			99
Blood urea mgm				15			21			24			27			17
Haematocrit %	39			40			39			38			37			

Calf 2

	Day of Experiment															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Diarrhoea					D1		D2									
Body weight lbs.		74		75		75		73		72		75		75		75
Plasma sodium m.eq	139			142			137			137			141			134
Plasma potassium m.eq	4.7			4.8			4.4			4.8			4.6			3.8
Plasma chloride m.eq	95			99			93			98			98			99
Haematocrit %	33			32			31			31			32			

Calf 3

	Day of Experiment															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Diarrhoea				D3	D3	D3	D3	D3	Died							
Body weight lbs.		69		75		69		63	62							
Plasma sodium m.eq	142			-			137									
Plasma potassium m.eq	5.1			-			6.0									
Plasma chloride m.eq	99			92			103									
Blood urea mgm				25			71									
Haematocrit %	53			46			59									

Calf 4 — Day of Experiment

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Diarrhoea					D3	D3	D2	D3	D2	D1				D1		
Body weight lbs.		64		64		63		62		79		78		78		78
Plasma sodium m.eq	142			142			133			129			134			134
Plasma potassium m.eq	5.1			4.9			4.2			4.5			4.8			4.2
Plasma chloride m.eq	97			98			102			97			97			99
Blood urea mgm				20			21			26			28			35
Haematocrit %	43			49			45				46		46			

Calf 5 — Day of Experiment

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Diarrhoea				D2	D3	D3	Died									
Body weight		69		74		67										
Plasma sodium m.eq	136			138												
Plasma potassium m.eq	4.8			4.7												
Plasma chloride m.eq	93			94												
Haematocrit %	45			40												

Calf 6 — Day of Experiment

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Diarrhoea				D1	D3	D2	D1	D3	D2				D1			
Body weight lbs.		65		70		67		65		64		63		64		61
Plasma sodium m.eq	139			139			135			136			137			135
Plasma potassium m.eq	5.1			5.0			4.4			4.6			5.2			3.9
Plasm chloride m.eq				27			24			26			18			28
Haematocrit %	45			45			44			44			44			

Calf 7

	Day of Experiment															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Diarrhoea							D1									
Body weight lbs.		74		78		76		78		78		79		77		77
Plasma sodium m.eq	142			139			140			140			140			137
Plasma potassium m.eq	4.8			4.4			4.7			4.2			4.0			4.0
Plasma chloride m.eq	97			92			97			99			98			99
Haematocrit %	28			26			28				29		30			

Calf 8

	Day of Experiment															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Diarrhoea					D3	D2	D2				D1					
Body weight lbs.		71		76		70		71		71		72		71		74
Plasma sodium m.eq	144			138			131			132			135			139
Plasma potassium m.eq	4.6			5.1			4.6			4.6			4.8			4.1
Plasma chloride m.eq	96			101			95			95			99			100
Blood urea mgm				20			22			31			20			28
Haematocrit %	33			32			33			33			31			

Calf 9

	Day of Experiment															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Diarrhoea			D2	D3	D3	D3	D3	D3	D3	D3	D3	D2	D3	D3	Died	
Body weight lbs.		84		84		77		74		73		71		65		
Plasma sodium m.eq	141			135			127			113			125			
Plasma potassium m.eq	4.8			4.8			4.9			5.4			5.0			
Plasma chloride m.eq	96			99			97			85			86			
Haematocrit %	41			40			48			43			47			

Calf 10

	Day of Experiment															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Diarrhoea				D2	D1	D3	D3	D1	D1							
Body weight lbs.		77		73		72		71		70		73		73		74
Plasma sodium m.eq	141			132			129			129			136			138
Plasma potassium m.eq	5.0			5.6			4.9			4.8			4.7			4.4
Plasma chloride m.eq	99			98			103			100			94			93
Haematocrit %	42			42			43			44			42			

Calf 11

	Day of Experiment															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Diarrhoea			D2	Died												
Body weight lbs.		74														
Plasma sodium m.eq	139															
Plasma potassium m.eq	4.9															
Plasma chloride m.eq	95															
Haematocrit %	56															
Plasma volume	2.6															

Calf 12

	Day of Experiment															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Diarrhoea		D2	D3	D3	D3	D3	D3	D2	D1	D1	D1	D1	D1	D1	Died	
Body weight lbs.		66		64		58		56		55		52		52	48	
Plasma sodium m.eq	137			129			127			120			135			
Plasma potassium m.eq	5.5			4.9			4.6			5.5			4.7			
Plasma chloride m.eq	98			100			89			93			93			
Haematocrit %	47			43			44			41			44			

Calf 13

Day of Experiment

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Diarrhoea				D1	D3	D2	D3	Died								
Body weight lbs.		65		68		64		57								
Plasma sodium m.eq	142			135			134									
Plasma potassium m.eq	5.0			4.7			4.9									
Plasma chloride m.eq	96			92			102									
Blood urea mgm				33			76									
Haematocrit %	50			45			61									

Calf 14

Day of Experiment

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Diarrhoea						D3		D2								
Body weight lbs.		79		85		80		81		80		80		78		79
Plasma sodium m.eq	144			136			135			132			146			131
Plasma potassium m.eq	5.0			4.8			5.6			4.6			4.6			4.2
Plasma chloride m.eq	98			94			99			101			96			92
Haematocrit %	49			46			46			50			53			

Calf 15

Day of Experiment

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Diarrhoea				D2	D2	D1	D3	D1	D1	D2		D2	D2			
Body weight lbs.		89		89		86		82		84		83		81		80
Plasma sodium m.eq	141			138			136			129			132			129
Plasma potassium m.eq	5.0			5.0			4.9			4.7			4.8			4.2
Plasma chloride m.eq	97			97			94			93			96			98
Haematocrit %	52			46			51			46			46			

Calf 16

Day of Experiment

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Diarrhoea		D1		D3	D3	Died										
Body weight lbs.		74		70												
Plasma sodium m.eq	139			126												
Plasma potassium m.eq	5.1			-												
Plasma chloride m.eq	95			-												
Blood urea mgm				43												
Haematocrit %	53			58												

Calf 17

Day of Experiment

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Diarrhoea					D1	D3	D3	D2	D2	D3	D2	D2				
Body weight lbs.		73		78		75		72		72		72		70		76
Plasma sodium m.eq	139			138			141			129			141			135
Plasma potassium m.eq	4.4			4.8			4.8			4.0			4.3			4.2
Plasma chloride m.eq	94			97			103			101			96			91
Blood urea mgm				20			52			31			22			25
Haematocrit %	45			41			44				44		43			

Calf 18

Day of Experiment

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Diarrhoea				D1		D1	D3	D3	D3		D1					
Body weight lbs.		75		78		78		75		75		75		75		75
Plasma sodium m.eq	145			141			138			129			146			138
Plasma potassium m.eq	5.1			5.2			4.6			4.6			4.6			4.5
Plasma chloride m.eq	95			98			97			99			95			99
Blood urea mgm				34			24			27			27			27
Haematocrit %	44			43			44				42		41			

Calf 19

Day of Experiment

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Diarrhoea					D2	D3	D3	D3	Died							
Body Weight lbs.		71		71		67		63	66							
Plasma sodium m.eq	137			136			131									
Plasma potassium m.eq	4.9			3.9			4.4									
Plasma chloride m.eq	98			101			102									
Blood urea mgm				15			66									
Haematocrit %	51			43			54									

Calf 20

Day of Experiment

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Diarrhoea					D3		D2	D2	D1							
Body weight lbs.		85		89		82		82		81		81		80		78
Plasma sodium m.eq	137			139			132			129			134			138
Plasma potassium m.eq	5.4			4.6			4.1			4.8			4.6			4.2
Plasma chloride m.eq	100			107			101			96			90			97
Blood urea mgm				31			33			22			20			22
Haematocrit %	50			50			52			51			53			

Calf 21

Day of Experiment

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Diarrhoea				D2		D2										
Body weight lbs.		66		68		58										
Plasma sodium m.eq	144			138												
Plasma potassium m.eq	4.9			4.7												
Plasma chloride m.eq	101			96												
Blood urea mgm				25												
Haematocrit %	36			38												

Calf 22

	Day of Experiment															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Diarrhoea			D1	D3	D3	D3	Died									
Body weight lbs.		71		69		64										
Plasma sodium m.eq	139			138												
Plasma potassium m.eq	5.2			4.3												
Plasma chloride m.eq	98			96												
Blood urea mgm				43												
Haematocrit %	47			48												

Calf 23

	Day of Experiment															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Diarrhoea					D3	D1										
Body weight lbs.		84		84		82		82		81		84		82		83
Plasma sodium m.eq	141			147			140			140			137			137
Plasma potassium m.eq	4.7			4.8			4.0			4.6			4.6			4.4
Plasma chloride m.eq	98			97			98			96			100			99
Blood urea mgm				33			17			29			20			28
Haematocrit %	35			37			32			35			33			

Calf 24

	Day of Experiment															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Diarrhoea		D1														
Body weight lbs.		80		82		81		82		82		83		83		86
Plasma sodium m.eq	144			-			140			140			145			144
Plasma potassium m.eq	5.2			-			4.6			4.6			4.6			4.4
Plasma chloride m.eq	99			100			94			97			98			98
Blood urea mgm				16			19			27			25			18
Haematocrit %	59			57			58				56		58			

Calf 25

	Day of Experiment															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Diarrhoea		D3	D3	D2	D2	D1	D3	D3	D3	D3	D3	D2	D3	D3	Died	
Body weight lbs.		76		72		70		66		63		59		56		
Plasma sodium m.eq	142			135			131			116			113			
Plasma potassium m.eq	5.7			4.6			5.1			5.6			5.5			
Plasma chloride m.eq	101			98			99			84			80			
Haematocrit %	46			41			44			43			46			

Calf 26

	Day of Experiment															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Diarrhoea					D3	D3	D3	Died								
Body weight lbs.		77		81		73		60								
Plasma sodium m.eq	144			151			122									
Plasma potassium m.eq	6.0			4.5			4.3									
Plasma chloride m.eq	104			101			-									
Haematocrit %	56			57			67									

Calf 27

	Day of Experiment															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Diarrhoea						D3	D1	D3	D1	D2		D1		D1		D1
Body weight lbs.		77		72		73		72		69		69		69		66
Plasma sodium m.eq	141			141			131			129			140			132
Plasma potassium m.eq	4.7			4.5			4.5			4.4			4.6			4.7
Plasma chloride m.eq	96			102			99			98			97			94
Blood urea mgm				20			27			22			25			37
Haematocrit %				33			33			35			35			

Calf 28

	Day of Experiment															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Diarrhoea			D2	D3	D3	D3	D3	D3	D3	D3		D1	D3	D3	D1	
Body weight lbs.		75		78		74		70		70		67		67		66
Plasma sodium m.eq	144			141			140			129			134			138
Plasma potassium m.eq	4.6			4.3			5.1			4.3			4.8			4.5
Plasma chloride m.eq	102			94			104			98			96			97
Blood urea mgm				23			29			31			23			25
Haematocrit %	48			45			47			45			48			

Calf 29

	Day of Experiment															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Diarrhoea							D1	D1								
Body weight lbs.		84		86		89		87		84		86		82		84
Plasma sodium m.eq	145			144			137			136			140			143
Plasma potassium m.eq	4.7			4.9			4.4			4.5			4.2			4.4
Plasma chloride m.eq	100			94			101			103			101			97
Blood urea mgm				29			27			31			34			32
Haematocrit %	34			35			36			37			39			

Calf 30

	Day of Experiment															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Diarrhoea		D1			D1	D1		D1								
Body weight lbs.		75		79		76		74		73		75		72		73
Plasma sodium m.eq	141			141			131			144			135			131
Plasma potassium m.eq	4.8			4.8			4.5			4.6			4.2			4.0
Plasma chloride m.eq	99			97			100			110			96			98
Blood urea mgm				22			24			29			20			30
Haematocrit %	44			34			38			41			40			

Plasma volumes of calves in Experiment 9

Calf No.	Day of Experiment 1	4	7	10	13	16
	Plasma Volume in Litres					
1	2.5	2.7	3.6*	2.8	2.6	2.9
2	2.3	1.9	2.3	2.0	1.9	1.9
3	1.9	2.1	1.5	-	-	-
4	2.4	-	2.7	-	1.9	2.2
5	2.1	2.1	-	-	-	-
6	2.2	2.0	2.5	2.7	1.9	2.1
7	2.3	3.1*	2.3	2.1	2.0	-
8	3.3	2.6	3.6	3.2	3.4	2.6
9	2.2	2.3	2.2	2.2	2.1	-
10	2.9	-	2.1	2.2	-	2.3
11	2.6	-	-	-	-	-
12	1.7	1.7	2.0	-	1.7	-
13	1.7	1.9	1.0	-	-	-
14	2.8	2.1	2.6	2.0	2.0	2.1
15	2.4	2.7	2.6	2.8	2.3	3.3*
16	2.7	1.6	-	-	-	-
17	2.2	2.0	-	2.2	2.4	2.2
18	3.0	2.6	3.0	2.9	2.8	-
19	1.9	1.8	1.4	-	-	-
20	3.1	-	3.3	3.4	2.7	-
21	2.1	1.6	-	-	-	-
22	2.3	1.9	-	-	-	-
23	2.8	2.5	2.8	2.4	2.1	2.6
24	2.3	2.1	2.2	1.7	1.9	1.9
25	1.8	-	1.9	1.7	1.5	-
26	2.7	2.4	1.6	-	-	-
27	2.5	2.2	2.6	-	2.2	2.1
28	2.2	1.6	2.0	2.1	2.3	-
29	2.8	2.9	-	2.5	2.5	2.5
30	2.6	2.4	3.1	2.9	3.0	2.5

*Believed to be high because of faulty injection of T.1824.

Calf 31

Day of Experiment

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Diarrhoea					D2	D2			D1		D2	D3		
Body weight lbs.		79		79		76		75		73		70		74
Plasma sodium m.eq	136						148		125		128			128
Plasma potassium m.eq	4.4						5.2		4.2		4.3			4.6
Plasma chloride m.eq	96						95		99		96			92
Blood urea mgm	8						15		19		25			38

Calf 32

Day of Experiment

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Diarrhoea														
Body weight lbs		70		74		75		75		75		75		78
Plasma sodium m.eq	144						133		133		133			133
Plasma potassium m.eq	5.6						4.9		4.4		4.7			4.2
Plasma chloride m.eq	93						91		97		93			90
Blood urea mgm	16						14		22		19			24

Calf 33

Day of Experiment

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Diarrhoea											D1			
Body weight lbs.		74		75		74		73		73		72		74
Plasma sodium m.eq	144						145		125		133			136
Plasma potassium m.eq	4.9						4.4		4.3		4.6			3.8
Plasma chloride m.eq	97						98		94		100			98
Blood urea mgm	12						39		24		25			24

Calf 34 — Day of Experiment

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Diarrhoea							D2	D2	D1			D1		
Body weight lbs.		76		81		78		77		76		76		77
Plasma sodium m.eq	136						148		128		128			128
Plasma potassium m.eq	4.2						5.1		5.1		4.6			4.3
Plasma chloride m.eq	96						94		92		94			94
Blood urea mgm	8						19		18		19			22

Calf 35 — Day of Experiment

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Diarrhoea						D1	D2	D2	D3	D3		D1		D1
Body weights lbs.		77		81		80		77		77		77		80
Plasma sodium m.eq	136						145		133		128			133
Plasma potassium m.eq	5.0						5.5		4.1		3.8			4.0
Plasma chloride m.eq	99						99		99		94			100
Blood urea mgm	16						25		-		22			22

Calf 36 — Day of Experiment

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Diarrhoea		D1												
Body weight lbs.		63		65		63		65		65		67		71
Plasma sodium m.eq	128						140		140		140			136
Plasma potassium m.eq	4.2						4.3		4.2		4.2			4.4
Plasma chloride m.eq	105						96		93		96			97
Blood urea mgm	34						19		19		24			22

Calf 37

Day of Experiment

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Diarrhoea							D2	D2					D1	
Body weight lbs.		71		71		70		66		74		63		68
Plasma sodium m.eq	133						120		120		120			128
Plasma potassium m.eq	4.8						4.2		4.4		4.5			4.4
Plasma chloride m.eq	101						93		93		93			93
Blood urea mgm	32						47		42		26			24

Calf 38

Day of Experiment

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Diarrhoea				D1		D2	D3	D1	D1	D1		D3	D2	D3
Body weight lbs.		68		71		70		68		69		62		64
Plasma sodium m.eq	128						133			128		125		125
Plasma potassium m.eq	4.9						3.9			3.8		4.2		3.6
Plasma chloride m.eq	97						95			95		90		95
Blood urea mgm	16						28			36		35		35

Calf 39

Day of Experiment

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Diarrhoea												D1		
Body weight lbs.		72		77		75		76		74		75		79
Plasma sodium m.eq	132						-		120		136			128
Plasma potassium m.eq	4.8						-		4.3		4.1			4.1
Plasma chloride m.eq	98						96		93		95			97
Blood urea mgm	13						19		25		25			22

Calf 40

	Day of Experiment													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Diarrhoea						D1	D3	D1	D2	D2	D1	D2		
Body weight lbs.		70		73		73		73		72		72		76
Plasma sodium m.eq	-						136		128		133			136
Plasma potassium m.eq	4.7						4.7		4.1		4.4			4.2
Plasma chloride m.eq	97						98		99		100			100
Blood urea mgm	13						26		28		31			35

Calf 41

	Day of Experiment													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Diarrhoea														
Body weight lbs.		75		76		74		70		73		70		74
Plasma sodium m.eq	145						136		136		128			136
Plasma potassium m.eq	4.4						4.3		4.7		4.7			4.3
Plasma chloride m.eq	97						96		95		97			100
Blood urea mgm	22						32		32		31			22

Calf 42

	Day of Experiment													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Diarrhoea					D1	D2	D2	Died						
Body weight lbs.		77		77		71		69						
Plasma sodium m.eq	136						-							
Plasma potassium m.eq	5.2						-							
Plasma chloride m.eq	92						-							
Blood urea mgm	13						-							

Calf 43 — Day of Experiment

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Diarrhoea					D1	D3	D3	D1					D2	D1
Body weight lbs.		67		71		70		67		64		63		67
Plasma sodium m.eq	132						128		120		120			120
Plasma potassium m.eq	5.6						5.4		4.6		4.9			4.4
Plasma chloride m.eq	94						92		88		88			90
Blood urea mgm	10						53		44		21			21

Calf 44 — Day of Experiment

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Diarrhoea							D3	D3	D2	D1	D3	D2	D2	D1
Body weight lbs.		78		80		79		76		76		71		74
Plasma sodium m.eq	136						145		128		125			120
Plasma potassium m.eq	4.8						4.8		4.8		4.4			4.4
Plasma chloride m.eq	99						93		99		96			95
Blood urea mgm	9						22		18		24			39

Calf 45 — Day of Experiment

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Diarrhoea						D2	D2	D2				D1	D1	D1
Body weight lbs.		71		75		74		72		73		72		75
Plasma sodium m.eq	132						136		120		133			136
Plasma potassium m.eq	5.4						4.9		4.0		5.3			4.4
Plasma chloride m.eq	96						95		94		95			92
Blood urea mgm	12						25		18		17			17

Calf 46

	Day of Experiment													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Diarrhoea		D1	D2	D1	D2	D1	D1							
Body weight lbs.		67		64		60		62		64		65		67
Plasma sodium m.eq	128						136		133		128			128
Plasma potassium m.eq	4.6						3.8		4.2		4.9			4.0
Plasma chloride m.eq	94						99		92		89			90
Blood urea mgm	16						26		18		17			19

Calf 47

	Day of Experiment													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Diarrhoea					D3	D3	Died							
Body weight lbs.		77		77		69								
Plasma sodium m.eq	136						145							
Plasma potassium m.eq	4.8						7.6							
Plasma chloride m.eq	98						103							
Blood urea mgm	43						100							

Calf 48

	Day of Experiment													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Diarrhoea			D2	D2	D2	D2	D2	D2	D2	D1	D3	D2	D2	D2
Body weight lbs.		64		64		60		60		56		55		59
Plasma sodium m.eq	128						128		136		125			125
Plasma potassium m.eq	4.1						4.8		4.0		3.2			3.9
Plasma chloride m.eq	96						95		99		96			106
Blood urea mgm	13						47		28		26			22

Calf 49

Day of Experiment

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Diarrhoea					D2	D1			D1					D1
Body weight lbs.		69		73		70		67		67		69		73
Plasma sodium m.eq	136						128		128		133			133
Plasma potassium m.eq	5.6						4.6		4.3		4.7			4.6
Plasma chloride m.eq	95						94		98		93			94
Blood urea mgm	24						35		26		19			15

Calf 50

Day of Experiment

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Diarrhoea		D2	D3	D1	D1	D3	D3	D2	D3	Died				
Body weight lbs.		62		61		59		58		52				
Plasma sodium m.eq	140						133		126					
Plasma potassium m.eq	5.0						4.1		7.2					
Plasma chloride m.eq	97						94		88					
Blood urea mgm	17						28		44					

Calf 51

Day of Experiment

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Diarrhoea								D1	D1	D1	D2			
Body weight lbs.		69		72		71		74		71		73		72
Plasma sodium m.eq	136						145		140		130			140
Plasma potassium m.eq	4.7						4.9		4.6		4.1			4.4
Plasma chloride m.eq	99						100		100		100			101
Blood urea mgm	14						21		29		28			39

Calf 52 — Day of Experiment

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Diarrhoea						D1		D1					D1	
Body weight lbs.		71		71		70		74		72		73		73
Plasma sodium m.eq	147						136		133		125			133
Plasma potassium m.eq	5.6						4.2		4.2		4.0			3.9
Plasma chloride m.eq	100						93		94		94			97
Blood urea mgm	15						11		14		22			21

Calf 53 — Day of Experiment

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Diarrhoea						D1	D1				D1	D1		D1
Body weight lbs.		57		62		60		62		60		62		62
Plasma sodium m.eq	144						136		133		128			133
Plasma potassium m.eq	4.8						4.6		4.0		4.6			4.3
Plasma chloride m.eq	103						95		94		99			102
Blood urea mgm	12						19		13		13			14

Calf 54 — Day of Experiment

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Diarrhoea		D1						D1				D3		D1
Body weight lbs.		75		78		78		76		74		74		74
Plasma sodium m.eq	140						133		128		128			125
Plasma potassium m.eq	4.4						4.6		4.3		4.4			4.4
Plasma chloride m.eq	99						99		97		95			97
Blood urea mgm	12						14		24		19			18

Calf 55 — Day of Experiment

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Diarrhoea														
Body weight lbs.		78		80		75		83		81		81		85
Plasma sodium m.eq	136						145		136		145			133
Plasma potassium m.eq	4.2						5.4		4.5		3.7			4.4
Plasma chloride m.eq	102						94		94		93			98
Blood urea mgm	14						27		22		11			26

Calf 56 — Day of Experiment

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Diarrhoea				D2	D2	D2	D2		D1	D1				
Body weight lbs.		76		80		75		75		75		75		78
Plasma sodium m.eq	-						140		136		136			128
Plasma potassium m.eq	-						4.6		4.5		4.8			4.3
Plasma chloride m.eq	95						93		96		97			90
Blood urea mgm	-						31		21		15			15

Calf 57 — Day of Experiment

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Diarrhoea						D1	D1					D1		D1
Body weight lbs.		60		64		63		63		64		63		68
Plasma sodium m.eq	136						128		136		128			
Plasma potassium m.eq	4.0						4.2		4.6		4.7			
Plasma chloride m.eq	101						104		101		98			
Blood urea mgm	18						15		14		19			

Calf 58

	Day of Experiment													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Diarrhoea						D1	D3	D2	D3	D2		D2	D1	D1
Body weight lbs.		77		80		80		77		71		65		67
Plasma sodium m.eq	132						150		136		136			125
Plasma potassium m.eq	4.8						5.4		4.2		5.0			4.8
Plasma chloride m.eq	93						97		98		96			95
Blood urea mgm	11						39		39		36			64

Calf 59

	Day of Experiment													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Diarrhoea					D1	D1								
Body weight lbs.		72		79		78		78		78		81		83
Plasma sodium m.eq	136						133		128		136			136
Plasma potassium m.eq	4.1						4.8		3.8		4.0			4.1
Plasma chloride m.eq	92						94		95		96			96
Blood urea mgm	21						33		22		25			24

Calf 60

	Day of Experiment													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Diarrhoea		D1				D1						D3		D3
Body weight lbs.		76		78		77		76		76		77		81
Plasma sodium m.eq	128						136		136		-			136
Plasma potassium m.eq	5.0						4.8		4.6		-			4.6
Plasma chloride m.eq	97						96		95		-			104
Blood urea mgm	10						11		11		-			10