RELATIONSHIP BETWEEN PRODUCTION PERFORMANCE AND RESPONSES OF BLOOD CHEMISTRY TO DIFFERENT PHYSIOLOGICAL STATES IN DAIRY CATTLE.

by

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ABSTRACT

The aim of the research presented in this thesis was to study the changes that occur in blood chemistry as a result of some common physiological factors (age, late pregnancy, early lactation, starvation, and stress due to ACTH injection) and the relationship of such changes with production indices (fertility, milk production and growth rate) in dairy cattle. The potential use of erythrocyte magnesium (EMg) as an indicator of body Mg status was studied by the experimental induction of hypomagnesaemia in young calves. A magnesium load test which could be used to determine body Mg status in young calves was also developed.

Since current methods of metabolic profile testing use mainly post-partum observations, a method that utilised and/or combined changes in blood components 8 weeks before and 8 weeks after parturition could be more informative. This approach could aid in early detection of any blood chemistry abnormalities which could affect production performance.

Blood chemistry changes in late pregnancy and early lactation were studied in two herds. The blood protein components (PCV, Hb, RCC, TPP, albumin and globulin) and plasma Mg concentrations decreased ($P<0.05$) while plasma Na and WCC's increased ($P<0.05$) towards calving. PCV, Hb, MCH, erythrocyte Na (ENa) and plasma creatinine concentrations decreased ($P<0.05$) while TPP, globulin, BUN, plasma, ENa and erythrocyte K(EK) concentrations increased ($P<0.05$) after calving. The changes in concentrations of the various parameters before and after calving were not significantly different between herds or age groups with the exception of Hb

and PCV levels which showed greater decreases in cows over 4 years than in those under 4 years.

Multiple linear regression analyses based on slopes and means of both pre-partum and post-partum periods accounted for 63% to 78.8% of the variation in services per conception, 44% to 65% of that in days open and 53% to 82% of the variation in milk production rank in both herds.

Changes in blood chemistry of heifers aged approximately 11 months occurred during a 40 hr starvation period with significant ($P<0.05$) between animal variation (except for plasma inorganic phosphorus, Na and K). Changes in plasma Mg, EMg and EK (decrease) and in BUN (increase) concentrations were correlated ($P<0.05$) with growth rate. A multiple linear regression equation with changes in plasma Mg, EMg, PCV and albumin as independent variables and growth rate as dependent variable yielded a multiple coefficient of determination of 0.79.

Blood chemistry changes occurred between 0 and 4 or 6 hours after an injection of 100 iu ACTH in heifers aged approximately 14 months and showed repeatable significant ($P<0.05$) between animal variations. A multiple linear regression equation with changes in plasma glucose, WCC, BUN, absolute eosinophil count and plasma Na concentrations as independent variables and growth rate as dependent variable yielded multiple coefficients of determination of 0.71 and 0.78 in repeat experiments.

Blood samples were collected monthly from 10 dairy heifers between 2 and 13 months of age. Regression equations based on the concentrations of plasma albumin and Ca at 7 months, plasma

globulin at 13 months, overall means (of 12 monthly estimations) of Hb, PCV and plasma albumin and changes in Ca and PK between 7 and 13 months of age accounted for 43% to 89% of the variation in growth rate. Similarly blood samples were collected monthly from 10 dairy heifers between 12 and 24 months of age, and regression analyses based on the concentrations of TPP and plasma globulin at 12 and 18 months of age accounted for 75% to 84% of the variation in growth rate. Similarly multiple regression equations based on the concentrations of TPP and plasma globulin at 12 months of age and plasma globulin and plasma Mg at 24 months of age and the overall mean concentrations of TPP, Hb and plasma Mg accounted for 64 to 72% of the variation in milk production rank at first lactation.

Hypomagnesaemia was induced in 4 experimental calves 1-2 weeks old by feeding 2 litres of skimmed milk (containing 1g of urea and 1g of KCl) twice a day and with ad libitum access to barley straw containing 5% urea, 10% soyabean oil and 5% KCl. The control calves received the same diet but were supplemented with 1g magnesium oxide daily. EMg concentrations decreased with age in both groups and also decreased following the onset of hypomagnesaemia in the experimental calves. The hypomagnesaemia was accompanied by significant ($P<0.05$) decreases in thyroid gland activity (FTI), plasma Ca, inorganic phosphorus, alkaline phosphatase concentrations and RCC's. Intramuscular injection of 40mg/kg b.wt of $MgSO_4$ solution increased both plasma Mg ($P<0.05$) and EMg concentrations after 4 hours in both hypomagnesaemic and normomagnesaemic calves but the changes in EMg were small and not significant in either group. The amount of Mg excreted in urine 4 and 24 hours after the $MgSO_4$ load was higher ($P<0.01$) in the control than in the hypomagnesaemic calves and could be used as a method of determining Mg status in young calves.

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GLOSSARY OF ABBREVIATIONS

AP	=	alkaline phosphatase
AST	=	aspartate aminotransferase (SGOT)
b	=	regression coefficient
BUN	=	blood urea nitrogen
B.wt	=	body weight
Ca	=	calcium
EK	=	erythrocyte potassium
EMg	=	erythrocyte magnesium
ENa	=	erythrocyte sodium
Fig	=	figure
FTI	=	free thyroxine index
g/L	=	grams per litre
Glob	=	globulin
G.R.	=	growth rate
Hb	=	Haemoglobin
HK	=	high potassium
iu	=	international units
LK	=	low potassium
MCH	=	mean corpuscular haemoglobin
MCHC	=	mean corpuscular haemoglobin concentration
MCV	=	mean corpuscular volume
mmol/l	=	millimoles/litre
MPT	=	metabolic profile testing
P	=	probability
PCV	=	packed cell volume
Pi	=	plasma inorganic phosphate
PK	=	plasma potassium

PMg	-	plasma magnesium
PNa	-	plasma sodium
P.R.	-	production rank
r	-	simple correlation coefficient
RCC	-	red cell count
SEM	-	standard error of the mean
SEB	-	standard error of the regression coefficient
SEE	-	standard error of the estimate
s/c	-	services/conception
*	-	$P<0.05$
**	-	$P<0.01$
TPP	-	Total plasma protein
µl	-	microlitre
WCC	-	white cell count
WBK	-	whole blood potassium
WBMg	-	whole blood magnesium
WBNa	-	whole blood sodium

STATEMENT OF SOURCE.

I certify that the work presented in this thesis is the result of original research by the author, except as otherwise acknowledged. The material presented has not been submitted either in whole or in part for a degree at this or any other University.

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CHAPTER 1

1. GENERAL INTRODUCTION

In terms of gross marginal profit per acre milk is one of the most attractive farming enterprises today (Collins 1979). Every effort is made to secure high yields at minimum cost and this is likely to increase in importance because high yielding cows are advantageous in terms of financial returns and in efficiency of protein conversion (Coward 1969). With increasing milk production, pathological phenomena become more manifest in the metabolic processes and in organs such as the uterus, the ovaries and the udder (Sommer 1975). According to Sommer (1975) metabolic disorders associated with high milk production occur immediately before or after calving.

However, farmers face a practical problem in that they are unable to predict which cows are likely to succumb to metabolic disorders or production inefficiency. In many instances actual clinical illness or other overt conditions are terminal manifestations of degenerative processes which have been in operation for sometime (Sommer 1975).

Several health monitoring procedures utilizing blood profiles have been developed with a view to improving the health status of dairy cows. However most of the methods do not become operative until postpartum (Payne, Dew, Manston and Faulks 1970, Stevens 1975) and thus cannot be used to detect animals with abnormal blood profiles before parturition. Sampling cows 8 weeks before

calving has proved very useful in detecting those cows likely to develop metabolic disorders postpartum in West Germany (Sommer 1975). Thus a method of monitoring blood profiles of dairy cows in late pregnancy and early lactation could be useful in identifying animals with metabolic abnormalities likely to affect their productive performances postpartum. Such a method may be of great importance to farmers who normally rely on irregularities of oestrus and multiple services per conception in absence of obvious organic irregularities (Delange 1950) to recognise functional infertility which results in uneconomically high calving to conception and calving to calving intervals. If blood profiles in cows before or immediately after calving bore a relationship to subsequent productive and reproductive performance this could form a basis for early identification and correction of abnormalities and perhaps for selection and culling of breeding stock.

In addition to helping identify those metabolic abnormalities likely to impair productive performance blood profiling has the potential of identifying animals with superior qualities in terms of growth rate or milk production (Rowlands, Payne, Dew and Manston 1974). There is evidence of individual variation in the blood composition of growing animals over short and long periods of time (Payne, Rowlands, Manston, Dew and Byrne 1973b, Rowlands et al. 1974, Kitchenham, Rowlands, Manston and Baldry 1977, Manston, Kitchenham and Baldry 1977). This suggests that not only might calves be screened early in life but also that animals might be bred to have certain desirable blood profile characteristics. Thus the idea that limited growth rate in calves

may be detected by blood analysis suggests that a test on growing stock might be devised.

Various workers (Arthaud, Schultze, Koch and Arthaud 1959, Payne et al. 1973b, Rowlands et al. 1974, Kitchenham et al. 1977) have reported the relationships between growth rate and blood chemistry. These published results have shown some inconsistency and it has been suggested that this variability is due to effects of [illegible] (Payne et al. 1973b, Rowlands et al. 1974). However despite the lack of agreement on the relationships between blood chemistry, growth rate and environment, the tendency to maintain high concentrations of certain blood constituents can be inherited (Rowlands et al. 1974). The evidence that the variation of many blood intermediary metabolites in domestic animals is under genetic control (Rowlands et al. 1974, Freeman, Kelly, Ledet, Evans, Appel, Mass and Pearson 1978) has stimulated interest in studies on the relationship between blood metabolite levels and animal performance under different physiological states (Hart, Bines, Balch and Cowie 1975, Bindon and Piper 1976, Tilakaratne, Alliston, Carr, Land and Osmond 1980, Land 1981). Some of these studies have suggested that animals of different productive performance can be identified early in life. Strains of sheep with high reproductive performance were found to have higher levels of gonadotrophic hormones in peripheral plasma at a young age than the strain of sheep with low levels of reproductive activity (Bindon and Piper 1976). On the basis that one characteristic of a good cow is the ability to maintain

production when in an overall energy deficit it has been shown that induction of a negative energy balance by fasting can reveal relevant physiological covariations (Broster, Broster and Smith 1969, Tilakaratne et al. 1980). These workers found that calves of high genetic merit utilized fat reserves (high blood FFA levels) more than body protein (low BUN levels) for energy, while calves of low genetic merit utilized body proteins (high BUN levels) more than fat reserves (low blood FFA levels) for energy.

The ability of an animal to withstand stress to which it is not adapted, depends on the ability of the adrenal glands to increase and maintain elevated plasma corticoids during exposure to stress (Cope 1972). Studies have shown that animals with an impaired ability to tolerate stress at parturition and during early lactation have high mortality rates (Trimberger, Tyrell, Morrow, Reid, Wright, Shipe, Merrill, Looshi, Coppock, Moore and Gordon 1972). Thus a method of assessing an animal's ability to withstand stress could be of great importance to farmers, since animals which are able to adapt easily to stressful conditions should be better able to direct their energy to agricultural performance (Schultze 1959).
Thus the aims of the studies described in this thesis were:

1. To review the literature on metabolic profile testing in dairy cattle, its usefulness and limitations.

2. To investigate an alternative approach to the classical metabolic profile testing in dairy cows by examining changes that occur in the blood chemistry 8 weeks before and 8 weeks after parturition, and the relationship of such changes to production indices (fertility and milk yield).

3. To investigate the effects of a short period of starvation on the blood chemistry of young replacement heifers in an attempt to identify changes that might be used to predict growth rate and milk production.

4. To investigate the response of blood chemistry to ACTH injection in young replacement dairy heifers and the relationships of the response to growth rate and subsequent milk production.

5. To investigate any possible relationship between blood chemistry and growth rate and future production potential of young replacement dairy calves (2-13 months) and heifers (12-24 months).

6. To investigate whether:

   i) EMg concentration was a better indicator of Mg status in calves than PMg concentration.

   ii) The response to a Mg load could be used to indicate Mg status in calves.

2. LITERATURE REVIEW

2.1. Metabolic profile testing (MPT)

Severe strains may be imposed on the milking cow's metabolism. These strains may increase the risk of imbalances in the input/output relationship and result in an increased incidence of production diseases (e.g. metabolic disorders)(Payne et al. 1970, Rowlands and Manston 1976). Therefore regular monitoring of the metabolic health of a dairy herd is required for early diagnosis and correction of the disease conditions and dietary imbalances which are associated with increased lactation demands (Payne et al. 1970). Various forms of monitoring metabolic parameters in dairy herd health have been developed by different workers.

(a) Types of herd metabolic profile tests

(i) Mini profile test

This is principally aimed at establishing the adequacy of the energy and protein intake of a dairy herd throughout lactation by monitoring concentrations of plasma glucose, serum urea and serum albumin (Blowey, Wood and Davis 1973). Herds are visited monthly and cows between 4 and 10 weeks after calving are sampled. This eliminates the effect of stage of lactation and aims at the correction of feeding imbalances during early lactation in order to coincide with the period of conception.

(ii) Individual preventive examination

This is a health check on each individual high-producing cow prior to calving (Sommer 1975). From each cow a blood sample is collected about 8 weeks before calving and the total cholesterol level and serum aspartate aminotransferase activity determined. These parameters are considered to indicate the state of energy balance and liver efficiency of each animal, and are used to predict susceptibility of each cow to the "production syndrome" i.e. metritis, retained placenta, hypocalcaemia, the downer cow syndrome and mastitis.

This concept of dairy herd monitoring emphasises the importance of the individual animal's response to its diet and its production demands, and has found support as a prelude to a more classical profile approach.

(iii) Method of using marker cows

This method entails blood sampling of the same marker cows within a herd at regular four weekly intervals throughout the year (Merrall 1976). The samples are subjected to the full profile analysis described by Payne et al. (1970). The results are correlated with the quality of feed available, the stage of plant growth and the stage of lactation of the marker cows. This method of herd health monitoring is suitable for use in herds wherein cows calve down within a short period.

(iv) Classical metabolic profile testing (MPT)

This was developed by Payne et al. (1970) in order to

discover the causes of high incidences of certain disease in so-called problem herds. The test is essentially a "herd test" and is based on assessment of blood chemistry in three groups of cows, i.e. 7 dry cows, 7 high-yielding cows and 7 cows with medium milk production.

(b) Uses of metabolic profile tests

(i) To monitor the metabolic state of a dairy herd.

(ii) To assess the adequacy of the dietary intake for production (Kronfeld, Donoghue, Copp, Steams and Engle 1982).

(iii) To elucidate the nature of existing clinical diseases and detect the presence of developing imbalances that are likely to affect production efficiency (Payne et al. 1970, Collins 1979).

iv) To identify those individuals which may possess a "superior metabolism" in coping with sub-optimal conditions. It may also be used to detect those blood constituents whose concentrations could be used as a "predictive marker" of production performance regardless of the environment (Rowlands and Manston 1976)

c) Blood parameters measured in metabolic profile tests

The blood parameters measured in the MPT (Payne et al. 1970) are:

Plasma glucose	Serum calcium
Blood urea nitrogen	Serum inorganic phosphate
Total plasma protein	Serum magnesium
Serum albumin	Serum sodium
Serum globulin	Serum potassium
Hemoglobin	Packed cell volume

Red cell counts and white cell counts are also examined in modifications of MPT used in the United States of America (Stevens 1975).

(i) Plasma glucose

Plasma glucose is commonly determined in the MPT of dairy herds due to its positive relationship with dietary energy intake (Hewett 1974, Herdt 1981). Decreased plasma glucose concentrations have been associated with an increased incidence of clinical ketosis (Herdt 1981) digestive disorders and poor milk yield (Wilson and Medd 1977), calf scours (Gardner and MacDonald 1975 , Zamet, Colenbranden, Erb, Chew and Callahan 1979) and infertility (McClure 1965, McClure 1966).

The blood glucose concentration has been reported to vary between individual animals (Rowlands and Manston 1976, Henricson Jonsson and Pehrson 1977) and to show diurnal variation in lactating cows (Coggin and Field 1977). The extracellular glucose concentration is hormonally regulated and its removal is positively correlated to plasma insulin levels (Basset, Weston and Hogan 1971). The proportion of roughage in the diet as well as glucogenic substrates have been shown to have an effect on hormones regulating glucose concentration in plasma (Trenkle 1971). However the regulation of extracellular glucose and insulin is controlled by complex neurohormonal factors in response to digestive substrates in the blood (Basset 1974, Evans, Buchanan-smith, Macleod and Stone 1975).

(ii) Blood urea nitrogen

Blood urea has a half-life of only a few hours (Cacimano and Leng 1967), thus it can only be used for detecting short-term

changes in nutritional status. Abnormal mean herd blood urea concentrations are not associated with specific clinical disease, however levels of urea in the blood can give information on dietary protein content and its utilization (Herdt 1981). The blood urea concentration is inversely related to blood glucose concentration (Gardner and MacDonald 1975). This is partly due to the dependency of rumen microbial growth on energy availability (Sykes 1977).

Dietary protein is degraded in the rumen to produce ammonia which is incorporated into amino acids which are then used for microbial protein synthesis. The rate of protein degradation is dependent on the rate of microbial growth (Tamminga 1979). While microbial protein synthesis is directly related to the microbial growth rate (Stern and Hoover 1979) this is dependent on energy availability (Sykes 1977). Thus diets rich in rumen degradable protein but poor in energy will produce a high rumen ammonia concentration of which the excess is absorbed through the rumen epithelium and converted to urea in the liver thus elevating blood urea levels. Diet rich in energy and poor in rumen degradable protein lowers rumen ammonia levels and as a result blood urea diffuses into the rumen where it is converted to ammonia for microbial synthesis (Houpt 1970), hence reducing blood urea levels. Thus blood urea can serve as an indicator of the balance between rumen degradable protein and energy supply (Herdt 1981).

(iii) Total plasma proteins

These are composed mostly of albumin and globulins both of which are normally analysed in the MPT.

(iv) Plasma albumin

Plasma albumin plays an important role in maintaining blood osmotic pressure (Rooney 1957, Kaneko and Cornelius 1970) and plays some part in the replenishing of amino acids utilized by the body as well as in [illegible] (Kon and Cowie 1961). While changes in blood urea concentrations reflect day to day changes in dietary protein, depression of serum albumin reflects a long-term protein deficiency (Manston, Russell, Dew and Payne 1975).

The homeostatic regulatory mechanisms controlling albumin production and catabolism are not well understood (Herdt 1981). Dietary factors other than total protein consumption and certain non-dietary factors may influence serum albumin concentration. Thus a careful ration evaluation and nutritional consultation must accompany any dietary recommendation based on serum albumin levels (Herdt 1981).

(v) Plasma globulin

Serum globulins increase with age and this possibly occurs due to exposure to various infections which stimulate the production of immunoglobulins (Larson and Touchberry 1959,

Dimopoullos 1961). Serum globulins are negatively correlated with albumin concentration (Rowlands, Manston, Pocock and Dew 1975), thus when one alters there is a compensatory increase or decrease in the other fraction in order to stabilize the serum osmotic pressure (Rooney 1957, Kaneko and Cornelius 1970).

(iv) Packed cell volume (PCV) and haemoglobin (Hb)

PCV and Hb values provide an estimate of the size of the circulating red blood cell pool (Herdt 1981). A decrease in these parameters indicates a degree of anaemia which has been associated with infertility in dairy herds (Hewett 1974, Rowlands and Manston 1976, Morrow 1978). The exact cause of anaemia in dairy herds is often difficult to define. However, deficiences of iron, copper or cobalt (Schalm, Jain and Carroll 1975) or chronic protein deficiency (Manston et al. 1975) are known to cause anaemia in dairy cows. Chronic internal parasitism must also be considered as a possible cause of anaemia in dairy cattle (Herdt 1981). Thus in herds with anaemia a thorough clinical and nutritional evaluation would be necessary.

(vii) Plasma calcium (Ca)

Plasma Ca concentration is commonly determined in dairy herds MPT because of the frequency of clinical problems associated with hypocalcemia in dairy cows (Herdt 1981). It is under homeostatic control by the parathyroid and thyroid glands

(thyroid C cells) (Payne and Sansom 1966, Adams, Stout and Kradel 1978). Through this homeostatic mechanism the cow regulates its intestinal calcium absorption and bone resorption or accretion to match its needs (Manston 1967, Picard 1977) and maintains serum Ca within normally fixed limits (Sansom 1969, Capen and Martin 1977).

Serum albumin concentration is an important factor influencing serum Ca (Herdt 1981) as 40% of bovine serum Ca is normally bound to albumin (Blum, Kamberg, Johnston and Kronfeld 1972). However a reduction in serum albumin level can reduce the total serum Ca without affecting the more functional ionized Ca (Davidson and Henry 1974). Thus serum Ca concentrations on an individual animal or herd basis should be interpreted with reference to serum albumin level (Herdt 1981).

(viii) Plasma magnesium (Mg)

Serum Mg is one of the most useful mineral determinations for the dairy herd MPT because both typical and atypical signs of hypomagnesaemic tetany (e.g. mid-lactation paresis, sudden deaths and poor production) occur in herds with low serum Mg (Payne et al 1970, Payne 1973a, Young 1978, Herdt 1981). It is not under rigid hormonal control and this leads to a great variation in the observed values in serum (Sansom 1973). Serum Mg concentrations depend on the input-output balance of Mg (Picard 1977).

Sutherland, Bell, McSporran and Carthew (1986) found that herd magnesium status could be determined either by measurements of serum Mg or urinary Mg fractional clearance ratio (FCR) or creatinine corrected urinary Mg concentration but suggested that the latter two measurements were more sensitive as predictors of a positive production response to Mg supplementation in dairy cows.

Plasma magnesium is partially correlated with blood glucose and albumin concentrations (Payne, Rowlands, Manston, Dew and Parker 1974). The correlation with albumin could be due to the binding of Mg on that protein (Wilson 1964). The correlation with glucose may be an indirect one through energy supply. Energy is necessary for rumen microbial protein synthesis from ammonia (Ammerman, Chicco, Moore, Van Walleghem and Arrington 1971), thus a low energy supply will result in reduced microbial protein synthesis leading to ammonia build up (Gardner and MacDonald 1975). High ammonia concentration in the rumen depress Mg absorption from the gut in dairy cows (Henry et al 1977).

(ix) Plasma inorganic phosphate (Pi)

Phosphorus is required in large amounts for milk production and exists in the blood as organic and inorganic fractions (Picard 1977, Herdt 1981). The plasma Pi concentration is not under any hormonal control thus its levels may fluctuate depending on the balance between phosphorus intake and outflow (Hewett 1974, Picard 1977). Considerable variation in plasma Pi concentrations may occur within classes and within and between

erds (Gartner, Ryley and Beattie 1966, Mylrea and Bayfield 1968). his is probably due to sampling variation and dietary factors oggin and Field 1977). Abnormal plasma Pi values (low or high) ave been associated with infertility in dairy cows (Mylrea and ayfield 1968, Morrow 1969).

x) Plasma sodium (Na)

Plasma Na concentration is the least variable of the MPT arameters (Payne et al. 1970). Thus even small falls in mean oncentration may exceed the 95% confidence limits and may ndicate an inadequate dietary intake of this element (Payne t al. 1970, Payne 1977).

xi) Plasma potassium (K)

Studies on the K requirements of various species have hown that the presence of K in the diet influences the general erformance of the animal, including growth rate, (Telle, reston, Kintner and Pfander 1964, Roberts and Driedger 1966). otassium-deficient diets produce specific signs associated with oor performance (low growth rate and poor body condition) in animals (Roberts and Driedger 1966). The K content of most rains is relatively lower than that in roughages, hence K eficiency can easily occur in a herd where high concentrate ations are in common use (Pradham and Hemken 1968).

(d) Selection of animals to be subjected to MPT

The selection of animals to be tested depends on the conditions on the farm. In "normal" herds no particular animals are prefered however in "problem" herds, animals with an abnormality are of value in diagnosing the problems of that particular animal and as such the problem for the herd (Collins 1979, Herdt 1981).

(e) Sampling sites

Differences in blood component concentrations exist among different blood sampling sites for example tail (coccygeal blood vessels) blood may be higher in Pi and K, lower in Ca, Mg, PCV and Hb concentrations than jugular blood (Rowlands et al 1975). Thus one site of sampling should be consistently used for any particular laboratory.

The coccygeal vessels may be preferred to the jugular vein because of convenience, i.e. reduced animal excitement and stability of metabolites (Herdt 1981). In beef cattle the blood values for Hb, PCV, K, Pi, TPP, albumin and globulin were elevated by excitement (Gartner, Ryley and Beattie 1965).

(f) Factors affecting blood parameter concentrations

Some parameters determined in the MPT may be affected by the following factors:

1. Milk yield
2. Stage of lactation
3. Stage of pregnancy
4. Age
5. Herd of origin
6. Season of the year
7. Parturition
8. Number of lactation

Milk yield

Milk yield has been shown to be negatively correlated with PCV, Hb, and Pi concentrations (Lane, Campbell and Krause 1968, Hewett 1974, Payne et al 1974, Whitlock, Little and Rowlands 1974). The fall of Hb and PCV with increase in milk yield could be due to an inadequate supply of energy, certain amino acids and trace elements necessary for the formation of Hb as a result of increased demand made by the high milk yield (Hewett 1974).

Serum Mg concentration has been shown to increase with milk yield (Hewett 1974, Manston et al 1975) however Kitchenham, Rowlands and Shorbagi (1975) observed a negative correlation between milk yield and plasma Mg concentration. The serum Ca concentration has been reported to decrease with milk yield (Rowlands et al. 1975, Claypool 1976, McAdam and O'Dell 1982). However Payne and Leach (1964) and Hewett (1974) did not find any effect of milk yield on serum Ca concentrations.

et al. 1975). The rise in BUN concentrations early in lactation has been attributed to variations in protein intake (Hewett 1974), and inadequate feed intake resulting in body tissue catabolism (Broster et al. 1969, Herdt 1981, Roberts, Reid, Rowlands and Patterson 1981).

Blood glucose concentrations are lower in lactating cows than in dry cows (Pehrson 1971), are low in early lactation (Rowlands et al. 1975, Herdt 1981) and then rise in the later stages of lactation (Rowlands et al. 1975). In early lactation, plasma Na concentrations were found to be lower than in dry cows (Murtuza et al. 1979). Total plasma protein concentrations were found to rise to a peak in the second month and then fell up to the 8th month of lactation (Hewett 1974). However, Rowlands et al. (1975) did not find any stage of lactation effect on TPP levels. Albumin and globulin concentrations fall before parturition and return to prepartum levels within two to three weeks after calving (Amiel 1970, Rowlands, Manston, Stark, Russell, Collins and Collins 1980). In the case of albumin the rate of return to prepartum levels has been shown to be dependent on protein intake (Manston et al. 1975). These workers found that plasma albumin concentrations returned to precalving levels faster in cows receiving a high protein intake than in those on a low protein diet intake.

Red blood cell counts increase after calving followed by a fall over 3 weeks to below pre-calving levels (Ferguson, Irwin and Beach 1941, Zamet et al. 1979). Leukocyte counts drop sharply after calving (Ferguson et al. 1941, Merrill and Smith 1954, Straub, Oscar, Schalm, Hughes and Thailen 1957). Lymphocyte

and eosinophil numbers increase and neutrophils fall while monocytes and basophils do not show any definite change upto 10 days post-partum (Merrill and Smith 1954). An absolute monocytosis from parturition up to 5 days post-partum was observed by Straub et al. (1957). No effect of the stage of lactation or milk yield on total leukocyte counts was found by Hewett (1974).

Stage of pregnancy

The levels of PCV, Hb, TPP, albumin, globulin and glucose decrease in the late stages of pregnancy (Larson 1958, Hewett 1974, Herdt 1981). The fall in PCV, Hb and glucose concentrations after drying off have been attributed to a decrease in feed intake and high demand for nutrients by the rapidly developing foetus (Hewett 1974, Herdt 1981).

Stage of pregnancy has been reported to have no effect on plasma Mg, Ca, Pi, Na and K concentrations in dairy cows (Belyea et al 1975, Parker and Blowey 1976, Rao, Prasad, Krishna and Rao 1981).

Total leukocyte counts increase in the last 2 weeks of gestation (Straub et al. 1959, Zamet et al. 1979).

Age

The blood levels of PCV, Hb, RCC, Pi, WCC, BUN, and glucose have been reported to decrease with age (Payne and Leach 1964, Gartner, et al. 1966, Hewett 1974, Kitchenham et al. 1975). A significant fall in PCV and Hb concentrations has been observed in the first 19 months of life (Gartner et al. 1966). The PCV's

and Hb concentrations were found to be higher in heifers than in older cows (Hewett 1974). Serum levels of Pi have been reported to show a marked decrease with age up to approximately 4 to 5 years (Payne and Leach 1964, Lane et al. 1968). At birth calves have higher serum Pi concentrations than their dams (Sato and Imamura 1980, Jagos, Dvorak and Boulda 1981).

Increasing age is associated with a slight decrease in serum Ca concentration in cows (Payne and Leach 1964, Gartner et al. 1966). This may be due to a decreasing ability to mobilize Ca from both the alimentary tract and skeleton with age (Moodie 1961, Picard 1977). Total leukocyte counts decrease with age and this has been shown to be due to a decrease in circulating lymphocytes (Hewett 1974, Noonan, Cross, Reynolds and Murphree 1978). At birth, calves have very high blood glucose concentrations and this decreases as the calves mature to adult animals (Jarret, Jones and Potter 1964, Nicolai and Stewart 1967, Kitchenham et al. 1975). The decrease in plasma glucose concentration with age was found to be due to a decrease in glucose entry rate into the extracellular space (Jarret et al. 1964).

The concentrations of TPP and globulins increase with age, the increase being mainly due to increases in gammaglobulins (Larson and Trouchberry 1959, Gartner et al. 1966, Kitchenham et al. 1975). The reported effects of age on albumin and PMg concentrations vary, and decreases (Gartner et al. 1966, Kitchenham et al. 1975) and increases (Little 1974, Jagos et al. 1981) have all been reported.

Herd of origin

Most of the blood parameters determined in MPT differ significantly between herds (Payne et al. 1974, Claypool 1976, Lee, Twardock, Bubar, Hall and Davis 1978). These differences have been attributed to differences in feeding programs (Payne et al. 1974, Claypool 1976). The concentrations of PNa and PK do not vary greatly between herds or classes of stock (Mylrea and Bayfield 1968), probably because they show small variation between individual animals (Gartner et al. 1966, Payne et al. 1974) and types of diet (Ruppanner, Norman, Adams, Addis, Lofgreen, Clark and Dunbar 1978).

Season of the year

The effects of season of the year on MPT parameters are mainly due to different feeding regimes (Claypool 1976). Most of the blood components (PCV, Hb, TPP, BUN, albumin, Mg, Ca, glucose, and Pi) are higher in summer than in winter (Russoff, Johnston and Branton 1954, Payne et al. 1974, Claypool 1976).

The high PCV's and concentrations of Hb, TPP, albumin and BUN in summer have been attributed to a higher protein intake during this time (Payne et al. 1970; Rowlands et al. 1975). Plasma Ca concentrations were found to be low during winter when animals were fed silage and high when fed alfalfa (Claypool 1976). However no change in plasma Ca concentrations due to season was observed by Russoff and Piery (1946).

Low plasma Na concentrations were commonly seen in summer (Sansom 1973). PK and globulin concentrations do not show any seasonal variations (Roubicek and Ray 1972).

Lactation number

Lactation number has been shown to have significant effects on TPP, PCV, Hb, albumin, globulins, RCC, PMg, and Pi concentrations (Payne and Leach 1964, Lane et al. 1968, Herdt 1981). The values for PCV, Hb, Mg, Pi, glucose and WCC have been reported to decrease with increase in the number of lactations (Lane et al. 1968, Henricson et al. 1977, Herdt 1981). The concentrations of TPP and globulin increase steadily with an increase in lactation number while albumin falls in later lactations (Herdt 1981).

Parturition

The blood concentrations of Pi, Ca, albumin, globulin and TPP decrease near parturition (Blum et al. 1972, Jagos et al. 1981, Rao et al. 1981). Plasma Ca concentrations fall prior (24 hours) to parturition with the lowest level occurring at the time of calving especially in multiparous cows (Moodie 1968, Jagos et al. 1981, McAdam and O'Dell 1982). This hypocalcemia is partly due to the drain of Ca into milk and a decrease Ca absorption which occurs at or around calving due to decreased feed intake and ruminal stasis occuring at this time (Moodie and Robertson 1962, Moodie 1968, Picard 1977). The greater decrease in plasma Ca in older cows has been attributed to reduced Ca mobilization from the skeleton causing them to depend on a continuous Ca absorption from the gut (Moodie and Robertson 1962, Picard 1977). The decrease in TPP and albumin and globulin concentrations towards parturition is due to their transfer from the blood into colostrum (Larson 1958, Pierce and Feinsten 1965, Williams and Miller 1975).

The concentration of plasma glucose, Mg and Hb and levels of PCV, RCC and WCC increase (24 hours) prior to parturition (Merrill and Smith 1954, Straub et al. 1959, Blum et al. 1972, Kelly 1977). The increase in plasma glucose concentration and WCC prior to parturition has been attributed to the stress of parturition and elevated serum glucocorticoids (Edgerton and Hafs 1973). The increase in RCC, PCV and Hb concentrations at the time of calving could be a result of a combination of reduced water intake and contraction of the spleen forcing the sequestered erythrocytes into the blood stream (Straub et al. 1959; Zamet et al. 1979). Plasma Na and K concentrations do not change at parturition (Dishington 1965).

(g) Limitations of Metabolic Profile Testing

A number of factors limit the usefulness of blood profiles in either problem situations or routine dairy herd management. Some of these factors may be overcome in the future while others are inherent (Adams et al. 1978).
They are as follows:

1. Sampling problems;
2. Correct choice of animals to be sampled;
3. Low correlations with feed intake;
4. Inconsistent patterns in disease syndromes;
5. Difficulties in interpretation.

(1) Sampling problems

Blood constituents may vary with the site of sampling so one site of sampling must be chosen for any particular laboratory.

The sampling should be done with minimum excitement, since this may elevate PCV, and Hb, K, Pi, TPP, albumin and globulin concentrations (Gartner et al. 1966). Thus coccygeal blood vessels appear to be the best site for MPT sample collection because of convenience, reduced animal restraint and excitement and stability of metabolites (Herdt 1981).

(ii) Choice of animals

The choice of animals to be sampled has not been resolved. However many workers follow schemes similar to those of the English workers (Payne et al. 1970, Payne et al 1973a).

(iii) Low correlations with nutrient intake

Homeostatic mechanisms and various nutrient interrelationships limit the usefulness of blood profiles in assessing nutritional status (Adams et al. 1978). However under farm conditions some significant but low correlations may exist between some blood metabolites and nutrient intake (Adams et al. 1978).

The values for PCV, WCC and concentrations of Hb, albumin, TPP, PMg, BUN and Pi are affected by type of feed (Roubicek and Ray 1972, Kitchenham et al. 1975, Sykes 1977, Herdt 1981). Low PCV's and Hb, TPP and albumin concentrations occur in herds receiving low protein diets (Roubicek and Ray 1972, Manston et al. 1976) and in herds with decreased feed intakes in the later stages of lactation (Hewett 1974). Plasma albumin concentrations do not change immediately with change in protein intake (Manston et al. 1976). These workers observed a delay of up to two months

and concluded that this delay showed that low serum albumin and Hb concentration could be useful indices of a long standing protein deficit in lactating cows. Low serum albumin values can also occur due to gastrointestinal tract parasites (Mulligan 1972), liver damage (Payne et al. 1974, Rowlands and Manston 1983) or increased globulin levels (Manston et al. 1975).

Although BUN concentration is directly related to protein level in the diet, the relationship is valid only if energy intake is not limited (Sykes 1977). In cases of energy deficiency gluconeogenesis of amino acids from the muscles occurs, leading to increased BUN concentrations (Graham 1968, Prior, Scott, Laster and Campion 1979). High plasma glucose concentrations are associated with low BUN concentrations (Gardner and MacDonald 1975).

There is an inverse relationship between dietary Ca and dietary energy intake and serum Pi concentrations (Hewett 1974, Kitchenham et al. 1976). However when these dietary variables are equal, serum Pi is related to dietary phosphorus intake (Kitchenham et al. 1976). Because of these and other dietary factors it is doubtful that variations in serum Pi within the normal range are a sensitive indicator of phosphorus intake (Herdt 1981). Plasma Mg concentration is directly influenced by dietary Mg intake (Economides, Miller, Topps, Gelman and Keith 1973, Herdt 1981). This is partly due to a non-rigid homeostatic mechanism regulating PMg which allow its levels to fluctuate with dietary levels (Herdt 1981). The greatest portion of body Mg is in the skeleton and immobilizable (Blaxter, Graham and Wainman 1956,

Payne 1977), thus animals have to depend on current feed intake and extracellular fluid Mg for daily needs (Herd 1966). High dietary Ca (Moodie and Robertson 1962) K and nitrogen (Parker and Blowey 1976) reduced Mg availability.

Dietary Ca does not have much influence on plasma Ca. In some experiments a high Ca diet elevated plasma Ca concentrations (Herdt 1981) while in others it reduced it or there was no effect at all (McAdam and O'Dell 1982). Blood glucose concentration has been shown to have some positive relationship with energy intake by several workers (Story and Rook 1962, Hewett 1974), however other workers (Annison 1960, Pehrson 1971) did not observe any relationship between blood glucose concentration and energy intake. It has been shown that blood glucose concentration could give an indication of the energy status of animals especially where a gross rather than a marginal dietary deficiency exists (Kelly 1977, Wilson and Medd 1977). The blood glucose concentions are affected by levels of starch and roughage in the diet (Trenkle 1970, Evans et al. 1975). A high starch diet yields more propionate which is readily converted to glucose thus resulting in increased glucose (Evans et al. 1975). The level of roughage in the diet has an effect on the neurohormonal regulation of extracellular glucose (Trenkle 1970). Sheep fed a low roughage diet were found to have a higher blood glucose concentration than those fed a high roughage diet (Evans et al. 1975). Although blood glucose levels have been reported to have a positive relationship with feeding intensity by many workers (Story and Rook 1962, Hewett 1974), Coggin and Field 1977, Herdt 1981) a few workers (Little 1972, Economides et al. 1973) did not observe any relationship between feeding intensity and blood glucose concentration.

Salt treatment has no effect on PNa and PK levels (McAdam and O'Dell 1982), however Sansom (1973) observed high PK levels in animals on pastures heavily fertilized with potassium fertilizers.

(iv) Inconsistent patterns in disease

A wide range of abnormalities in a blood profile may occur in any type of production syndrome. Abnormalities are not always striking or consistent among herds suffering from a high incidence of a particular disorder (Adams et al. 1978).

Both high and low herd mean serum concentrations of calcium have been associated with milk fever and the downer cow syndrome (Payne et al. 1970, Adams et al. 1978). Herds with a high serum Ca and Pi concentrations during the dry period were found to have a high incidence of milk fever (Payne et al. 1970). Low PMg concentrations have been found to result into both typical signs of hypomagnasaemic tetany as well as atypical signs such as midlactation paresis and sudden deaths (Payne et al 1970, Payne et al 1973a, Herdt 1981).

Low plasma glucose concentration has been associated with increased clinical ketosis (Schultze 1971, Wilson and Medd 1977) while others have not confirmed this observation (Parker and Blowey 1976). Low blood glucose levels have sometimes also been associated with digestive disorders (Wilson and Medd 1979). Low blood glucose levels have also been associated with infertility in dairy cows (McClure 1965, McClure 1966, McClure 1968) fatty liver (Zamet et al. 1979), high incidence of calf

scours and poor general herd health (i.e. increased bulk milk cell counts, severe mastitis, unresponsive milk fever and increased incidence of retained fetal membranes (Gardner and MacDonald 1975).

Anaemia may show up in many blood profiles from herds with infertility problems but not consistently (Adams et al. 1978). Abnormal concentrations (high and low) of serum Pi have been associated with infertility problems in cows (Payne et al. 1964, Hewett 1974, Rao et al. 1981). Thus a phosphorus supplement should be critically evaluated in response to low or high serum Pi levels (Herdt 1981). Cows requiring more services per conception have been shown to have lower serum albumin and higher globulin concentrations than those conceiving to first service (Amiel 1970, Rowlands, Little and Kitchenham 1977, Rowlands et al. 1980). However other workers did not observe any relationship between serum albumin concentrations and fertility (Hewett 1974, Parker and Blowey 1976). High serum K concentrations have also been associated with both low fertility (Hewett 1974) and high fertility (Rowlands et al. 1977).

Thus the wide range of abnormalities that may occur in a problem herd makes it difficult to use blood profiles in predicting the susceptibility of a herd to individual problems. However problem herds usually have a higher incidence of blood profile abnormalities (Adams et al. 1978) and thus, profiling in a general way may indicate the presence of a potential problem.

(v) Difficulties in interpretation

One of the main problems relating to interpretation is the establishment of normal or expected values for a given area or population and also for individual laboratories since values established by laboratories in widely separated areas differ considerably for some parameters (Payne et al. 1973a, Morrow 1975, Adams et al. 1978). The interpretation of the results can be complicated by the fact that some parameters are affected by production level, stage of lactation, season of the year and age. However some of these difficulties can be overcome by grouping results from dry cows and cows in various production levels separately (Adams et al. 1978, Rowlands 1980).

2.2. The effect of starvation on the blood chemistry of cattle

In practice a short fast sometimes occurs when animals are yarded for various reasons (e.g. drenching, marking, sales) or in adverse weather. In most cases the period without food is less than 24 hours but occasionally, it may be longer. A short fast can cause significant changes in the blood chemistry of cattle.

The plasma concentrations of Ca, Mg and creatinine decrease during starvation (Robertson, Paren, Barden and Marr 1960, Herd 1966, Stufflebeam, Blakely, Lasley, Thompson and Mayer 1969). The decrease in PMg occurs within 24 hours while that of plasma Ca occurs 48 hours after the onset of starvation (Robertson et al. 1960, Herd 1966), and the lowest values of PMg are

observed 1 to 2 days after the re-introduction of feed (Herd 1966). The levels of plasma Ca return quickly to the pre-starvation levels on re-feeding (Halse 1958, Herd 1966). This quick recovery of plasma Ca has been attributed to the immediately available Ca reserve of about 6-10 g (Payne 1963) in the cow due to mobilization of Ca from the skeleton (Herd 1966). The tendency of PMg to remain low on re-feeding is because a cow has limited Mg reserves and has to depend on dietary Mg intake (Herd 1966). Therefore it may take several days following the re-introduction of feed before the PMg status of lactating cows is restored to normal. The decrease in plasma creatinine levels during starvation has been attributed to the reduced energy intake (Stufflebeam et al. 1969).

PCV and Hb, TPP, albumin, globulin, Pi, and BUN concentrations increase with starvation (Pehrson 1966, Tilakaratne et al. 1980). The increase in PCV and Hb, TPP, albumin and globulin concentrations has been associated with the dehydration that occurs during starvation (Pehrson 1966, Rumsey and Bond 1976). The concentration of plasma Pi decreases initially and then increases with starvation. The initial decline has been attributed to reduced phosphorus absorption from the gut (White, Christian and Williams 1956) and the rise after the initial fall has been associated with increased tissue (especially protein) catabolism (Dale, Goberdahn and Brody 1954, White et al. 1956; Rumsey and Bond 1976). During starvation the organic phosphorus compounds, phosphatides, nucleic acids, nucleotides, phosphocreatinine and the hexose phosphates in the body tissues are catabolised with

release of Pi into the blood (Dale, Goberdahn and Brody 1954, White et al. 1956).

Starvation changes both the origin and the nature of energy yielding material. The normal base rich diet of plant origin is supplanted by a base poor diet, the origin of which is the animal's body tissue (Dale et al. 1954, Tilakaratne et al. 1980) and this leads to a rise in BUN and FFA concentrations (Dalton 1967, Tilakaratne et al. 1980).

Calves of high genetic merit were found to have higher plasma FFA concentrations than those of low genetic merit during starvation (Tilakaratne et al. 1980). These workers concluded that calves of high genetic merit utilized body fat reserves more than body tissue proteins while low genetic merit calves utilized tissue proteins more than body fat reserves. Thus from their report it appears that animals of good genetic merit may be identified by subjecting them to short periods of starvation and monitoring the metabolic response.

Varying effects of fasting on plasma glucose concentration in animals have been reported in the literature. Some workers have reported an increase in blood glucose levels during fasting (Robertson and Thin 1953, Robertson et al. 1960) while others (Pehrson 1966, Ballard and Abdalla 1968, Baird, Heitzmann and Hibbitt 1972) reported a decrease in blood glucose during starvation.

More recently, Xing, Mackenzie, McCutcheon, Wilson and Flux (1988) found that 8-18 day old dairy calves with a high breeding index for milk production had significantly higher plasma glucose

concentrations after an over night fast compared to a group of similar calves with low breeding index. They suggested that the results were consistent with higher plasma glucose concentrations in the former calves being genetically determined and that blood metabolite and hormone profiles in young Friesian calves might be used as an indicator of genetic merit.

During starvation, milk yield falls very rapidly in lactating cows and it has been suggested that this contributes to the maintenance of normal blood glucose concentrations (Robertson and Thin 1953, Robertson et al 1963). An initial fall in blood glucose concentrations followed by a rise to pre-starvation levels within 2 to 3 days of starvation was observed by Robertson and Thin (1953). Because of this phenomenon these workers found that cows were able to withstand a period of semi-starvation or a few days of complete starvation without developing any severe acetonaemia and concluded that liver glycogen reserves were sufficient to maintain blood glucose levels during the period. This has also been noted by Kronfeld and Simesen (1960) and Baird et al. (1972). Glycogenolysis was found to increase during starvation and it was concluded that normal blood glucose levels could be maintained while liver glycogen lasted, and a hypoglycaemia would develop when these stores fell below a certain threshold (Baird et al. 1972). Thus the fall in blood glucose concentrations reported by Pehrson (1966) and Baird et al. (1972) could have been due to depletion of liver glycogen reserves during starvation.

A significant increase in PNa with no significant changes in PK concentrations was observed during starvation in dairy

cows (Pehrson 1966). The dehydration that occurs during starvation should have elevated both PNa and PK levels. However since starvation is a form of stress it could lead to a decrease in PK concentrations as hypokalemia can theoretically arise from severe stress (Dale et al. 1954, Mulrow 1967, Paape, Desjardins, Guidry, Miller and Smith 1977).

2.3. Effect of ACTH injection on the blood chemistry of cattle

The normal response to a wide variety of physical and psychological stressors is the ACTH mediated synthesis and release into the blood circulation of large quantities of adrenal corticosteroids (Cope 1972, Sharyantar, Head, Wilcox and Thatcher 1975, Paape et al. 1977). The released corticosteroids then cause or permit physiological adjustments enabling the animal to tolerate stress (Cope 1972, Gwazadanskas, Paape, Peery and McGilliard 1980). An impaired ability to increase and/or to maintain elevated plasma corticosteroids during exposure to stress to which an animal is not adapted would thus reduce its tolerance to stress (Marple, Judge and Aberle 1972, Smith, Hansel and Coppock 1975). This may explain why lactating dairy cows experience the highest incidence of metabolic and infectious diseases during periods of chronic stress such as that due to imbalanced diet (Trimberger et al. 1972, Smith et al. 1975, Morrow 1976), high environmental temperature (Sharyanfar et al. 1972), early stages of lactation (McDowell and McDonald 1965) and parturition (Trimberger et al. 1972). During chronic stress conditions it has been suggested that the continued stimulation of the adrenal glands by ACTH could possibly result in adrenal fatigue (i.e. reduced synthesis and

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release of adrenal corticosteroids) and hence contribute to a number of the diseases associated with chronic stress (Cope 1972, Gwazadanskas et al 1980). Since not all animals develop diseases during chronic stress conditions (Smith et al. 1975), this may reflect individuality in adapting to stress.

The experimental administration of ACTH through its steroidogenic effect (Lipscomb and Nelson 1962) alters several blood components (Wegner and Stott 1972, Paape, Carrol, Kral, Miller and Desjardins 1974). Corticotropin induces secretion of adrenal glucocorticoids (Shayanfar et al. 1975, Paape et al. 1977) and mineralocorticoids (Crabbe, Reddy, Ross and Thorn 1959) which then induce changes in some blood constituents (Pehrson 1966, Wegner and Stott 1973, Paape et al. 1974) and in the numbers of circulating leukocytes.

Blood glucose and PNa concentrations and circulating leukocyte numbers increase after ACTH injection (Pehrson 1966, Wegner and Stott 1972, Paape et al. 1974). The increase in plasma glucose concentrations has been mainly attributed to impaired glucose utilization by cells (Butler 1969, Braun, Bergmann and Albert 1970) and to an increased rate of gluconeogenesis (Wilcke and Davis 1980). Corticotropin releases aldosterone as well as glucocorticoids from the adrenal cortex (Crabbe et al. 1959, Mulrow 1967), and the released aldosterone stimulates renal absorption of Na and excretion of K and Mg ions, thus resulting in elevated PNa and decreased PK and PMg concentrations. The increase in WCC's after ACTH administration has been shown to be due to release of neutrophils from the marginal pool resulting in an increase in circulating neutrophils

(Paape et al. 1974). It has also been found that corticosteroids reduce neutrophil stickiness and diapedesis which then results in release of neutrophils from the marginal pool (Paape, Kral, O'Brien and Schultze 1971). The numbers of circulating lymphocytes does not alter after ACTH administration although there is a decrease in their relative percentage i.e. relative lymphopenia (Pehrson and Wallin 1966). Both absolute numbers of eosinophils and differential count percentages decrease after ACTH administration (Merrill and Smith 1954, Pehrson and Wallin 1966).

Plasma Ca concentration decreased following ACTH administration in dairy heifers and this has been attributed to increased Ca excretion (Wegner and Stott 1972).

It is possible that cows which are good adaptors to stress may have a greater production potential i.e. their response to a stressor may be related to their production potential.

2.4. The relationship between blood chemistry and production potential, growth rate and genetic selection for production

The individuality of blood chemistry in cattle over long periods of time has been shown to be inheritable and hence might form a base for selection and breeding of superior animals (Payne et al. 1973b, Rowlands, Payne, Dew and Manston 1973). It has been found that for most blood or serum constitutents individuality accounted for 45% to 55% of the relevant total variance, especially for glucose, Hb, and K (Rowlands et al. 1973, Rowlands and Manston 1976). These workers found that Hb,

glucose and K had high heritability, Ca, albumin, Pi, and Mg moderate heritability and Na, urea and globulin had very low heritability. However estimates of heritability are probably influenced by the environment and the plane of nutrition since they differ between places and feeding systems (Rowlands et al. 1974). However despite the lack of agreement in detail about heritability, there is evidence to support the view that the tendency to maintain high concentrations of certain blood constitutents is inherited (Payne et al. 1973b, Rowlands et al. 1974).

Concentrations of blood constituents with high heritability have been reported to be highly correlated with growth rate (Payne et al. 1973b, Rowlands et al. 1974, Rowlands and Manston 1976). Growth rate was found to be related to blood glucose in young calves growing at an average of 0.5 or 0.56 kg/day (Payne et al. 1973b, Rowlands et al. 1974). However, this relationship was not observed in older calves growing at a faster rate (Arthaud et al. 1959, Rowlands et al 1973). Thus the correlation between growth rate and blood glucose seems to occur early in life and especially when energy is not a limiting factor (Payne et al. 1973b, Little, Kay, Manston, Rowlands and Stark 1977). It has been concluded that these correlations were unlikely to be useful in growing stock where there was improved efficiency of feed conversion (Little et al. 1977).

A positive correlation between albumin and Hb concentrations and growth rates and a negative correlation between

growth rate and serum K and Na has been reported (Roubicek and Ray 1972, Payne et al. 1973b, Rowlands et al. 1974, Rowlands and Manston 1976, Little et al. 1977). It was found that albumin and Na concentrations could be used to predict growth performance in later life whereas plasma K concentrations were not correlated with such performance (Rowlands and Manston 1976). This observation has also been reported by Kitchenham et al. (1977), who found that the relationship between growth rate and plasma albumin concentration tended to become more significant in later life whereas an observed relationship between growth rate and K concentrations developed earlier in life. It was suggested by these workers that the relationships seemed to be independent of the environment. Growth rate was also found to be negatively correlated with plasma globulin concentrations (Kitchenham et al. 1977). A positive relationship between growth rate and Hb concentration has been observed in early life and it was concluded that Hb was a useful parameter in predicting weight gain (Little et al. 1977). A high eosinophil count has also been shown to be related to fast growth rate and efficiency of gain in cattle (Schultze 1955, Schultze 1957). The level of circulating eosinophils has been shown to be negatively related to the amount of circulating adrenocortical hormone and consequently the animals ability to adjust to stressful conditions (Schultze 1955, Hopwood and Tibolla 1958). Because of this relationship it was concluded that the cows ability to adjust to adverse conditions with minimum expenditure of energy would enhance its ability to expend a maximum amount of energy in desired agricultural production (Schultze 1955, Schultze 1957).

The application of genetic techniques to the improvement of farm livestock is dependent upon the rate of change which can be offered. One factor affecting the rate of response to genetic selection is the ability to identify individuals which carry desirable alleles. However for reproduction and lactation this ability is severely restricted by the limitation of the expression of these traits to mature females (Land 1981). Thus if the expression of the favourable allelles could be measured conveniently early in life the above restriction could be alleviated.

There are indications that metabolite responses to changes in energy balance may be related to dairy merit (Tilakaratne et al. 1980). At any level of feeding it has been found that high yielding cows preferentially directed their energy towards milk production and away from the deposition of body tissues whereas the converse applied to low yielding cows (Broster et al. 1969). Thus it was suggested that variations in energy supply to the mammary gland could be responsible for the variations in milk yield observed in lactating cows (Broster et al. 1969). The partition of dietary energy towards milk production or body weight has been shown to be a result of inherited patterns of endocrine function and hormonal inter-relationships (Hart, Bines and Morant 1978). Thus energy availability (neither age nor sex-linked) for lactation may be a measure of good milk production (Land 1981). This is in accordance with the suggestion of Broster et al. (1969) that one of the characteristics of a good cow is its ability to cope with an overall energy deficit. Thus induction of a negative energy balance may reveal relevant physiological covariation.

2.5 Erythrocyte sodium (ENa), potassium (EK) and magnesium (EMg)

Species differences

It has been known for a long time that ENa, EK and EMg concentrations vary greatly between species (Eveleth 1937, Bernstein 1954). In the case of EK two distinct types (high and low) are found in cattle (Ellory and Tucker 1970, Christinaz and Schatamann 1972). Those with high EK (HK) are considerably less frequent than those with low EK (LK) in both sheep (Evans 1954) and cattle (Ellory and Tucker 1970, Christinaz and Schatamann 1972).

In cattle the relationship between concentrations of EMg and those of PMg varies in different reports. Low concentrations of EMg compared with those of PMg have been reported by some workers (Eveleth 1937, Salt 1950) while other workers have reported higher concentrations of EMg than PMg (Green and Macaskill 1978, Wise, Caldwell, Parish, Flipse and Hughes 1948).

The chemistry of erythrocyte Na, K and Mg.

Investigations of the membrane transport properties of HK and LK erythrocytes have shown that both types of cells have a cation pump which exchanges one intracellular Na ion for one K ion but the pump works approximately four times faster in HK cells (Tosteson 1963, Ellory and Tucker 1969). Cations also leak out of the cells, HK cells being relatively more "leaky" to Na than LK cells while the reverse is true for K. Both HK

and LK cells show appreciable Na exchange diffusion, however this process is more rapid in LK than HK cells (Tosteson 1963, Brewer, Eaton, Beck, Feitler and Shreffler 1968). The level of ATPase which catalyses the active process of the cation pump is four times greater in HK than in LK cells (Brewer et al. 1968, Ellory and Tucker 1969). It has been shown that HK cells contained more ATP than LK cells (Eaton 1967, Brewer et al. 1968) and it is concluded that the level of ATP in the cellsinfluencesENa and EK concentrations. The presence of Na and K ions has been found to stimulate the hydrolysis of ATP by ATPase thus providing energy for the maintenance of the concentration gradient across the erythrocyte membrane (Chau, Calabrese and Theil 1964).

The EK type has been linked with an antigen (termed L) on the erythrocyte membrane (Rasmussen and Hall 1966, Tucker and Ellory 1970). In a potential LK lamb at birth (when concentration of EK is high) the L antigen was found to be poorly developed and as the adult low EK value was reached the L antigen activity increased (Tucker and Ellory 1970).

More recently (Wolowyk and Ellory 1985), it has been found that in the red blood cells of lambs which are genotypically of the low potassium type, the intracellular sodium concentration increases and potassium decreases from 30 days before parturition onwards and these changes continue up to adult life. These changes occur partly by reduction in the number of sodium pumps per cell and later by the introduction into the circulation of cells with L antigen modified sodium pump.

Most (70-80%) of EMg is bound to organic phosphates (mostly ATP) and enzymes (Rose 1968, Bunn, Ransil and Caho 1971), and only 2-6% of the total EMg concentration is bound to the membranes (Carvalho, Sami and Pace 1963, Fukii, Sato and Hanzawa 1973). The intracellular Mg exists in two forms, unexchangeable Mg (bound Mg) and exchangeable Mg (unbound Mg) which can be depleted (Frater, Shirley, Simon and Shaw 1959, Care 1960, Elin, Armstrong and Singer1971). Reticulocytes and young erythrocytes have higher ATP and Mg concentrations than older erythrocytes (Bernstein 1959, Ginsburg, Smith, Ginsburg, Readon and Aikawa 1962, Bunn et al. 1971) hence their numbers in the circulation can influence the EMg concentration. EMg concentration has been found to decrease with the age of erythrocytes and this was associated with reduced rate of glycolysis (i.e. reduced metabolic activity) and ATP concentration in the older erythrocytes (Bernstein 1959). In rats it has been shown that a decrease in ATP concentration led to a decrease in erythrocyte membrane stability (Elin 1973).

There are variable reports on the uptake of Mg by erythrocytes both "in vivo" and "in vitro". Following intravenous Mg injection, a slight uptake of Mg by erythrocytes was found by McAleese, Bell and Forbes (1961) whereas a very marked uptake was observed by other workers (McDonald, Care and Nolan 1959). After addition of Mg to blood both slight Mg uptake (Ginsburg et al. 1962) and marked uptake (Tufts and Green 1937, Care, MacDonald and Nolan 1959) have been reported.

Genetics of erythrocyte Na, K and Mg concentrations

Erythrocyte K type in sheep and cattle has been shown to be genetically determined (Evans 1954, Evans and Mounib 1957, Ellory and Tucker 1970). The EK type inherited is controlled by a simple allelic pair of genes, the gene for LK being dominant to that of HK (Evans and King 1955, Ellory and Tucker (1970). However the dominance of the allele for LK is incomplete, the heterozygotes having higher K values than the homozygotes for LK (Evans 1954, Agar, Evans and Roberts 1972). However, the differences observed between breeds and sire groups and pairs of twins suggest that other genes may operate to alter the EK concentration in addition to the major pair (Taneja and Abichandani 1967, Rasmussen, Tucker, Ellory and Spooner 1974).

Earlier reports indicated that EMg levels were reflective of the Mg status of the animal at the time of erythropoiesis (Tufts and Greenberg 1937, Salt 1950, Elin et al 1971) in that erythrocytes produced during a period of low Mg status have low EMg levels and vice-versa. However, recent studies in man and mice have shown that EMg concentrations could be genetically determined. Studies between twins and families have shown a very high heritability (0.92) for EMg levels and greater similarities in EMg levels between monozygous than between dizygotic twins and between families than between unrelated people (Darlu, Henrotte, Benech, Francriquer, Pineau and Santarromana 1981, Darlu and Michotte 1981). Similar observations have been made in mice (Henrotte and Colombani 1981). Greater similarities in EMg levels were observed between inbred, genetically identical mice than between unrelated mice (Henrotte and Colombani 1981).

the relationships of erythrocyte Na, K and Mg with other physiological factors.

EK level has been associated with haemoglobin type (Evans, Ring, Cohen and Warren 1956, and the oxyhaemoglobin dissociation curve (Dawson and Evans 1962). These workers reported that within each haemoglobin type, blood from HK sheep had a curve displaced to the left of that of LK sheep. EK level is affected by haemoglobin type, individuals with the HBA types have higher EK levels than HBB animals with HB-AB animals being intermediate (Evans et al 1956).

EK level has also been shown to have some adaptive significance in sheep (Evans 1954) and cattle (Evans 1963b). Zebu type of cattle adapted to a subtropical environment have been shown to have a tendency to have a higher ENa, ENa+EK and lower EK levels than British breeds of cattle (Evans 1963b). EK levels were also found to be related to the coat score index developed by Tucker and Schleger (1960) (Evans 1963b). This worker postulated that differences in the regulation of the endocrine system (in particular the ratio of thyroxine to corticoids) may be responsible for the differences in respiration rate, growth rate, EK levels and other factors between Zebu breeds and British breeds of cattle.

There are a few reports which suggest that relationships exist between EK types and reproductive efficiency in sheep. LK animals were reported to have marginally better neonatal growth rate than HK animals (Watson and Khattab 1964) whilst other workers suggested that the HK type was associated with

reproductive advantage in Germany (Meyer, Lohse and Groning 1967). LK ewes with EK levels near the mode were found to have a better reproductive performance than ewes with EK values outside the mode (Turner and Koch, 1961). Low EK levels were associated with higher milk production than HK levels in cattle (Rasmussen et al. 1974).

In man and mice, EMg concentrations have been related to immune responsiveness and certain autoimmune diseases (e.g. myasthenia gravis) characterised by high blood antibody levels (Henrotte 1980, Henrotte, Hannoun and Dausset 1981). Healthy individuals with low EMg concentrations had higher humoral immune responses and lower cell-mediated immune responses than those with high EMg concentrations (Henrotte et al. 1981). In a group of inbred strains of mice selected according to their immuneresponse intensity, the high responders had lower EMg concentrations than the low responders (Henrotte, Mouton, Colombani and Franckriquer 1982).

Factors affecting erythrocyte Na, K and Mg concentration

EK and EMg concentrations remain relatively constant over a long period of time for any particular individual animal (Salt 1950, Evans and Phillipson 1957, Blunt and Evans 1963, Henrotte et al. 1982). However some factors have been shown to affect EK and EMg concentrations. These include:

(a) Diet

A dietary deficiency of K and/or Na has been shown to

result in a decreased concentration of these electrolytes in the erythrocytes of man and rat (Schwartz, Cohen and Wallance 1953, Agar et al. 1972). In acute Mg deficiency EMg concentrations do not fall as quickly or as much as PMg concentrations (Tufts and Greenberg 1937, Elin et al. 1971). However, in a long-term dietary Mg deficiency (small or profound) the EMg concentrations can decrease markedly (Tufts and Greenberg 1937, Salt 1950). Thus EMg concentrations may be of value in demonstrating a long-term dietary Mg deficiency in animals.

(b) Blood loss

Blood loss that causes a depression of the PCV can lead to an increase in EK and EMg concentrations and little or no change in ENa concentrations (Ginsburg et al. 1962, Evans 1963b, Blunt and Evans 1965, Timms and Murphy 1980). Anaemia caused by experimental infection of cattle by Babesia bigemina or by bleeding caused an increase in EK and a decrease in ENa concentrations (Timms and Murphy 1980). Bleeding led to a very marked increase in EMg concentrations (28.4 mmol/l) in the experimental animals in contrast to 7.8 mmol/l in the control animals (Ginsburg et al. 1962). Such changes in ion concentrations occuring in erythrocytes during blood loss have been shown to be due to production of reticulocytes and immature erythrocytes in which the concentration of Na is decreased while that of K and Mg is elevated (Ginsburg et al. 1962, Blunt and Evans 1965, Timms and Murphy 1980, Albers and Lejambre (1983). The disappearance of these immature erythrocytes from the circulation was shown to coincide with the return to more normal EK and EMg concentrations (Ginsburg et al. 1962, Timms and Murphy 1980).

These reports indicate that the concentrations of EK and EMg can vary depending on the proportions of the reticulocytes, immature and old erythrocytes in the circulation.

(c) Sex

Higher EK concentrations were found in the blood of rams compared to ewes (Evans 1961). This worker thought that steroid hormones might in some way modify the expression of the EK genes and thus the EK concentration might be an indirect reflection of the levels of steroids. Castration caused a significant drop in EK concentration (Evans 1961) which could be reversed by the injection of testosterone. No sex effect on EK concentration was found in both HK and LK types of cattle in Sweden (Christinaz and Schatamann 1972).

EMg and PMg concentrations were found to be lower and more variable in women at fertile age than in men or in post-menopausal women (Goldsmith 1971, Henrotte, Benech and Pineau 1980). The low EMg concentrations in the young women was attributed to less active erythropoiesis and suggested that steroid hormones could be involved (Henrotte et al 1980).

(d) Age

Calves were noted to have a higher whole blood K and Mg concentrations at birth than their dams, and these levels fell with increasing age, reaching that of adult cattle at 10 weeks of age (Wise et al. 1948). Whole blood K concentration predominated over Na concentration during the first 4 weeks of life while Na

concentrations predominated over K in the extracellular fraction and the level of whole blood Mg was higher than that of the plasma (Wise et al. 1948). In lambs the concentration of EK fell while that of Na increased with increasing age (Blenchner 1960, Blenchner 1962, Evans and Blunt 1961). In man and pigs EK concentration is lower in foetuses than in their dams and increases with age (Coulter, Ewan, Swenson, Alherne and Wyllie 1970) a reverse situation to that of other animals.

(e) Environment

It has been suggested that there may be a gene-environment interaction in determining haemoglobin and EK types and their frequency of occurrence (Agar et al. 1972). These workers also suggested that if such an interaction existed it was a complex one. In sheep a definite environmental influence on EK and ENa concentrations was found in the same breeds of animals with the same EK and ENa types (Darcel, Simpson and Avery 1961). However, no environmental effect on EK concentrations in sheep was observed when they were moved from their place of origin (Evans 1963b). No environmental effect on EMg concentrations has been reported in animals (Henrotte et al. 1981).

(f) Vanadate

Vanadate determines the rate of active transport of Na and K ions in erythrocytes (Cantley, Josephson, Warner, Yang, Sawa, Lechene and Guidett 1977, Macara 1980). In erythrocytes vanadate occurs in the +4 oxidation state (less potent inhibitor of cation pump)(Cantley et al. 1977) and is for most part bound to haemoglobin (Macara 1980). When vanadium (+5 oxidation state) is

taken up by the cell it is reduced to the +4 oxidation state and then binds to intracellular proteins (Cantley and Aisen 1979). This reduction depends on the cellular reducing agents NADH and glutathione which prevents vanadate inhibition of the cation pump by conversion of the +5 oxidation state (which is a more potent inhibitor of ATPase) to +4 oxidation state (Macara 1980). Thus the "energy state" i.e. level of reduced NADH and glutathione of erythrocytes, control the ATPase activity (hence ENa and EK concentrations) by regulating the balance of +4 and +5 oxidation states of vanadate.

(g) Developmental changes of erythrocytes

Within the life cycle of a single red cell developmental events may lead to a decrease in ATPase activity and hence changes in EK, ENa and EMg concentrations. For example as reticulocytes develop into erythrocytes, ATPase and metabolic activities decline (Bernstein 1959, Smith and Rozengurt 1978). The low EMg concentration in old erythrocytes was associated with a decrease in their metabolic activity with an increase in age (Bernstein 1959).

(h) Pregnancy

No significant differences in ENa and EK concentrations were observed between pregnant and non-pregnant goats (Blechner 1960). Significantly lower EMg values were found in normal pregnant women in the third trimester than in the normal population (non-pregnant)(Lim, Jacob, Dongs and Khoo 1969).

It was suggested by these workers that this could have been due to increased general fluid retention or occult (marginal) Mg deficiency possibly due to an increased Mg demand made by the foetus at this stage of pregnancy.

Application of ENa, EK and EMg determinations

High yielding cows were observed to have lower EK levels than low-yielding cows (Rasmussen et al. 1974) and this suggests that the gene(s) that control(s) EK levels might be related to milk production. Hence determination of EK concentrations might be of value in identifying animals with genetic potential for high milk production.

The relative values of PMg and EMg may be of value in indicating whether an observed hypomagnesaemia had arisen suddenly or gradually over a period of weeks or months (Salt 1950). In acute hypomagnesaemia the concentrations of PMg would be very low and those of EMg normal, while in chronic hypomagnesaemia both PMg and EMg concentrations would be low. When mild hypomagnesaemia is prolonged a profound decrease in EMg concentrations and slight decreases in PMg concentrations occur. Thus EMg estimations could be of value in diagnosing a prolonged marginal hypomagnesaemia which might not be detected by PMg determination.

MPT's have been developed and used in various forms but have not gained wide acceptance due to variation in interpretation and due to lack of consistent relationship with diseases and feed intake. They are also limited in that they do not

consider the period around calving (i.e. late pregnancy and early lactation) when most of the abnormalities of blood chemistry associated with production sydromes occur (Trimberger et al. 1972, Sommer 1975, Stevens 1975). Therefore a MPT based on changes in blood chemistry 8 weeks before and 8 weeks after calving may be more informative than the currently used MPT methods. Erythrocyte levels of Mg, Na and K as discussed above may also be important and therefore they should be considered for inclusion in the MPT.

CHAPTER 2

GENERAL MATERIALS AND METHODS

1. Experimental Animals

Friesian and Jersey cows from two dairy herds (herd I = 24 cows out of 200 cows) and (herd II = 63 cows out of 100 cows), aged 2.5 to 9 years were used in these studies. Both farms utilized irrigated rye grass and clover pastures during the dry winter period (April to November) to feed the lactating cows whilst in the summer months they were fed tropical pastures (mainly Rhodes, Kikuyu and green panic grasses) supplemented with meal mixtures (usually sorghum grain with protein supplements). The dry cows were grazed on the tropical grass pastures and were taken to the calving paddock in the last week of pregnancy in both herds.

The cows were observed twice daily during morning and afternoon milking periods for oestrus, were artificially inseminated and the dates of services recorded. Both herds utilized the Queensland Department of Primary Industries Dairy Division Herd Production Recording Scheme.

Ten calves (2 months old) and 10 heifers (1 year old) running at pasture in herd II were each blood sampled monthly for a period of one year.

2. Collection of Blood Samples

Blood samples unless stated otherwise were collected from the tail (coccygeal blood vessels) in the adult cows and heifers and from the jugular vein in the calves.

Blood was collected from each animal into three types of vials.

(a) 10 ml of blood into a vial containing 100 iu of lithium heparin for plasma biochemistry.

(b) 2.5 ml of blood into a vial containing 2.5 mg of sodium fluoride and 2.5 mg of potassium oxalate for glucose determination.

(c) 5.0 ml of blood into a vial containing 2mg of Dipotassium Ethylene Diamine Tetra Acetic Acid (EDTA) for differential cell counts, white cell counts, red cell counts and estimation of haematocrit (PCV) and haemoglobin concentration.

3. Preparation of Samples

Haemolysed blood was prepared by diluting 0.35 ml of the heparinized blood with 1.0 ml of double deionized water. After this the haemolysed blood was centrifuged at 3000 revolutions per minute (rpm) for 10 minutes to remove cell debris and the supernatant was then transferred into autoanalyser tubes. The remainder of the heparinized blood

sample was then centrifuged at 3000 rpm for 20 minutes and the plasma removed into 4.0 ml autoanalyser tubes. The plasma was used for plasma biochemistry and the remainder was stored at -20° C. Blood smears for differential cell counts were made within one hour after the blood collection. The fluoride oxalate blood was centrifuged at 3000 rpm for 10 minutes within one hour after blood collection and the plasma was used for detemination of plasma glucose concentration.

4. Haematological and Biochemical Techniques

Blood smears

Two blood smears for differential cell counts made from the EDTA blood were stained using Harlecos Diff Quik stain (AHS Australia Pty Ltd) which is a modification of the Wright stain technique.

Blood analyses

The blood samples were analysed for haematocrit (PCV), Hb, Pi, PNa, PK, plasma urea, creatinine, WCC, RCC, ENa, EK, EMg, alkaline phosphatase and Thyroxine (T3 and T4) by the following methods. These were done in duplicate.

Haematocrit (PCV)

This was determined by the micro-haematocrit method (Fisher 1962).

Haemoglobin (Hb)

Hb concentration was determined by the cyanmethaemoglobin method (Aculute, Ortho Diagnostics, Raritan, New Jersey).

Plasma glucose

The plasma glucose concentration was determined by the oxidation method (Boehringer and Soene GMBH, Mannheim).

Total plasma protein (TPP)

TPP concentration was determined by refractometry using an "Atago" refractometer (Instrumentation Lab. Inc. U.S.A.).

Plasma albumin and globulin

The plasma albumin concentration was determined by the Bromocresol Green (BCG) method (Boehringer and Soehne, GMBH, Mannheim). The plasma globulin concentration was found by subtracting the albumin concentration from that of TPP.

Magnesium (Mg) and calcium (Ca)

These were determined by atomic absorption spectroscopy using the method of Willis (1960). However the following modifications were made so as to enable the two determinations to be carried out on the same supernatant. 0.5ml of plasma was added to a test tube containing 4.5ml of a working solution containing 2500 ppm of strontium and 4 per cent trichloracetic acid (TCA), that is, 7.7g strontium chloride and 40g TCA/l. The test tubes were mixed thoroughly and allowed to stand for 5 minutes before centrifuging them at 3000 rpm for 5 minutes. The supernatant was then aspirated directly into the flame of a Techtron AA4 - atomic absorption spectrophotometer (Technicon Instruments Corp. Ardsley, N.Y.) and the concentration read at an absorbance of 2852^{A} for Mg determination and 4227^{A} for Ca determination.

Sodium (Na) and potassium (K)

These were determined in an emission flame photometer, Model 343, equipped with a dilutor (Instrumentation Laboratory Inc. Lexington, Mass, U.S.A.) according to the manufacturers' recommended procedure.

Plasma inorganic phosphate (Pi)

Into test tubes containing 2.0ml of TCA, 0.2ml of the sample plasma was added. These were mixed, left to stand for 10 minutes and then centrifuged for 10 minutes at 3000 rpm. 1.0 ml of the supernatant was pipetted into 2.0 ml of working

solution (containing equal parts of ammonium molybdate and ammonium vanadate). The colour (yellow) developed immediately and the absorbance was read in a spectrophotometer at 410 nm and the Pi concentration was estimated from the standard curve.

Plasma urea

The plasma urea concentration was determined by an enzymatic colorimetric method (Boehringer Mannheim, GMBH).

Plasma creatinine

This was determined in an automated analyser (multistat III-Instrumentation Lab. Inc., U.S.A.). Twenty µl of the standard and of the plasma samples were put into cells of a rotor containing 80 µl of the working solution (containing equal parts of 1.6 molar sodium hydroxide and picric acid -35 mmol/l). The machine was then standardized and the concentration in the samples was determined.

White cell count (WCC) and red cell count (RCC)

These were determined by an electronic coulter counter (Instrumentation Lab. Inc., U.S.A.).

Erythrocyte sodium and potassium (ENa and EK)

The concentration of ENa and EK was determined indirectly using the haemolysed blood in the same manner as that of the plasma Na and K in an emission flame photometer. The concentration in the erythrocytes was then calculated by the following formula:

$$ENa\ (mmol/l) = \frac{3.9 \times HBNa\ value - (1-PCV) \times PNa\ value}{PCV}$$

where 3.9 = the dilution factor

HBNa value = Na value in the haemolysed blood (mmol/l)

PNa value = Na value in plasma (mmol/l)

PCV = Packed cell volume (l/l)

The same formula was used for the calculation of EK concentrations.

Erythrocyte magnesium (EMg)

This was determined indirectly from the haemolysed blood using the same analytical procedure as that for plasma magnesium determination. The actual concentration in the erythrocytes was calculated using the formula described above for ENa and EK determinations.

Urine magnesium

The same analytical procedure as for plasma Mg determination was used for urine Mg determination.

Plasma alkaline phosphatase (AP)

This was determined in an automated analyser (Multistat III - Instrumentation Lab. Inc. U.S.A.). Five µl of sample and 35 µl of distilled water were put into rotor cuvettes containing 80 µl of working solution reagent (containing substrate i.e. Diethanolamine buffer - 1 mmol/l, pH 9.8 and MgCl2 0.5mmol/l). The machine was then standardized at 37°C and the concentrations in the samples were read at 405 nm.

Thyroxine

This was determined by "Magic" (Corning magnetic immunochemistries, Australia.) T4 (125) radioimmunoassay.

Into test tubes containing 100 µl of (I^{125}) T4 tracer 2.5 µl of the standard, control or sample were added followed by 500 µl of T4 antibody. The mixture was vortexed for 3 to 4 seconds and incubated for 15 minutes at 37°C, after which it was separated in "Magic" separation units for 3 minutes. The supernatant wasdecantedand the tubes blotted and each tube's radioactivity counted in a gamma counter for one minute. The level of T4 is given in ng/ml which is converted to umol/l (S.I. units) by multiplying by 12.9 (conversion factor).

Production rank (P.R.)

The P.R. is the cow's milk production variation from the herd average expressed as a percentage after the herd average is adjusted to 100 i.e. a cow with a P.R. of above 100 means that its milk production was above the herd average while a P.R. below 100 means that the cow's milk production was below the herd average (Wayne, Urquhart and Ortega 1977).

The P.R's used in this study were obtained from the monthly herd production recordings produced by the Queensland Department of Primary Industries Dairy Division Herd Production Recording Scheme. They are calculated using the method described by Wayne et al. (1977), which makes the following adjustment to the data to allow comparison of all milking cows in the herd on the sampling day;

(a) Age at calving.

(b) Age and breed combined

(c) Stage of lactation, and

(d) Season of the year.

CHAPTER 3

CHANGES IN BLOOD COMPONENTS OF DAIRY COWS IN LATE PREGNANCY AND EARLY LACTATION

INTRODUCTION

Some blood and plasma components show changes before and after calving in dairy cows (Amiel 1970, Manston et al. 1975, Rowlands et al. 1975, Rowlands et al. 1980). If consistent relationships were found between these changes and milk production and fertility then the changes could be of predictive value. Blood and plasma components that do not show any significant changes before or after calving may also be important in predicting milk production and fertility i.e. their stable levels might be used to predict which animals are likely to have either high or low milk production and good or poor fertility.

This study was undertaken to evaluate the changes that occur in late pregnancy and early lactation by initially utilizing pooled data in herd II (all age groups and calving periods), and then data grouped by age and calving period. Subsequently results in herd I were compared with those in herd II. The relationships between the observed changes and subsequent fertility and production were then examined.

In this section these changes are shown diagrammatically in three parts; pooled data, age group data (herd II) and herd of origin data (both herds). The regression coefficients for the respective blood components regressed on weeks were also used to show blood component changes occurring either before or after calving (both herds).

MATERIALS AND METHODS

Experimental animals

The number and management of the animals, the blood components measured and methods of analysis have been described in the section on general materials and methods.

The cows (herd II) were divided into two groups, group 1(<4 yrs-32 cows) and group 2(>4 yrs-30 cows) and were arranged into 4 calving groups according to season of calving, group 1 (December, January, February)(17 cows), group 2 (March, April and May)(15 cows), group 3 (June, July and August) (14 cows) and group 4 (September, October and November)(16 cows).

Statistical analysis

The means for each sampling week (pre-partum and post-partum) were found and compared for significant differences

by students T-test. The regression (slope) of each blood component on time (weeks pre-partum or post-partum) was calculated for each cow and the mean pre-partum and post-partum slopes estimated. Age, calving period and herd effects were estimated by analysis of variance using both pre-partum and post-partum results separately.

RESULTS

(a) Changes in blood components before and after calving for all ages and calving groups (Herd 11)

The trends of the blood components before and after calving are shown in figures 1a to 1w.

PCV, Hb and RCC mean values decreased significantly ($P<0.05$) towards calving and the lowest levels occurred in the last week of pregnancy. The levels increased slightly in the first week after parturition, and then fell steadily and significantly ($P<0.01$) upto 6 weeks post-partum. The MCV and MCHC showed no significant change either pre-partum or post-partum, however the mean MCH levels decreased significantly ($P<0.01$) from 1-7 weeks post-partum (Figs. 1a, 1b, 1q, 1r, 1s and 1t).

Plasma glucose concentrations did not show any significant trends in either pre-partum or post-partum periods, however the mean value at 6 weeks post-partum was higher ($P<0.05$) than those at 2, 3, 4 and 5 weeks post-partum (Fig. 1c).

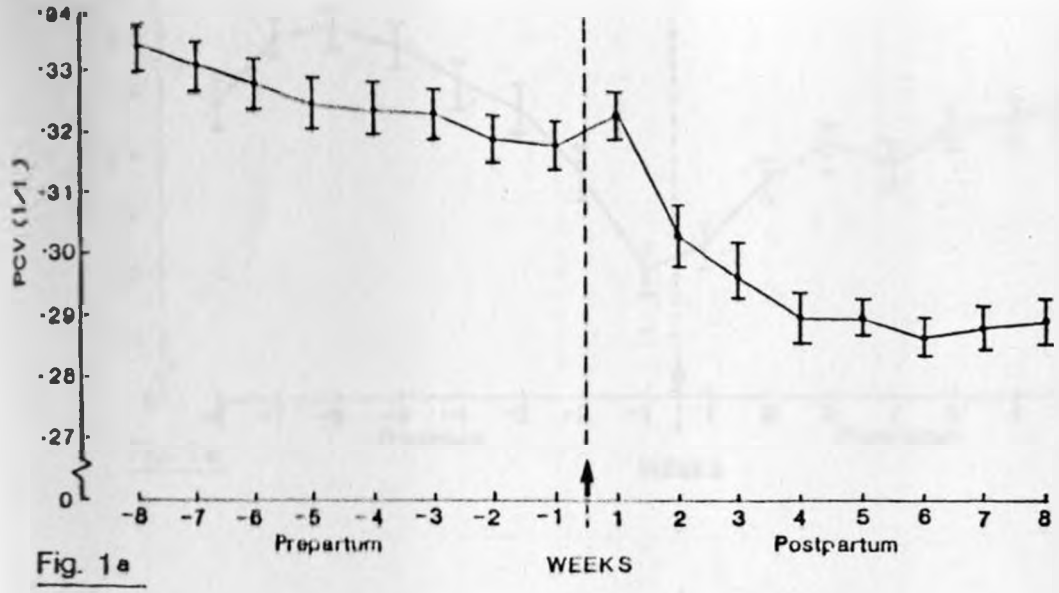

Fig. 1a

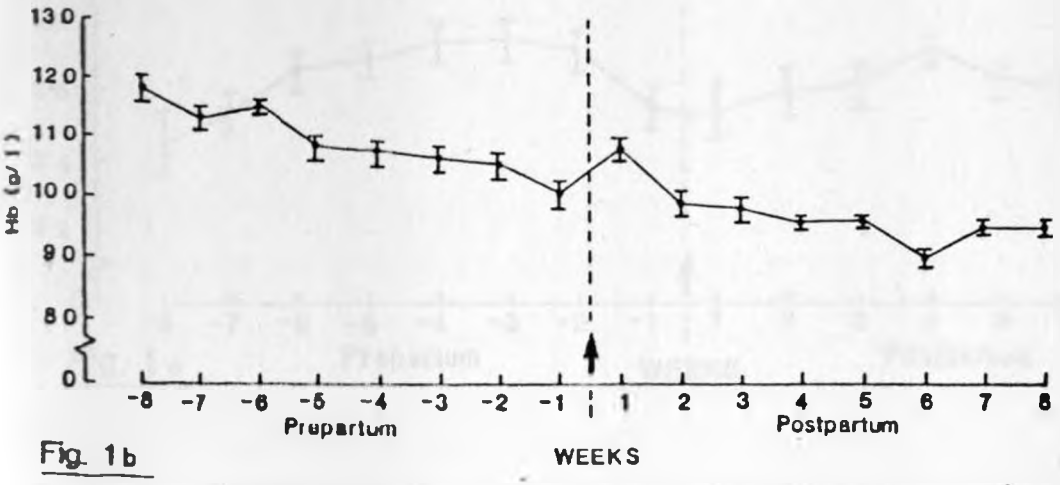

Fig. 1b

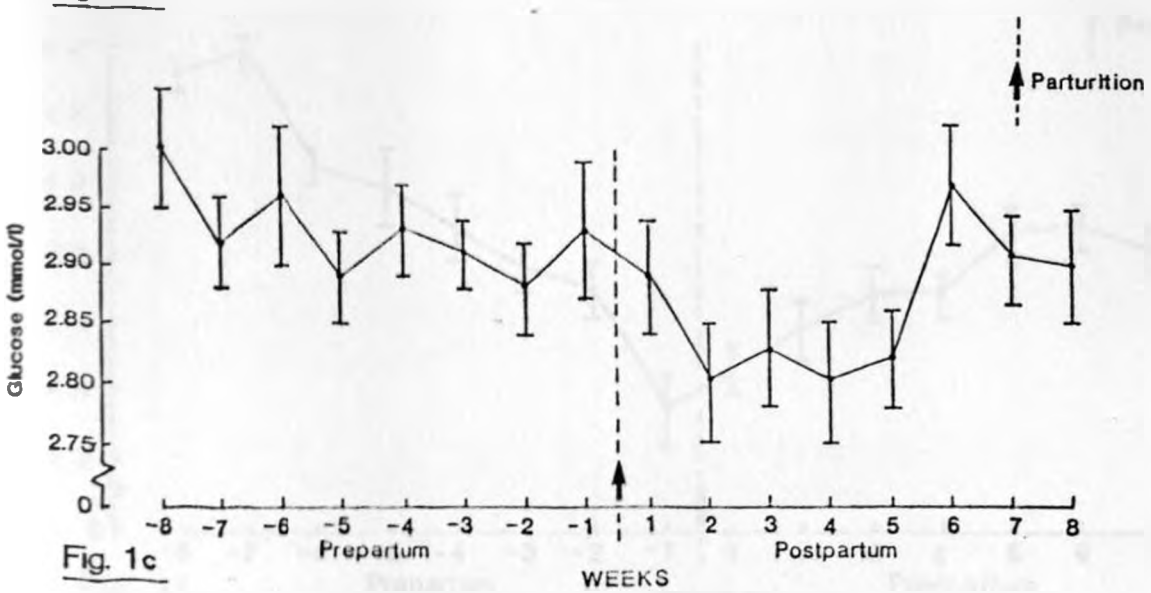

Fig. 1c

Figures 1a-1c Changes in PCV, Hb and plasma glucose (mean ±SEM) values in dairy cows 8 weeks before and 8 weeks after calving (pooled data - all age groups and calving periods)(Herd II)

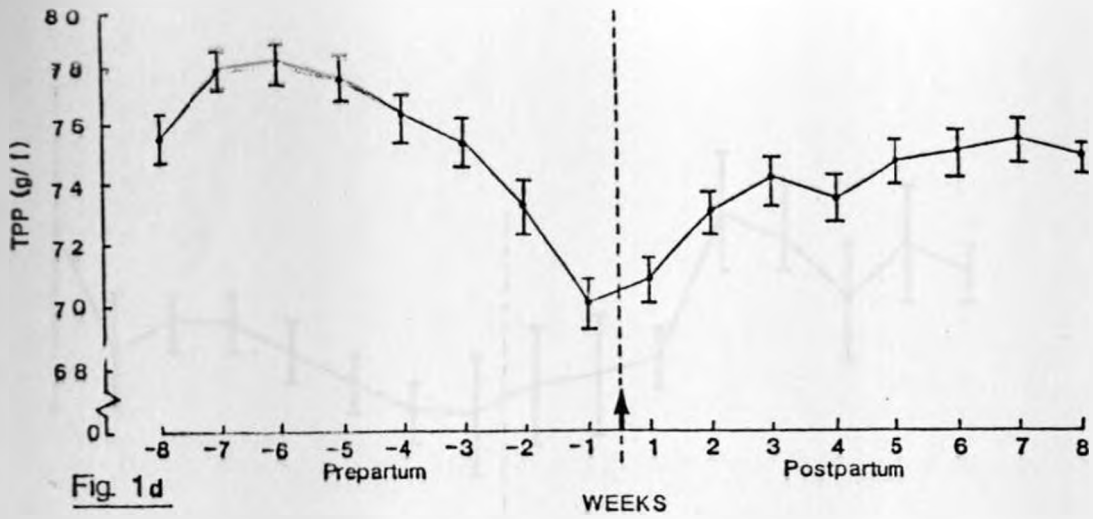

Fig. 1d

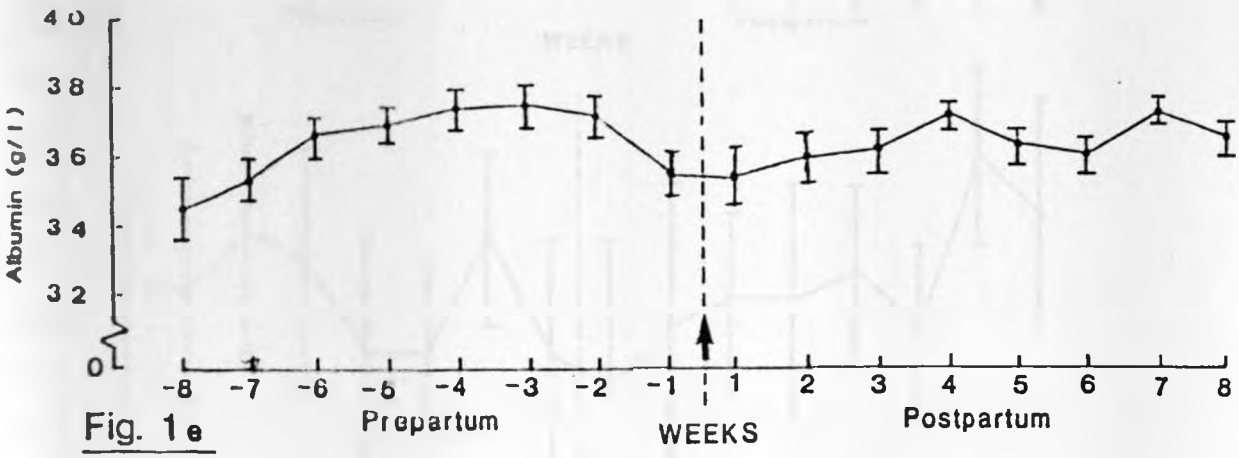

Fig. 1e

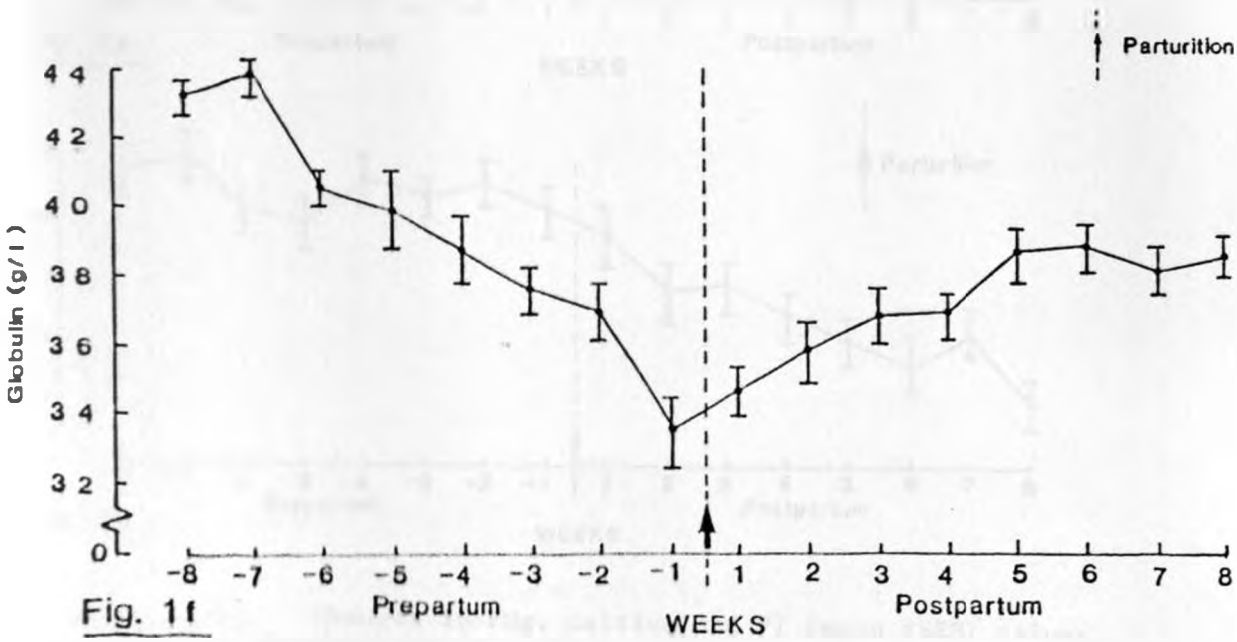

Fig. 1f

Figures 1d-1f. Changes in TPP, albumin and globulin (mean ± SEM) values in dairy cows 8 weeks before and 8 weeks after calving (pooled data - all age groups and calving periods) (Herd II)

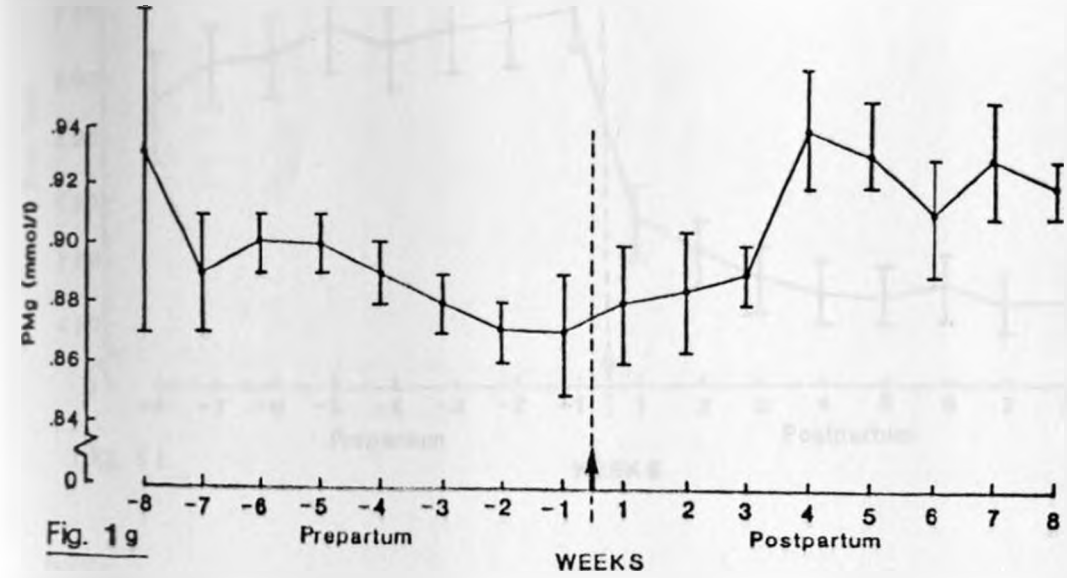

Fig. 1g

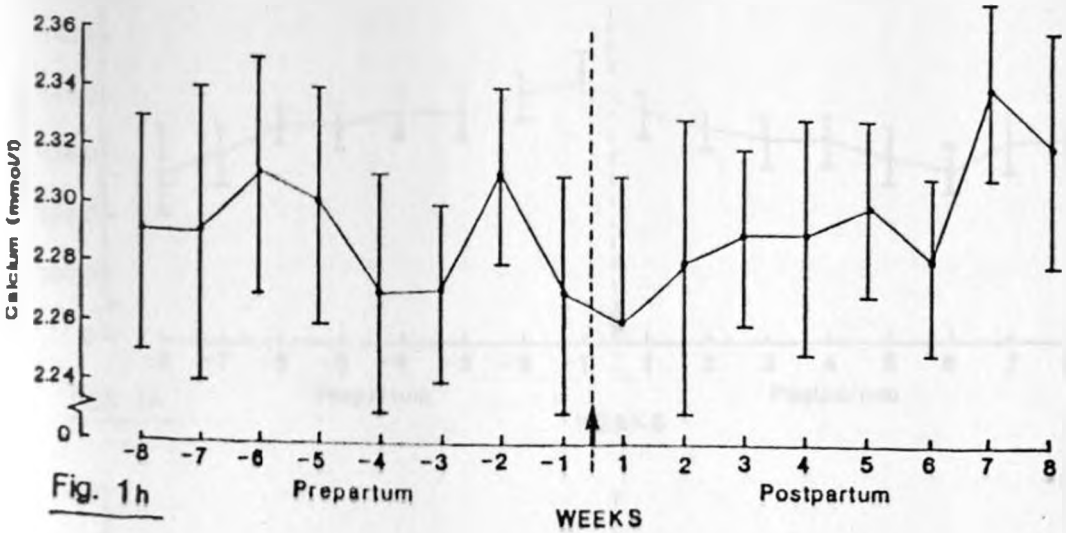

Fig. 1h

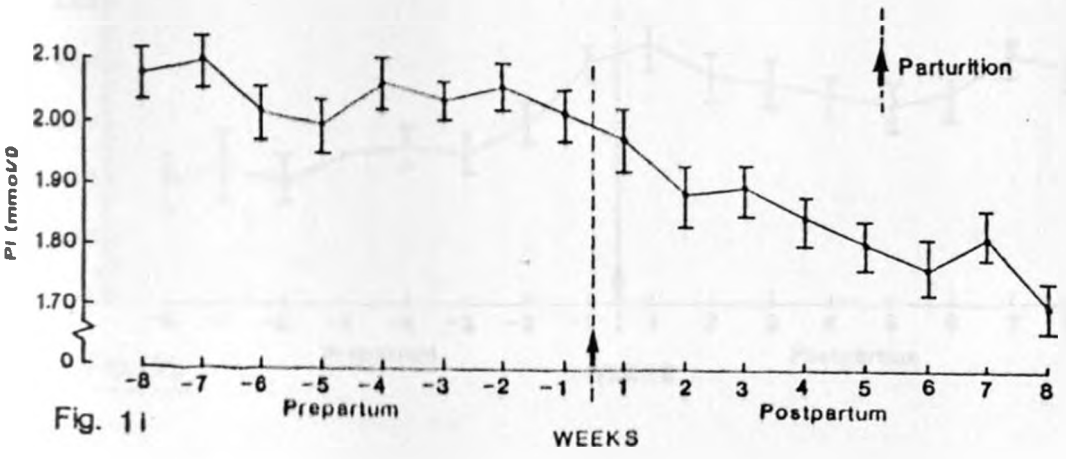

Fig. 1i

Figures 1g-1i. Changes in PMg, calcium and Pi (mean ±SEM) values in dairy cows 8 weeks before and 8 weeks after calving (pooled data - all age groups and calving periods) (Herd 11)

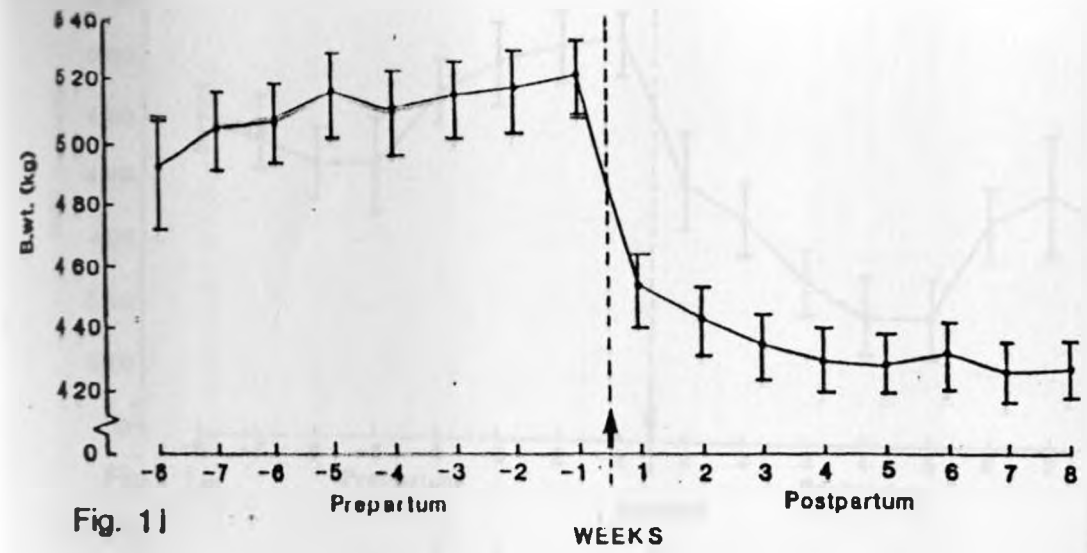

Fig. 1J

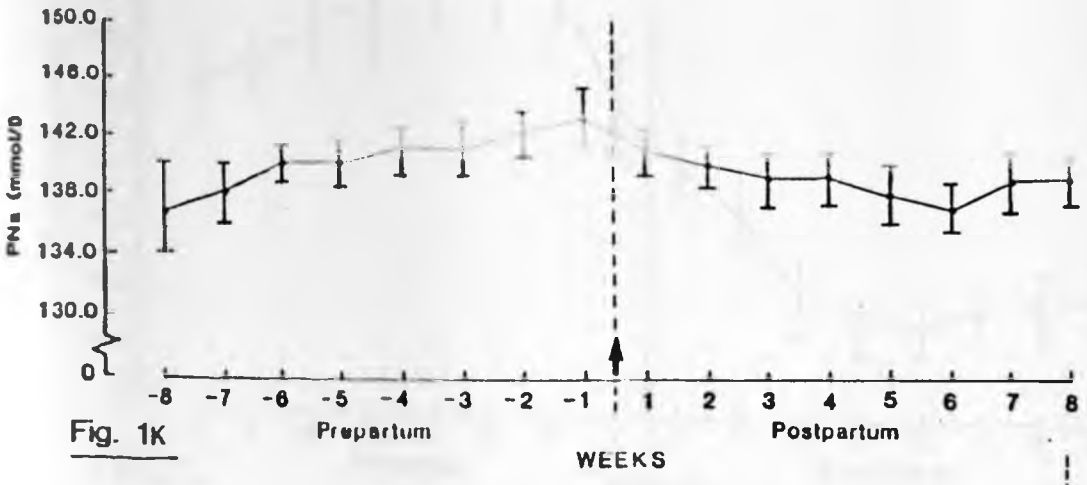

Fig. 1K

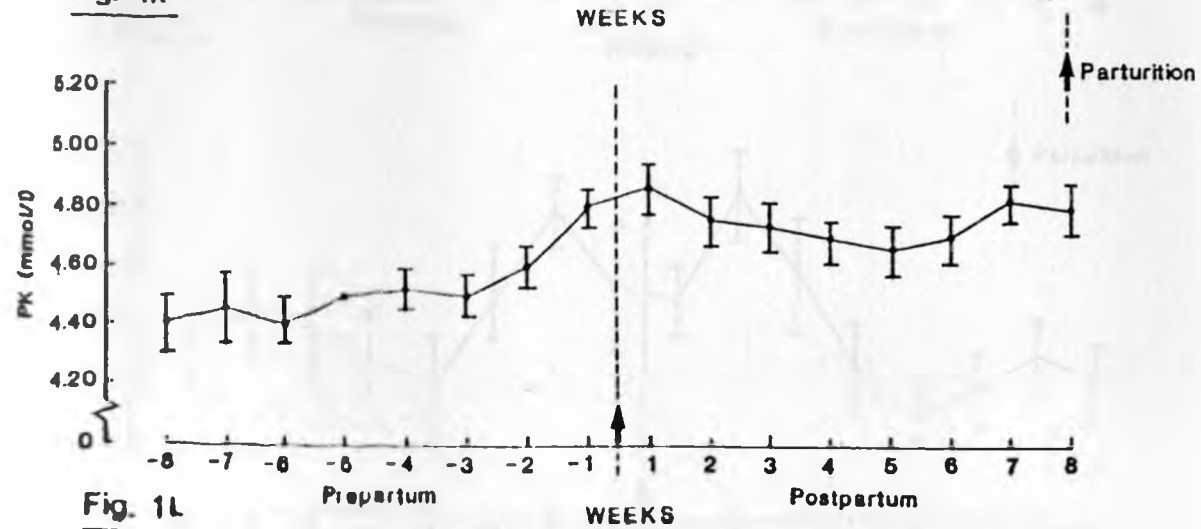

Fig. 1L

Figures 1J - 1L. Changes in body weight, PNa and PK (mean ± SEM) values in dairy cows 8 weeks before and 8 weeks after calving (pooled data - all age groups and calving periods) (Herd II)

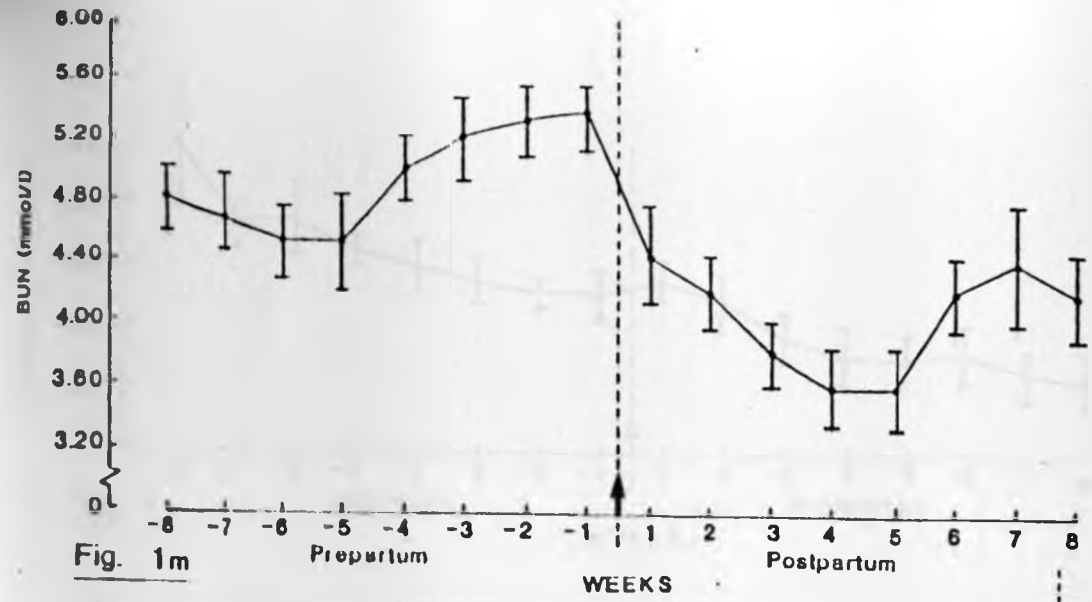

Fig. 1m

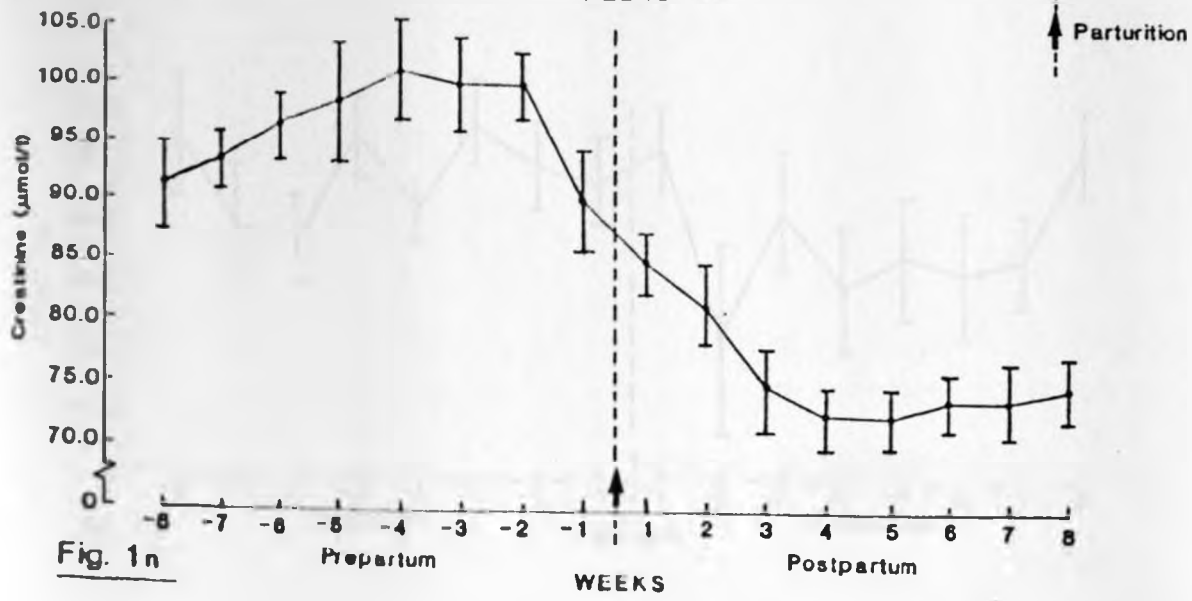

Fig. 1n

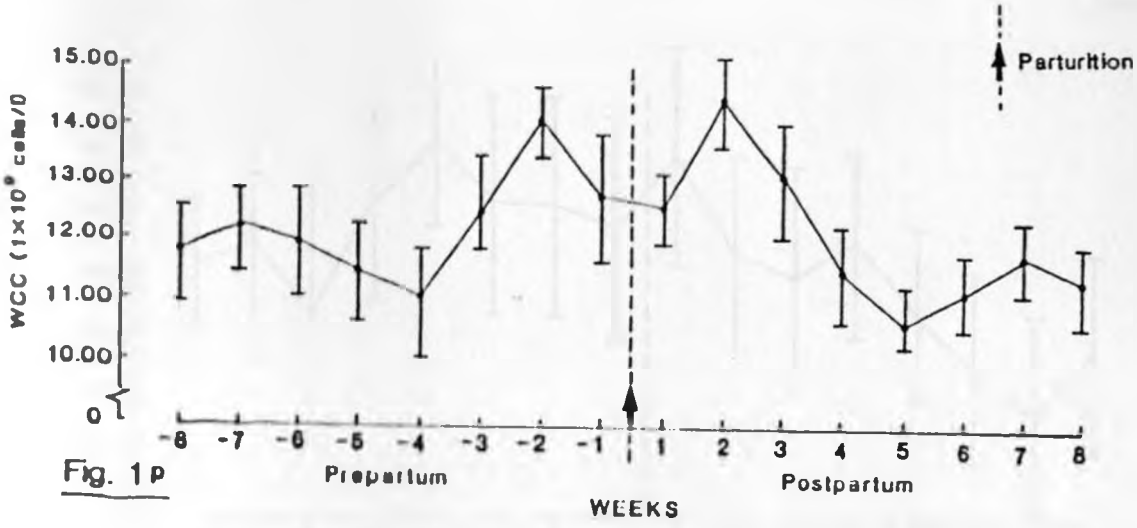

Fig. 1p

Figures 1m - 1p. Changes in BUN, creatinine and WCC (mean ± SEM) values in dairy cows 8 weeks before and 8 weeks after calving (pooled data - all age groups and calving periods) (Herd II)

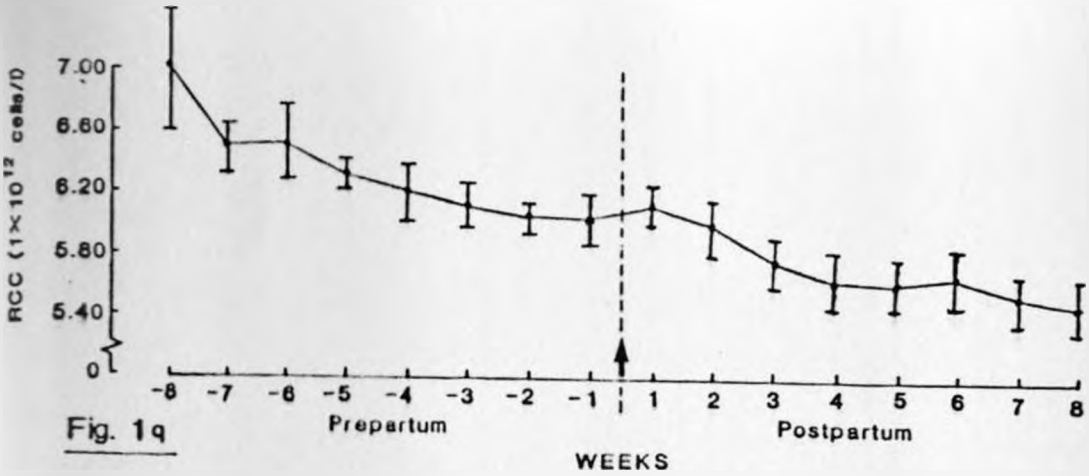

Fig. 1q

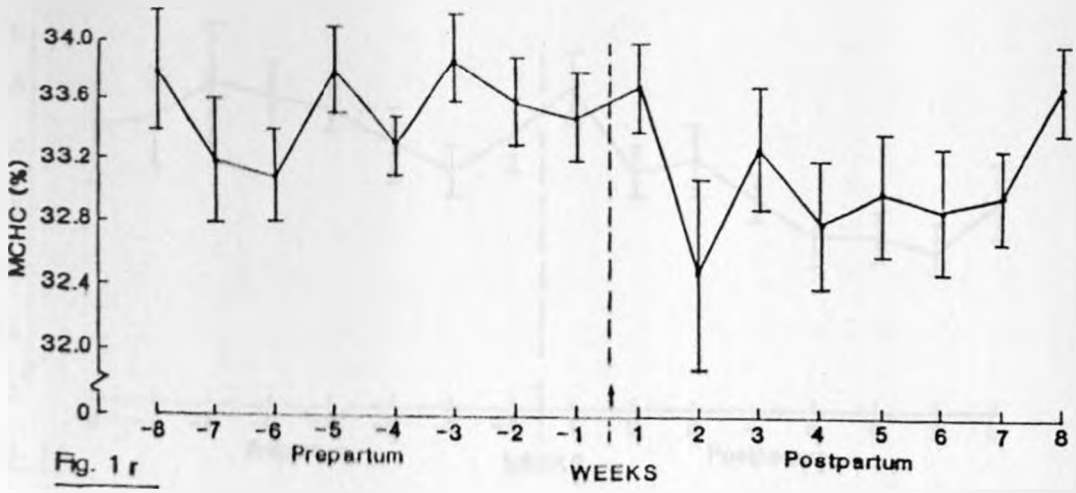

Fig. 1r

† Parturition

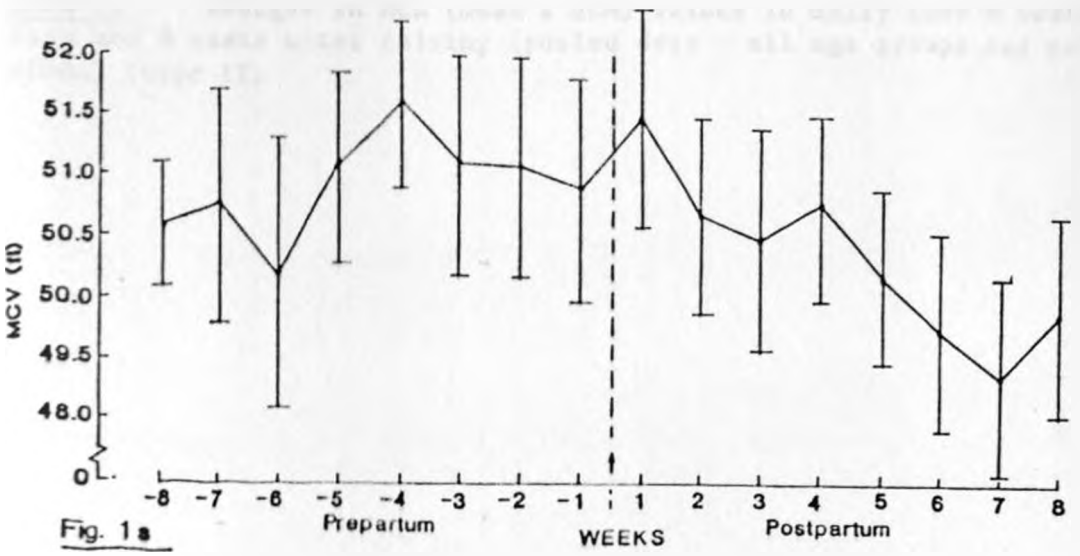

Fig. 1s

Figures 1q-1s. Changes in RCC, MCHC and MCV (mean ±SEM) values in dairy cows 8 weeks before and 8 weeks after calving (pooled data - all age groups and calving periods) (Herd II)

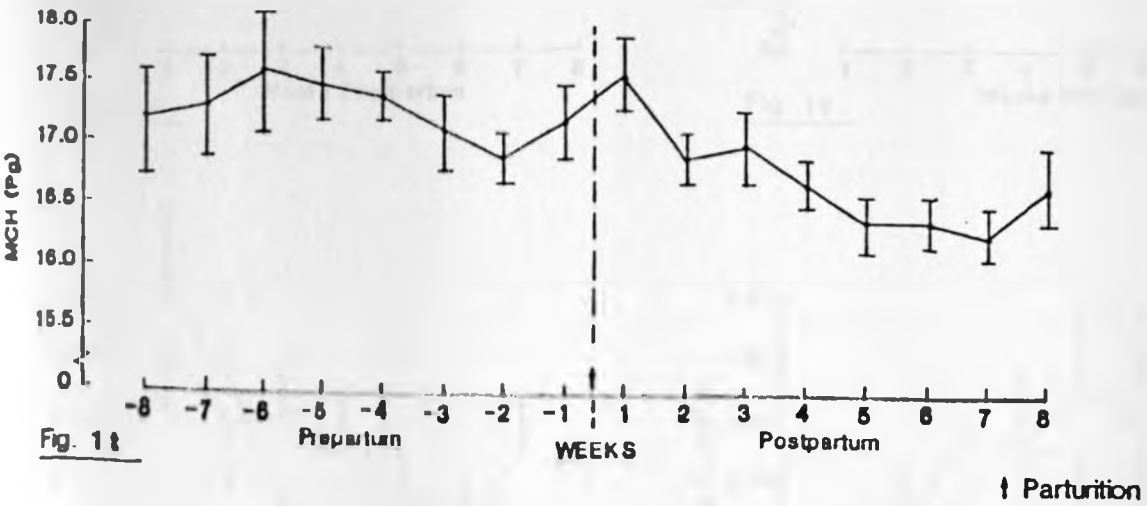

Figure 1t Changes in MCH (mean ± SEM) values in dairy cows 8 weeks before and 8 weeks after calving (pooled data - all age groups and calving periods) (Herd 11)

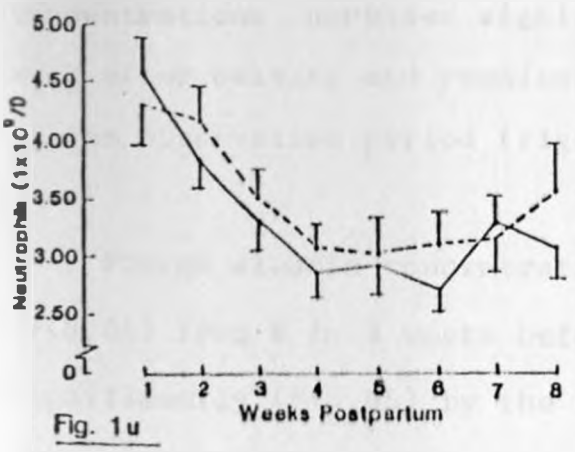

Fig. 1u

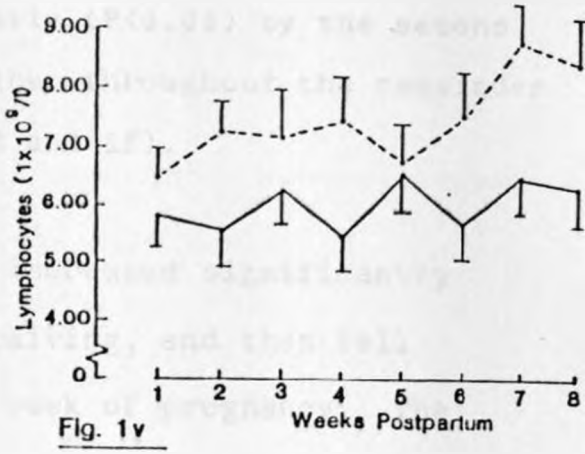

Fig. 1v

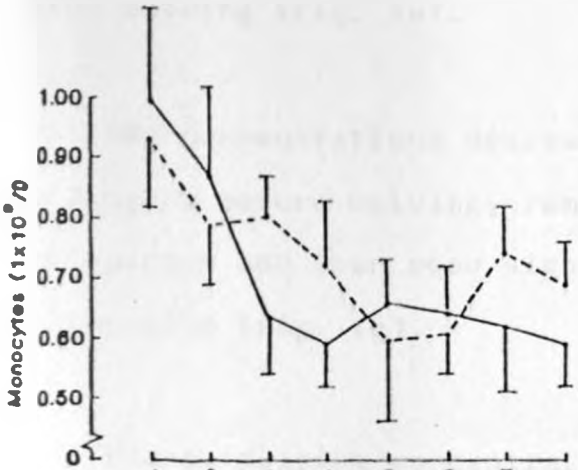

Fig. 1x

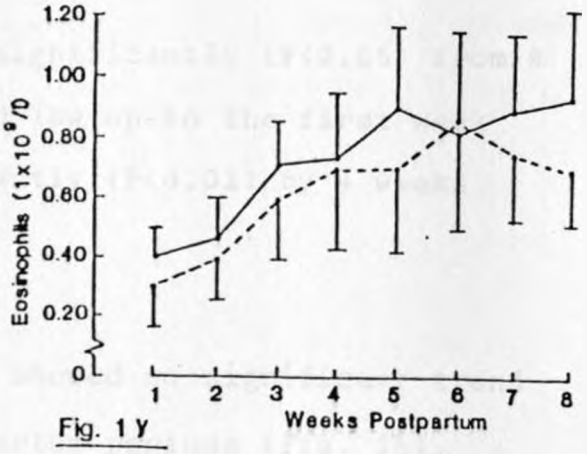

Fig. 1y

——— Old cows (> 4 yr)

- - - - Young cows (< 4 yr)

Figures 1u-1y. Changes in neutrophil, lymphocyte, monocyte and eosinophil (mean ± SEM) counts 8 weeks after calving according to age groups

TPP and plasma globulin concentrations decreased significantly ($P<0.01$) from 5 weeks to 1 week pre-partum, the lowest level occurring in the last week of pregnancy. Mean concentrations increased significantly ($P<0.05$) by the second week after calving and remained higher throughout the remainder of the observation period (Figs. 1d and 1f).

Plasma albumin concentrations increased significantly ($P<0.05$) from 8 to 3 weeks before calving, and then fell significantly ($P<0.05$) by the last week of pregnancy. The mean values increased to pre-partum values by the second week after calving (Fig. 1e).

PMg concentrations decreased significantly ($P<0.05$) from 8 to 2 weeks before calving, remained low up-to the first week post-partum and then rose significantly ($P<0.01$) by 4 weeks post-partum (Fig. 1g).

Plasma calcium concentrations showed no significant trend in either the pre-partum or post-partum periods (Fig. 1h).

Plasma Pi concentrations decreased significantly ($P<0.05$) in both the pre-partum and the post-partum periods. The post-partum mean concentrations were significantly ($P<0.05$) lower than the pre-partum mean concentrations (Fig. 1i).

PNa concentrations increased significantly ($P<0.01$) by the last week of pregnancy, and then decreased significantly ($P<0.01$) by the sixth week post-partum (fig. 1k).

PK concentrations showed no significant changes in either the pre-partum or post-partum periods. However the post-partum mean values were significantly ($P<0.05$) higher than the pre-partum mean values (Fig. 11).

BUN concentrations increased significantly ($P<0.01$) from 6 weeks to 1 week pre-partum, and fell significantly ($P<0.01$) between weeks 1 and 5 post-partum after which they increased ($P<0.05$) up to the end of the study (Fig. 1m).

Plasma creatinine concentrations increased from 8 to 3 weeks before calving and then fell steadily up to the fourth week after calving and increased thereafter up to the end of the observation period. The post-partum mean concentrations were significantly ($P<0.01$) lower than those in the pre-partum periods (Fig. 1n).

Total leucocytes rose significantly ($P<0.05$) from week 4 to week 2 before calving. After calving the mean values remained high up to the second week post-partum and then fell significantly ($P<0.01$) up to the fifth week post-partum (Fig. 1p).

Differential leucocyte counts were only measured in the post-partum period and the mean neutrophil counts were significantly ($P<0.01$) higher in the first two weeks post-partum than in the other weeks. The mean lymphocyte counts were significantly lower ($P<0.05$) in the first week after

calving, and did not show any significant trend after that. The mean monocytes values were significantly ($P<0.05$) higher in the first week after calving than in the other weeks and did not subsequently show any significant change. The lowest eosinophil counts were observed in the first week after calving, after which the mean values increased significantly ($P<0.01$) until the end of the observation period (Fig. 1u to 1y).

(b) Changes in blood components 8 weeks before and 8 weeks after calving according to age groups (Herd II).

The trends of blood component concentrations before and after calving for the age groups are shown in Appendix figures A1a to A1q. Table 1 shows the means and slopes of blood components which differed significantly ($P<0.05$) between the two age groups. With the exception of TPP, plasma globulin and BUN the trends of the blood components were similar in both age groups.

The decrease in both TPP and plasma globulin concentrations started seven weeks before calving in young cows and four weeks before calving in older cows (Figs. A1d and A1f). The mean BUN concentrations in the young cows at the start of the observations was about half that of the older cows. During the 8 weeks pre-partum the mean concentrations of BUN in the young cows increased significantly ($P<0.05$) so that by one week before calving the concentrations of the two groups were

Table 1 Blood components in which significant differences occurred between cows over 4 and under 4 years of age (Herd II)

Blood components	Under 4 years (n=32) Mean ± SEM	Over 4 years (n=30) Mean ± SEM
PCV (l/l)(pre)	0.32 ± 0.004	0.30 ± 0.004
Globulin (g/l)(pre)	33.5 ± 1.80	38.8 ± 1.40
WCC(10^9cells/l)(pre)	12.89 ± 0.40	9.03 ± 0.35
PCV (slope)(post)	-0.23 ± 0.06	-0.47 ± 0.08
Hb (slope)(post)	-0.08 ± 0.04	-0.27 ± 0.07

Pre = pre-partum

post = post-partum

All differences significant ($P<0.05$).

similar and remained so up to the end of the observation period of 8 weeks post-partum (Fig. A1a).

The pre-partum overall mean PCV's and WCC's and concentrations of plasma globulin and Ca were significantly ($P<0.05$) higher in the young cows than in the older cows (Table 1). The post-partum PCV and Hb slopes (decrease) were significantly ($P<0.05$) higher in the older cows than in the young cows.

(c) Changes in blood components before and after calving according to calving periods (Herd II).

The mean values and mean slopes of the blood components for the four calving periods are shown in Tables 2 to 5. The pre-partum mean values differed significantly between the calving periods for PCV, Hb, albumin, globulin, PNa, PK and BUN ($P<0.01$) and for TPP and plasma creatinine ($P<0.05$) (Table 2). The mean PCV's and Hb concentrations were higher in calving periods 1 and 4 than in calving period 3, however the slopes did not differ significantly between the calving period groups (Table 3). The pre-partum mean concentrations of TPP and plasma globulins were highest in calving period 1 and lowest in calving period 4. The albumin mean values were lowest in calving period group 3 and their slopes differed significantly ($P<0.05$) between the calving periods (Table 2). The PNa and PK pre-partum overall mean concentrations were highest in period 1

Table 2. Mean pre-partum concentrations of blood components in 62 cows according to calving period (Herd 11)

	Periods				F value
	1	2	3	4	(df=3,60)
PCV (l/l)	0.34	0.32	0.31	0.33	5.76*
Hb (g/l)	111	104	103	114	6.12**
Glucose (mmol/l)	3.08	2.91	2.85	2.89	2.02
TPP (g/l)	81.0	77.0	75.0	72.3	5.89*
Albumin (g/l)	36.0	35.6	24.9	38.5	11.54**
Globulin (g/l)	44.7	41.5	40.2	33.7	7.94**
PMg (mmol/l)	0.86	0.89	0.85	0.87	0.85
Ca (mmol/l)	2.19	2.26	2.34	2.38	2.49
Pi (mmol/l)	2.04	2.04	2.01	2.14	0.74
WCC($\times 10^9$cells/l)	12.26	12.68	14.06	11.81	1.74
RCC ($\times 10^{12}$cells/l)	6.77	6.32	6.03	6.47	2.42
PNa (mmol/l)	141.9	139.1	140.5	139.6	6.86**
PK (mmol/l)	4.59	4.28	4.50	4.53	5.09**
BUN (mmol/l)	4.17	4.35	6.78	5.26	6.66**
Creatinine (µmol/L)	99.9	96.4	94.8	111.8	4.07*

Table 3 Pre-partum slopes of blood components of 62 cows grouped according to the calving periods (Herd II)

Blood component	Periods 1	2	3	4	F value (df=3,60)
PCV	-0.01	-0.03	-0.11	-0.32	0.70
Hb	-0.01	-0.02	-0.05	-0.04	0.04
Glucose	0.03	0.02	0.08	-0.03	0.96
TPP	-0.07	-0.07	-0.19	-0.13	1.34
Albumin	-0.03	-0.06	0.01	0.01	3.82*
Globulin	-0.08	-0.11	-0.18	-0.14	0.63
PMg	0.01	-0.10	-0.01	0.02	0.85
Ca	0.03	-0.05	-0.02	0.03	2.04
Pi	-0.83	0.77	-0.30	-0.83	0.84
WCC	0.25	0.52	0.24	0.25	1.92
RCC	0.01	-0.09	-0.11	-0.01	0.79
PNa	0.56	0.36	0.38	0.56	1.50
PK	-0.01	0.03	0.03	-0.01	1.06
BUN	0.11	0.20	0.58	0.21	21.5**
Creatinine	1.29	1.34	1.80	1.49	5.48*

Table 4 Mean post-partum concentrations of blood components in 62 cows grouped according to calving periods (Herd II)

Blood components	Periods 1	2	3	4	F value (df=3,60)
PCV (l/l)	0.31	0.30	0.28	0.30	3.38*
Hb (g/l)	105	95	94	102	4.67*
Glucose(mmol/l)	2.87	2.87	2.91	2.80	0.47
TPP (g/l)	77.1	75.0	75.2	71.1	6.71**
Albumin (g/l)	37.3	36.6	36.3	37.2	1.93
Globulin (g/l)	39.1	38.5	38.7	33.7	6.64**
PMg (mmol/l)	0.90	0.83	0.91	0.91	2.74
Ca (mmol/l)	2.46	2.09	2.21	2.24	12.98**
Pi (mmol/l)	1.89	1.71	1.86	1.88	2.53
WCC($x10^9$cells/l)	11.26	11.07	10.91	11.34	1.02
RCC($x10^{12}$cells/l)	6.24	6.43	6.12	6.09	0.98
PNa (mmol/l)	41.2	150.6	138.2	141.4	0.70
PK (mmol/l)	8.00	4.80	4.75	4.64	1.63
BUN (mmol/l)	3.36	3.47	3.57	4.25	1.88
Creatinine (umol/l)	71.1	74.3	81.4	73.2	2.24

* $P<0.05$

** $P<0.01$

Table 5. The post-partum slopes of blood components of 62 cows grouped according to calving periods (Herd II)

Blood component	Periods 1	2	3	4	F value (df=3,60)
PCV	-0.84	-0.23	-0.18	-0.47	6.92**
Hb	-0.26	-0.08	-0.08	-0.18	3.66*
Glucose	0.008	0.008	0.004	0.025	0.34
TPP	0.01	0.07	0.07	0.04	1.46
Albumin	-0.01	-0.02	0.01	-0.02	0.40
Globulin	0.02	0.10	0.05	0.07	0.22
PHg	0.001	0.002	0.012	0.01	0.50
Ca	0.02	0.02	-0.01	0.02	1.20
Pi	-0.05	-0.05	-0.01	-0.02	1.75
WCC	-0.11	-0.54	-0.08	-0.53	1.62
RCC	0.009	0.06	-0.07	-0.002	1.04
PNa	-0.45	0.72	-0.39	-0.29	0.73
PK	0.03	0.06	-0.006	0.02	0.81
BUN	0.27	-0.06	-0.03	-0.06	0.92
Creatinine	-2.18	-1.96	-2.83	-2.70	0.27

while BUN concentrations were highest in period 3 and plasma creatinine overall mean concentrations were lowest in period 3. The pre-partum BUN and plasma creatinine slopes differed significantly between the calving period groups (BUN $P<0.01$, plasma creatinine $P<0.05$). The mean concentrations of BUN decreased in period 1 and increased in periods 3 and 4 towards calving. The plasma creatinine concentrations increased in periods 1 and 4 and decreased in period 3 towards calving (Table 2).

The mean post-partum concentrations differed significantly between calving periods for PCV ($P<0.05$), Hb, TPP, globulin and Ca ($P<0.01$) (Table 4). The PCV's and Hb mean concentrations were highest in period 1 and lowest in period 3. The mean concentrations of TPP and globulin were highest in period 1 and lowest in 4, while mean concentrations for Ca were highest in period 1 and lowest in 2. The post-partum PCV and Hb slopes differed significantly between the calving periods (Hb, $P<0.05$; PCV, $P<0.01$), and they were greater in periods 1 and least in 3 (Table 5).

(d) Changes in blood components before and after calving according to herds

The trends of the blood components before and after calving according to herds are shown in Appendix figures A2a to A2q and Tables 6 to 8.

Table 6. The overall pre-partum and post-partum means (± SEM) and slopes (± SEB) of blood components for herd I (pooled data).

Blood Parameter	Pre-partum Mean ± SEM	Pre-partum Slope ± SEB	Post-partum Mean ± SEM	Post-partum Slope ± SEB
PCV (l/l)	0.34 ± 0.004	-0.13 ± 0.01	0.28 ± 0.003	-0.43 ± 0.04
Hb (g/l)	110 ± 2.7	-0.17 ± 0.01	95 ± 2.0	-0.15 ± 0.04
Glucose (mmol/l)	2.86 ± 0.06	-0.04 ± 0.02	2.99 ± 0.05	-0.09 ± 0.01
TPP (g/l)	77.3 ± 2.00	-0.14 ± 0.04	73.8 ± 3.00	0.60 ± 0.01
Albumin (g/l)	37.0 ± 0.70	0.08 ± 0.02	36.0 ± 0.70	0.03 ± 0.01
Globulin (g/l)	37.5 ± 1.20	-0.14 ± 0.03	35.6 ± 1.00	0.08 ± 0.02
PMg (mmol/l)	0.91 ± 0.01	-0.01 ± 0.01	0.95 ± 0.02	0.01 ± 0.01
Ca (mmol/l)	2.26 ± 0.05	0.01 ± 0.10	2.26 ± 0.04	-0.04 ± 0.01
Pi (mmol/l)	2.26 ± 0.03	-0.01 ± 0.01	1.80 ± 0.03	-0.02 ± 0.02
EMg (mmol/l)	1.10 ± 0.03	-0.03 ± 0.01	1.24 ± 0.24	-0.04 ± 0.03
ENa (mmol/l)	108.3 ± 3.19	2.61 ± 1.38	88.3 ± 6.75	-3.08 ± 1.62
EK (mmol/l)	29.2 ± 1.27	-1.70 ± 0.40	25.1 ± 1.59	-2.57 ± 0.99
WCC ($\times 10^9$ cells/l)	9.38 ± 0.84	0.02 ± 0.33	9.69 ± 0.67	-0.48 ± 0.19
RCC ($\times 10^{12}$ cells/l)	6.23 ± 0.15	-0.06 ± 0.02	5.35 ± 0.17	-0.20 ± 0.05
PNa (mmol/l)	141.9 ± 0.70	0.49 ± 0.19	137.6 ± 0.58	-0.06 ± 0.24
PK (mmol/l)	4.46 ± 0.07	0.01 ± 0.01	4.56 ± 0.07	0.03 ± 0.01
BUN (mmol/l)	6.58 ± 0.37	0.05 ± 0.01	4.02 ± 0.39	-0.16 ± 0.03
Creatinine (umol/l)	105 ± 4.10	0.38 ± 0.02	85.1 ± 2.11	-1.81 ± 0.45

Table 7. The overall pre-partum and post-partum means (± SEM) and slopes (± SEB) of blood components for herd II (pooled data).

Parameter	Pre-partum Mean ± SEM	Pre-partum Slope ± SEB	Post-partum Mean ± SEM	Post-partum Slope ± SEB
[illegible] (l/l)	0.34 ± 0.004	-0.12 ± 0.01	0.29 ± 0.004	-0.41 ± 0.03
[illegible] (g/l)	106 ± 1.3	-0.20 ± 0.07	94 ± 1.5	-0.15 ± 0.02
[illegible]cose (mmol/l)	2.85 ± 0.03	0.03 ± 0.03	2.82 ± 0.04	0.02 ± 0.01
[illegible] (g/l)	76.3 ± 1.50	-0.12 ± 0.03	73.5 ± 3.00	0.05 ± 0.01
[illegible]umin (g/l)	35.1 ± 6.00	0.04 ± 0.01	37.3 ± 3.00	0.30 ± 0.02
[illegible]mulin (g/l)	39.2 ± 7.00	-0.14 ± 0.03	36.7 ± 5.00	0.07 ± 0.02
[illegible] (mmol/l)	0.88 ± 0.01	-0.01 ± 0.01	0.93 ± 0.05	0.01 ± 0.01
[illegible] (mmol/l)	2.32 ± 0.03	-0.02 ± 0.01	2.22 ± 0.04	0.02 ± 0.01
[illegible] (mmol/l)	2.03 ± 0.03	-0.02 ± 0.01	1.80 ± 0.03	-0.03 ± 0.01
[illegible] (mmol/l)	1.10 ± 0.02	-0.03 ± 0.01	1.07 ± 0.01	-0.04 ± 0.01
[illegible] (mmol/l)	110.1 ± 4.12	2.95 ± 1.37	85.5 ± 7.73	3.74 ± 1.54
[illegible] (mmol/l)	30.3 ± 0.31	-0.10 ± 0.04	28.4 ± 0.37	-0.20 ± 0.04
[illegible] (10^9 cells/l)	12.45 ± 0.38	0.08 ± 0.15	11.8 ± 0.42	-0.38 ± 0.12
[illegible] (10^{12} cells/s)	6.55 ± 0.10	-0.08 ± 0.03	5.94 ± 0.09	-0.09 ± 0.03
[illegible] (mmol/l)	140.3 ± 0.29	0.48 ± 0.12	139.1 ± 0.46	-0.46 ± 0.11
[illegible] (mmol/l)	4.44 ± 0.03	-0.01 ± 0.02	4.77 ± 0.05	0.01 ± 0.01
[illegible] (mmol/l)	5.40 ± 0.34	0.11 ± 0.33	3.73 ± 0.12	-0.12 ± 0.03
[illegible]tinine (mol/l)	101.9 ± 3.12	0.54 ± 0.03	75.2 ± 1.22	-2.57 ± 0.45

Table 8. The means (± SEM) for blood components which showed significant differences between herd I and herd II

Blood component	Herd I (n=23)	Herd II (n=62)	Sig. of diff
PCV (l/l) (pre)	0.34 ± 0.004	0.32 ± 0.004	**
Hb (g/l) (pre)	115 ± 2.7	106 ± 1.3	**
BUN (mmol/l)(pre)	6.58 ± 0.37	5.40 ± 0.34	*
Albumin (g/l) (pre)	37.7 ± 0.70	35.1 ± 0.80	*
WCC($\times 10^9$cells/l)(pre)	9.38 0.84	12.45 0.38	**
(post)	9.69 0.67	11.78 0.42	*
Ca (mmol/l)(pre)	2.46 0.06	2.26 0.04	*

* P<0.05 ** P<0.01

pre = Pre-partum

post = Post-partum

Sig. of diff = Significance of difference

The general trends of the blood components analysed were similar in both herds for both pre-partum and post-partum periods. However, the overall mean concentrations of some blood components differed significantly ($P<0.05$) between the two herds (Table 8). The pre-partum mean PCV's and concentrations of Hb ($P<0.01$), albumin, Ca and BUN ($P<0.05$) were higher in herd I than in herd II. These blood components also tended to be higher in herd I than in herd II in the post-partum period. WCC's in both pre-partum and post-partum periods were significantly ($P< 0.05$) higher in herd II than in herd I (Fig. A2K).

DISCUSSION

(a) Changes in blood and plasma components 8 weeks before and 8 weeks after calving

Mean PCV's and Hb concentrations decreased towards calving with the lowest level occurring at the last week of pregnancy. Similar results were reported by Hewett (1974). Hb contains globin whose production could be influenced by the factors which affect the reserves in the protein pool (Roubicek and Ray 1972). This might partially explain the decrease in PCV and Hb concentrations towards calving, when there is increased protein demand for foetal development and colostrum production.

The slight increase in PCV and Hb concentrations in the first week post-partum has been associated with temporary dehydration that occurs after calving (Holman 1954, Hewett 1974). The decrease in PCV and Hb values in early lactation has been attributed to increased water consumption and increased blood volume associated with the onset of lactogenesis (Van Soest et al. 1954, Whitlock et al. 1974). Thus since there is increased blood volume with lactogenesis then the rate of decrease of PCV and Hb concentrations could be related to production.

The decrease in PCV and Hb concentrations with stage of lactation has been reported previously (Kossila 1970, Hewett 1974, Manston et al. 1975). However Patterson, Shrode, Kunkel, Leighton and Kupel (1960) and Fisher (1962) did not observe the negative relationship between PCV and/or Hb concentrations and stage of lactation. These varying results could be due to differing protein status during pregnancy. PCV and Hb values were found by Manston et al. (1975) to be affected by long-term protein status. They found that cows with a low protein supply during pregnancy showed a greater decline in PCV and Hb concentrations after calving than those receiving a high protein diet during pregnancy.

The significantly lowered MCH values observed post-partum were probably due to the significant decrease in Hb concentrations without a corresponding decrease in RCC. The decrease in both

MCH and Hb concentrations may also have been due to the reported inverse relationship between blood copper concentrations and milk production (Kappel, Ingraham, Morgan, Babcock and Stat 1984). Copper is secreted into the milk throughout lactation, however the secretion is highest in early lactation and is greatest in high producting cows (Amiel 1970, Kappel et al. 1984). Thus high yielding cows should have a greater decrease in MCH and Hb concentrations than low yielding cows during early lactation. Low blood copper concentrations lead to a decrease in protohaem and haemoglobin synthesis (Gallagher 1957). Unfortunately blood copper concentrations were not determined in the present study.

Plasma glucose concentrations showed no significant change before or after calving as has been observed previously (Rook and Line 1961, Prior et al. 1979, Rao et al. 1981). Lactating cows have been observed to adapt to reduced energy intake by decreasing daily milk yield (Rook and Line, 1961). Cows in marginal or inadequate energy balance maintain lower plasma glucose concentrations than cows which receive adequate or excess energy intake (Parker and Blowey 1976). This suggests that plasma glucose concentrations could be important in indicating cows likely to have reduced milk production and possibly poor fertility. Low plasma glucose concentrations have been associated with low fertility in dairy cows (McClure 1965, McClure 1968).

The TPP concentrations decreased towards calving with lowest values occurring at the last week of pregnancy. This decrease in TPP concentrations towards calving has been shown to be due to transfer of immunoglobulins to colostrum (Larson 1958, Williams and Miller 1975). The increase in TPP concentrations after calving have been shown to be due to reduced globulin transfer to milk (Williams and Miller 1979). Since the changes in TPP concentrations before and after calving are due to mainly changes in plasma globulin concentrations it is suggested that changes in plasma globulin concentrations should be used in predicting fertility and milk production instead of changes in TPP concentrations.

The plasma albumin concentrations decreased significantly in the last two weeks of pregnancy as previously reported (Dixon, Weigle and Varques 1961, Little 1974). This has been shown to be due to transfer of albumin from the blood to colostrum (Dixon et al. 1961, Murphy and Pattee 1964). The low albumin concentrations observed in the first week post-partum probably were due to increased dilution as a result of increased blood volume and loss from the blood into extracellular fluids including urine and milk (Whitlock et al. 1974). Maximum synthesis of milk proteins in the udder occurs a few days after parturition (Larson 1958) and plasma albumin is utilized to replenish some of the amino acids utilized by the udder (Kon and Cowie 1961). This may partly account for the low albumin concentrations in the first week of lactation. Cows that

maintain stable albumin concentrations have been shown to have better fertility than those cows which show changes in albumin concentrations (Rowlands et al. 1977, Rowlands et al. 1980). The changes in plasma albumin concentrations before and after calving could be a useful parameter in predicting fertility and milk production since they may reflect the cows protein status and ability to maintain stable albumin concentrations.

The decrease in plasma globulin concentrations towards parturition is mainly due to selective uptake of IgG1 by specific IgG1 receptors on the mammary alveolar epithelial cells (Pierce and Feinstein 1965). The plasma immunoglobulins are composed mainly of IgG1 and IgG2 which are approximately equal at 10mg/ml (Williams and Miller 1979), and transfer of IgG1 to colostrum can lead to a fall of plasma globulins of up to 45% (Murphy and Pattee 1964, Williams and Miller 1979). Some IgM also gets into colostrum from the blood (Williams and Miller 1979). The rapid increase of plasma globulin after parturition is due to an increased plasma immunoglobulin concentration as their transfer into milk is greatly reduced following parturition (Larson et al. 1959, Williams and Miller 1979). The mean value and changes of plasma globulin may indicate inflammatory processes (e.g. metritis, endometritis, mastitis) which can impair both fertility and milk production. High mean globulin concentrations were found to be associated with poor fertility in dairy cows (Rowlands et al. (1980). Hence plasma globulin values and changes may be useful parameters in predicting fertility and milk production.

The decrease in PMg concentrations towards parturition as previously reported (Murtuza et al. (1979) could have been due to the increased Mg demand for foetal growth in the last trimester of pregnancy (Lim et al. 1969). An increase (Hewett 1974, Rowland et al. 1975), decrease (Kossila 1970, Payne et al. 1974) and no change (Parker et al. 1976, Rao et al. 1981) in PMg concentrations with stage of lactation have all been reported. These varying observations of PMg changes are difficult to explain, however they could reflect differing Mg intake concentrations for different herds. Since Mg is involved in most of the energy synthesis processes (Wacker 1965, Pike 1967), the change in PMg concentrations may be of value in predicting fertility and milk production in dairy cows.

The mean Ca concentrations showed no significant changes either pre-partum or post-partum as previously reported (Payne and Leach 1964). Mean Ca concentrations may affect uterine involution (Ward 1967, Silva and Noakes 1984) hence they could be of value in predicting fertility in dairy cows.

The decrease in Pi concentrations observed towards calving has previously been noted (Hackett, Gazalar and Busted 1957, Rao et al. 1981), and could be due to increased utilization of phosphorus with an enhanced carbohydrate metabolism of pregnancy (Hackett et al. 1957). The decrease in Pi concentrations after calving have been attributed to an increased phosphorus utilization during lactation (Herdt 1981). The concentrations of Pi have been associated with fertility in dairy cows by previous workers (Amiel 1970, Hewett 1974, Herdt 1981). Therefore both mean Pi concentrations and changes

could be important in predicting fertility and milk production.

The increase in PNa concentrations near parturition has been previously reported (Murtuza et al. 1979). However, a negative correlation of PNa with month of pregnancy was reported by Lane et al. (1968). These workers also noted a positive correlation between PNa concentrations and the month of lactation. A decrease in PNa concentrations with stage of lactation has been observed by some workers (Hewett 1974) while no effect of stage of lactation on PNa values was reported by others (Kitchenham, Rowlands and Shorbagi 1975b).

In this study the findings observed in PK concentrations were similar to those of Murtuza et al. (1979). However Lane et al. (1968) observed a positive correlation between PK concentrations and the month of pregnancy and a negative correlation with stage of lactation. High PK concentrations were found to be associated with poor fertility (Hewett 1974). Therefore PK concentrations may be of value in predicting fertility in dairy cows.

The increase in BUN concentrations pre-partum has previously been reported (Prior et al. 1976). This increase was probably due to insufficient energy intake, which leads to increased deamination of amino acids in the liver and hence elevation of BUN concentrations (Treacher 1977, Prior 1979). The decrease in BUN concentrations in the first few weeks post-partum was similar to that reported by Rowlands et al. (1975) and was probably due to increased utilization of amino acid nitrogen for milk production When the utilization of dietary protein is optimal few amino acids remain to be deaminated and the concentrations of BUN remain low

(Treacher 1977).

The rise in BUN concentrations observed 5 weeks post-partum corresponded to the period of peak milk yield (Rowlands et al. 1975) in which cows undergo a period of energy deficit (Roberts et al. 1981). In this period of energy deficit cows mobilize body reserves (fat deposits, body proteins) for milk production leading to a rise in BUN concentrations (Broster et al. 1969). The rise in BUN concentrations in early lactation may reflect a cow's ability to utilize body reserves for milk production. The cows that utilize more body proteins than fat reserves (i.e. high BUN concentrations) will have lower milk yields than those which utilize more fat reserves than body proteins (i.e. low BUN concentrations) since one gram of fat gives more energy for milk production than one gram of protein (Webster 1978).

The increase in plasma creatinine concentrations observed before paturition could have been due to the increase in body weight. The amount of creatinine in the muscles or body is described as the creatinine coefficient and is directly related to body weight (Barakat and Abdalla 1961, DeGroot et al. 1969). Most of the creatinine in the body (98%) is present in the skeletal muscles where it stores energy in the form of phosphocreatine (Kertz et al. 1970, Tyler 1972). The daily creatinine production from phosphocreatine is directly related to the plasma creatinine concentrations (Finco and Duncan 1976). According to Eubenberger (1982), high plasma creatine concentrations in animals in late pregnancy and early lactation are physiologically normal.

The decrease in plasma creatinine concentrations after calving was probably due to loss in body weight (mass) which occurred after calving. Loss in body weight (mass) has been reported to cause a decrease in the creatinine coefficient and plasma creatinine concentrations (Barakat and Abdalla 1961, Doolan, Alsen and Meil 1962). Dairy cows undergo body weight losses in early lactation (Fig. 1j) during the period of energy deficit (Roberts et al. 1981). Since the decrease in body weight may be reflective of a cow's ability to utilize body reserves for milk production, the decrease in plasma creatinine concentrations after calving which is closely related to body weight loss (Barakat and Abdalla 1961) may as well reflect the cow's ability to utilize body reserves for milk production.

The observation of a leucocytosis around calving is similar to previous reports (Morris 1954, Straub et al. 1958, Zamet et al. 1979) and has been attributed to the stress of parturition. The leucocytosis observed in the first 2 weeks after calving in this study was probably due to stress of parturition and the onset of lactation (Morris 1954, Straub et al. 1958). Lactating cows that are not able to adapt to prolonged stress have low fertility and milk production (Thatcher 1975). The blood leucocyte counts of an animal are related to its adaptability to stress (Paape et al. 1974a), thus the mean values and the changes of total and differential leucocyte numbers may show which animals are able to withstand the stress of early lactation and hence are likely to have better fertility and milk production.

(b) Changes in blood and plasma constituents according to age groups

The lower PCV, Hb and RCC concentrations in older cows have been observed previously (Rowlands et al. 1974, Noonan et al. 1978) and are probably due to an increase in plasma volume with age (Noonan et al. 1978). The greater decrease in PCV and Hb concentrations observed after calving in the older cows could have been due to a greater loss of protein and copper from the blood into milk in these higher producing cows. The higher plasma globulin concentrations in older cows was as expected since globulin concentrations increase with age (Larson and Touchberry 1959, Gartner et al. 1966). The earlier decrease in plasma globulins in young cows than in the older cows has also been previously observed (Williams and Miller 1975). These workers found that IgG1 concentrations decreased earlier in young cows (4 weeks before calving) as compared to a late decrease in older cows (2 weeks before calving). They suggested that young pregnant cows should be vaccinated earlier than older cows if the maximum amount of immunoglobulins is to be transferred into colostrum. The lower Ca concentrations in older cows (Fig. A1h) probably reflect a reduced ability to mobilize Ca from both the alimentary tract and the skeleton as suggested by Moodie (1961). The higher rate of increase in BUN concentrations in young cows than in the older cows towards calving is difficult to explain. However, it could be that older cows utilize dietary proteins more efficiently than young cows, since concentrations of BUN for any given amount of dietary nitrogen depends on the amount of amino acid nitrogen which is used for productive purposes (Sykes 1977). The higher

number of blood leucocytes observed in young cows in this study was also observed by Hewett (1974) who found that heifers had significantly higher leucocyte counts than older cows. The decrease in WCC with age has been attributed to the decrease in circulating lymphocyte numbers with age (Bhannasiri, Bogart and Krueger 1961, Conner, la Bell, Fysler and Wennacott 1967).

(c) Changes in blood components according to calving periods

The higher mean PCV's, RCC's and the concentrations of Hb, TPP and plasma albumin observed in cows calving in the hotter months (October to February) may have been due to a relative dehydration that may occuring during hot weather (Amiel 1970, Noonan et al. 1978, Nawaz and Nawaz 1983). The lower PCV and Hb concentrations in cold months could have been due to low copper levels in pastures and reduced copper availability in the soil in the cold months as has been previously noted in Queensland (Donaldson et al. 1964, Alexander et al. 1967).

The high BUN concentrations and slopes observed in periods 3 and 4 corresponded with the period of utilization of nitrogen-fertilised irrigated pastures which probably had higher protein values. The concentrations of BUN are affected by level of protein intake (Payne et al. 1970, Rowlands et al. 1974) and increase with high protein intakes (Sykes 1977). The period of utilization of the irrigated pastures also corresponded with high plasma creatinine concentrations, hence this might mean that high nutritive (high energy and protein concentrations)

feeds can lead to high plasma creatinine concentrations as previously suggested (Stufflebeam et al. 1969). The lack of decrease in albumin concentrations towards calving in periods 3 and 4 probably indicate that transfer of albumin into colostrum can be compensated for by a higher protein intake. Higher protein intakes lead to higher plasma albumin concentrations (Rowlands et al. 1974).

The post-partum decrease in PCV and Hb concentrations was greatest in period 1 when the cows were grazing on tropical pastures (probably low in nutritional values). The rate of decrease of PCV and Hb concentrations has been shown to be greatest in cows which had a low protein intake during late pregnancy (Manston et al. 1975). Thus cows calving in period 1 may have had a lower protein intake during late pregnancy.

(d) Changes in blood components according to herds

Herds differ one from another in husbandry practices and nutrition and this can be reflected in their blood profile patterns. However in this study the blood components analysed did not show any marked differences in their mean concentrations. The differences observed for PCV, Hb, Albumin and BUN mean concentrations probably reflected differences in the concentrations of protein intake during late pregnancy. Haemoglobin, plasma albumin and BUN concentrations are directly related to dietary protein intake (Prewitt et al. 1971, Manston et al. 1975).

Plasma Ca concentrations have been reported to vary markedly between herds (Payne et al. 1973a, Payne et al. 1974, Claypool

1976). The differences in mean Ca concentrations between the herds could have been due to different levels of Ca intake (Claypool et al. 1976, Pickard 1977).

The high WCC's in herd II were probably due to the smaller proportion of old cows (average age 5 yrs) as compared to herd I (average age 7 yrs) since WCC concentrations decrease with age (Hewett 1974, Conner et al. 1978).

The results of this study show that the pre-partum and post-partum mean concentrations and slopes were similar for most of the blood components for each age group, calving period and herd. This suggests that such changes can be used to predict milk production or fertility in a herd composed of different age groups and at any period of the year. Should the blood components that showed significant mean concentrations and slopes between age groups and calving periods be important in prediction equations for production and fertility then these factors should be considered when such predictions are being formulated or made.

CHAPTER 4

CHANGES IN ERYTHROCYTE Mg (EMg), Na (ENa) AND K (EK) CONCENTRATIONS 8 WEEKS BEFORE AND 8 WEEKS AFTER CALVING IN DAIRY COWS

INTRODUCTION

Irrespective of their breed, cattle (Ellory and Tucker 1970, Christinaz and Schatamann 1972) and sheep (Evans and King 1955, Evans et al. 1956, Evans and Mounib 1957) fall into two classes in respect of EK concentrations. The majority of cattle have low K (LK) cells and a minority high K (HK) cells (Ellory and Tucker 1970). The EK type in any individual animal in both sheep and cattle is genetically determined with the mode of inheritance being controlled by a simple allelic pair (Evans and King 1955, Kidwell, Bohman, Wade, Haverland and Hunter 1959, Ellory and Tucker 1970). The gene for LK being dominant over the gene for the HK type (Evans and king 1955, Evans and Mounib 1957, Kidwell et al. 1959, Ellory and Tucker 1970).

Several investigations (Tosteson 1963, Tosteson 1968, Tosteson, Cook and Blount 1965; Tosteson, Andreoli, Tieffenberg and Cook 1968) have elucidated the physiological basis for the differences in EK (and ENa) content. The salient features seem to be a high number of Na-K pump sites per unit of cell membrane surface in HK cells and a low number in LK cells, and a higher Na-K ATPase activity in the HK cells than in the LK cells.

In addition it was found that the permeability of the membrane towards K and Na is different in the two types of cells, the HK cells being relatively more permeable to Na^+ ions and less permeable to K^+ ions than the LK cells, and the reverse being true for LK cells (Tosteson and Hoffman 1960).

Recent studies in man and mice have shown that EMg levels could be genetically determined (Darlu et al. 1981, Henrotte and Colombani 1981) and may be related to antibody production (Henrotte et al. 1982). This suggests that studies on the relationship between EMg concentration and production indices (milk production, fertility and growth rate) may be worth investigating in dairy cows.

EK concentrations have been shown to have some adaptive significance in sheep (Evans 1954) and cattle (Evans 1963) and were related to the coat score index developed by Turner and Schleger (1960). It was postulated that differences in the balance of endocrine factors may be responsible for the differences in the EK concentrations between different breeds of cattle (Evans 1963). Since EK concentrations may be related to endocrine function (Evans 1963), they may therefore be related to production indices in cattle. Better reproductive performance was found in LK ewes with EK concentrations near the mode than ewes with EK values outside the mode (Turner and Koch 1961).

This work was undertaken to study the effects of late pregnancy and early lactation and age on EMg, ENa and EK concentrations in dairy cattle. A greater understanding of these effects could assist in determining the usefulness of mean levels or changes in these parameters as predictors of fertility and milk production in dairy cattle.

MATERIALS AND METHODS

EMg, EK and ENa concentrations were determined on weekly blood samples from cows in herds I and II by methods described previously in the General Materials and Methods. The samples were collected over the last 8 weeks of pregnancy and the first 8 weeks of lactation and animals were grouped according to age and calving period as described in Chapter 3. They were also grouped according to EK concentration after it was found that the distribution of EK concentrations was bimodal. Eleven calves (progeny of some of the experimental cows) were also sampled at one week of age (to compare their mean EMg, ENa and EK concentrations with that of their dams). Similarly 10 six-month-old replacement calves and 13 replacement yearling heifers from herd II were sampled to observe the effect of age on EMg, ENa and EK concentrations.

The mean weekly concentrations of each parameter in each group of animals was determined and compared using student's T-test. The pooled data (pre-partum and post-partum), age groups and calving period data was analysed by analysis of

variance, and correlations between erythrocyte cation concentrations and other blood components were found using the statistical program of Anderson (1981). The mean (± SEM) concentrations of the blood components used in the correlation analyses are shown in Table 9.

RESULTS

(a) Changes in EMg, ENa and EK concentrations 8 weeks before and 8 weeks after calving and with age in dairy cows

The mean EMg, ENa and EK concentrations 8 weeks before and 8 weeks after calving for the pooled data (herd II), for each age group and for each calving period are shown in Figures 2 to 6 and Table 10.

The correlations between ENa and EK concentrations are shown in Figure 5a, while the frequency distributions of ENa and EK concentrations are presented in Figures 5b and 5c. The means (± SEM) for EMg, ENa and EK concentrations for the different age groups are shown in Table 11 and Figure 6.

The mean EMg concentrations did not show any significant change before and after calving in the pooled data (Fig. 2a), however, they increased significantly ($P<0.05$) after calving from week 1 to week 5 in the older cows (>4 years old) (Fig. 3a). The overall mean concentrations for the young mature cows (≤4 years) was significantly ($P<0.05$) lower than that of the older cows (>4 years)(Fig. 3a). The overall mean EMg

Table 8: The overall mean concentrations (± SEM) of PMg, PNa, PK, blood Mg, Na and K, EMg, ENa, EK, PCV, Hb, RCC, MCV, MCH, and MCHC used in the correlation analysis (n = 416)

Variable	Mean ± SEM
Hb (g/l)	108 ± 1.7
PCV (l/l)	8.32 ± 0.004
PMg (mmol/l)	0.88 ± 0.02
PNa (mmol/l)	138.8 ± 0.64
PK (mmol/l)	4.40 ± 0.06
RCC ($1x10^{12}$cells/l)	6.28 ± 0.12
Whole blood Mg (mmol/l)	0.98 ± 0.02
Whole blood Na (mmol/l)	126.6 ± 1.42
Whole blood K (mmol/l)	14.0 ± 0.83
EMg (mmol/l)	1.16 ± 0.04
ENa (mmol/l)	104.0 ± 3.03
EK (mmol/l)	36.9 ± 3.00
*MCV (fl)	51.5 ± 0.92
*MCH (pg)	17.0 ± 0.31
* MCHC (%)	33.3 ± 0.33

* determined by standard methods (Schalm, Jain and Carrol 1975).

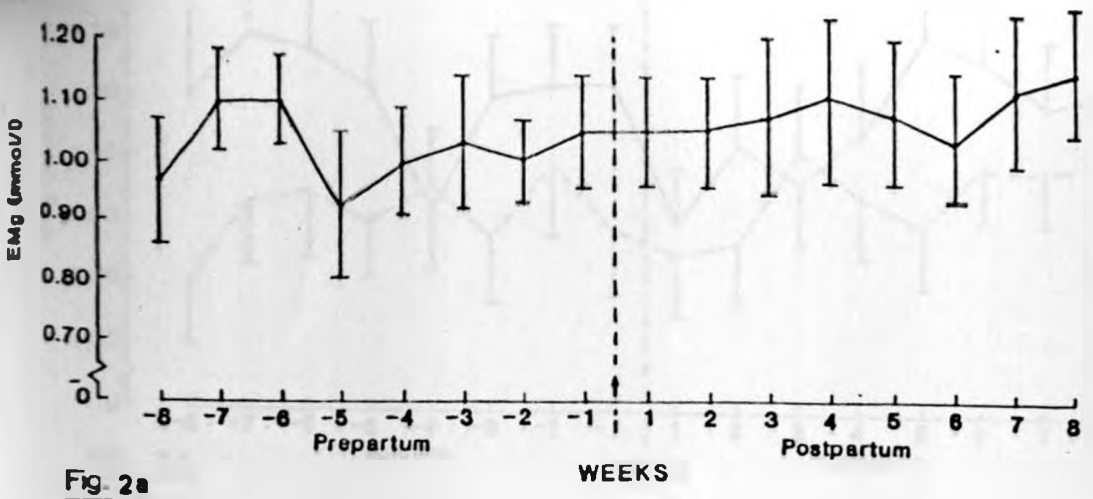

Fig. 2a

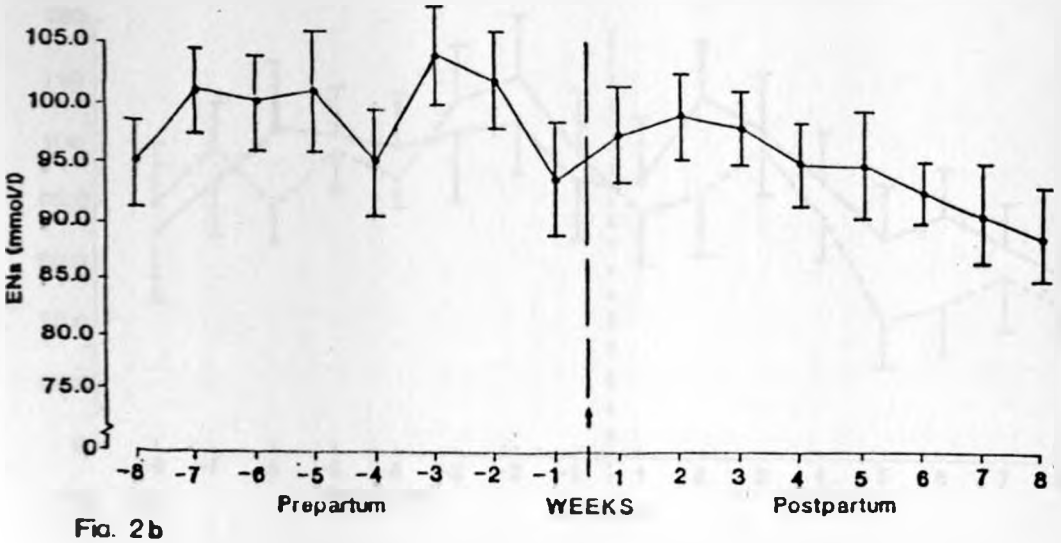

Fig. 2b

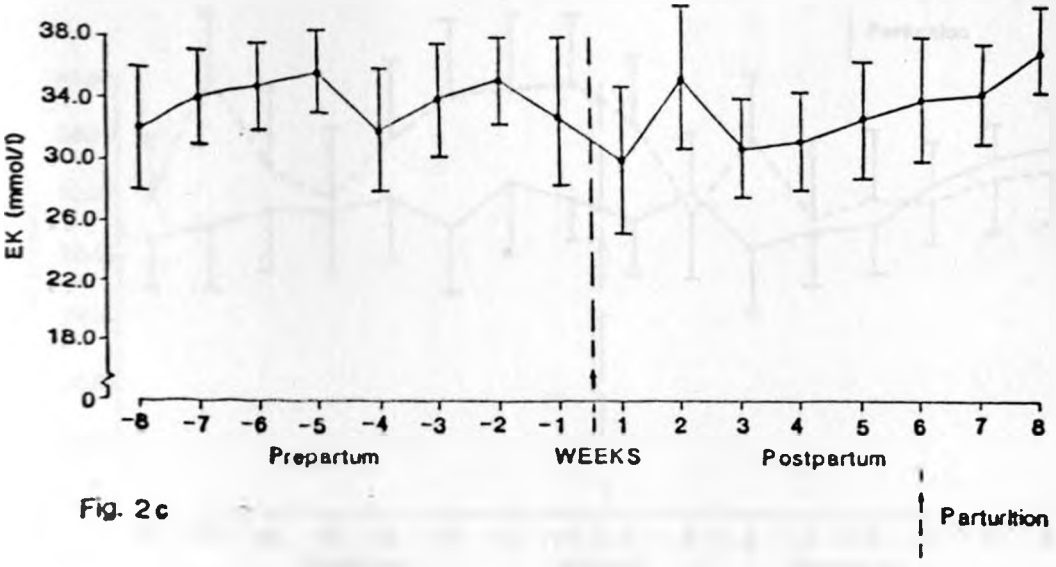

Fig. 2c

Figures 2a-2c. Changes in erythrocyte magnesium, sodium and potassium (mean ± SEM) values in dairy cows 8 weeks before and 8 weeks after calving (pooled data from cows in herd 11)

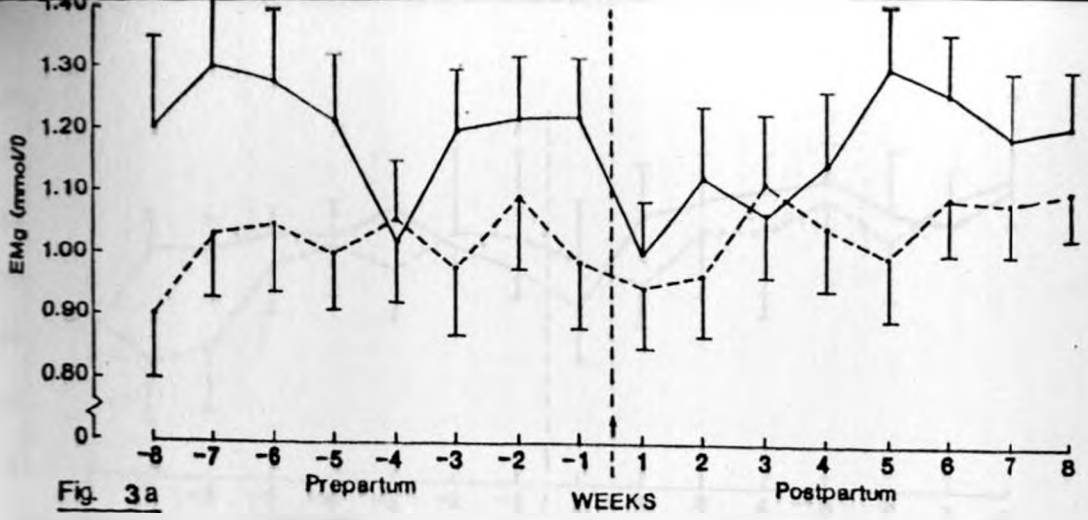

Fig. 3a

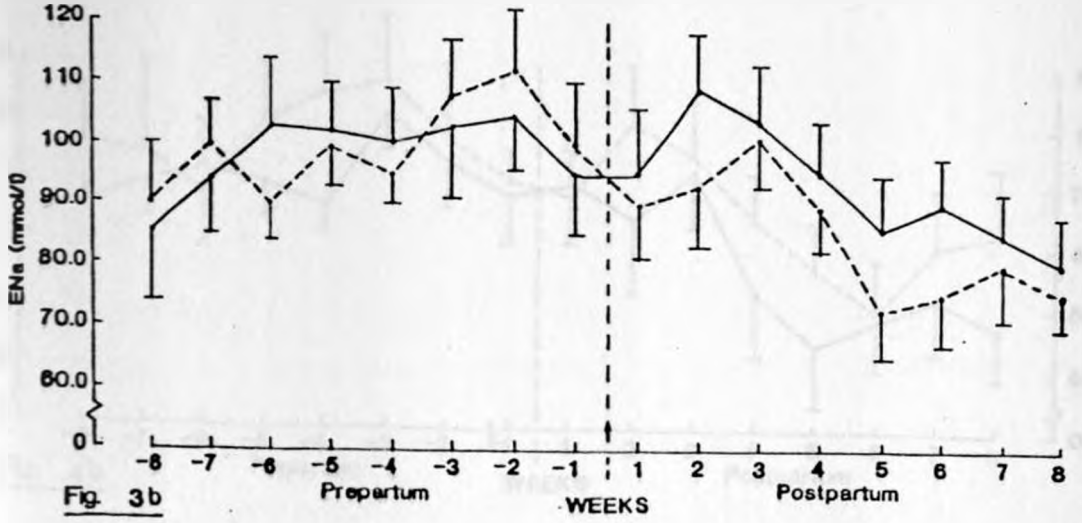

Fig. 3b

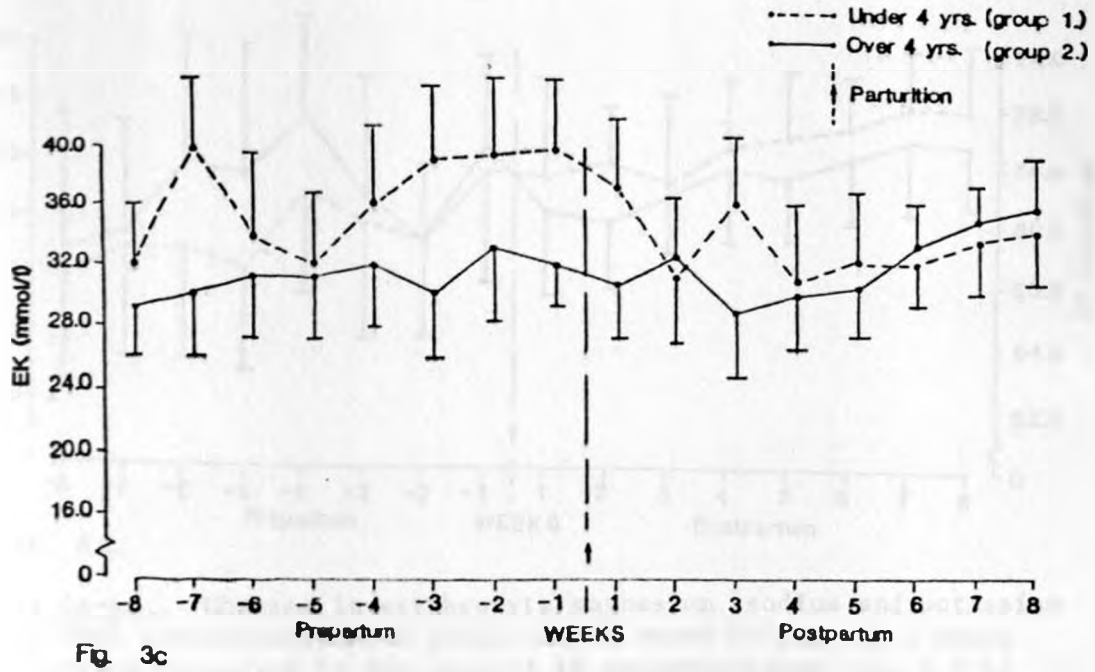

Fig. 3c

Figures 3a-3c. Changes in erythrocyte magnesium, sodium and potassium (mean ± SEM) concentrations in dairy cows 8 weeks before and 8 weeks after calving according to age groups (Herd 11)

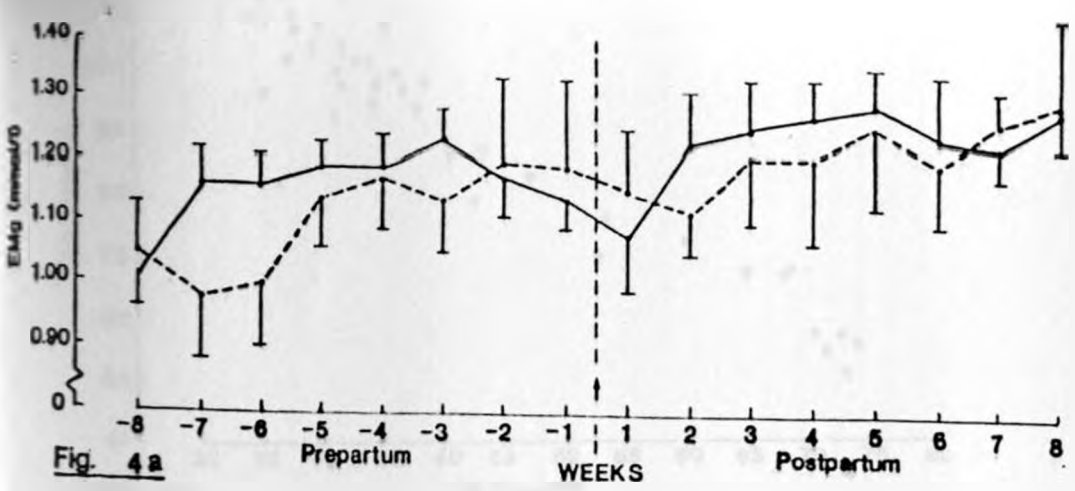

Fig. 4a

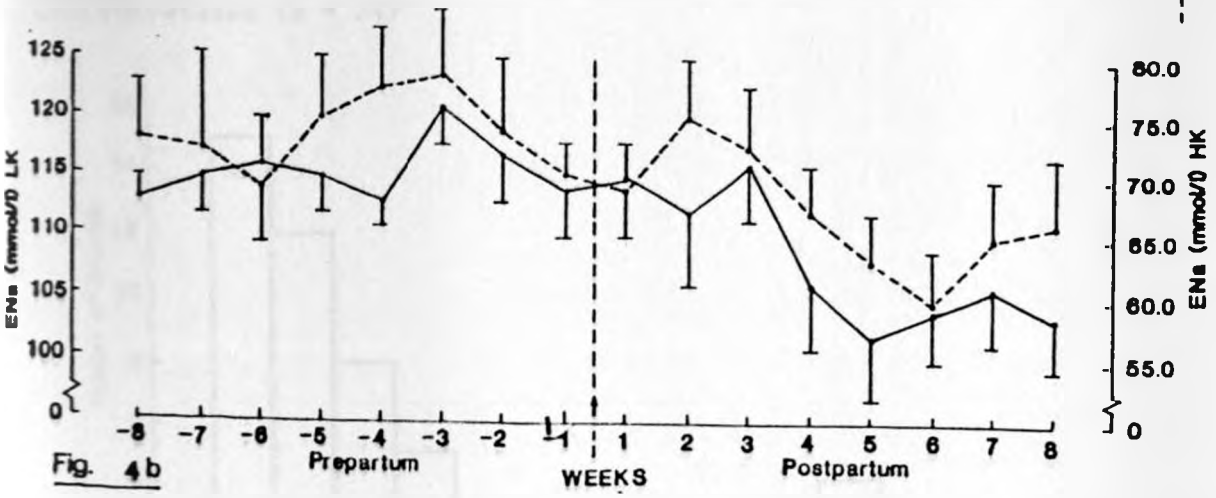

Fig. 4b

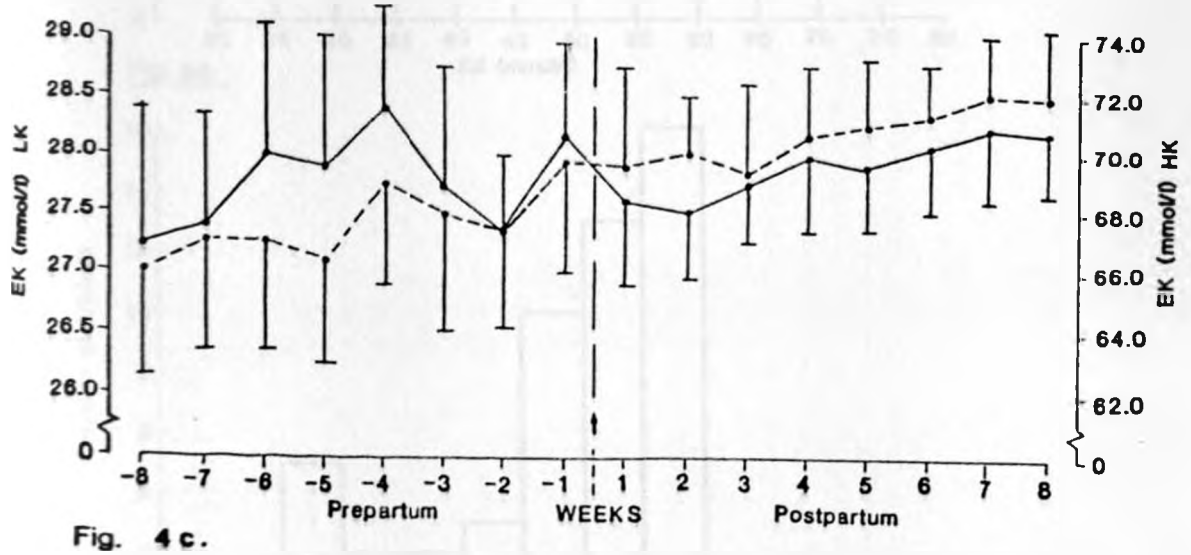

Fig. 4c.

Figures 4a-4c. Changes in erythrocyte magnesium, sodium and potassium (mean ± SEM) concentrations in dairy cows 8 weeks before and 8 weeks after calving according to the type of EK concentrations (LK, n = 43 and HK, n = 11)

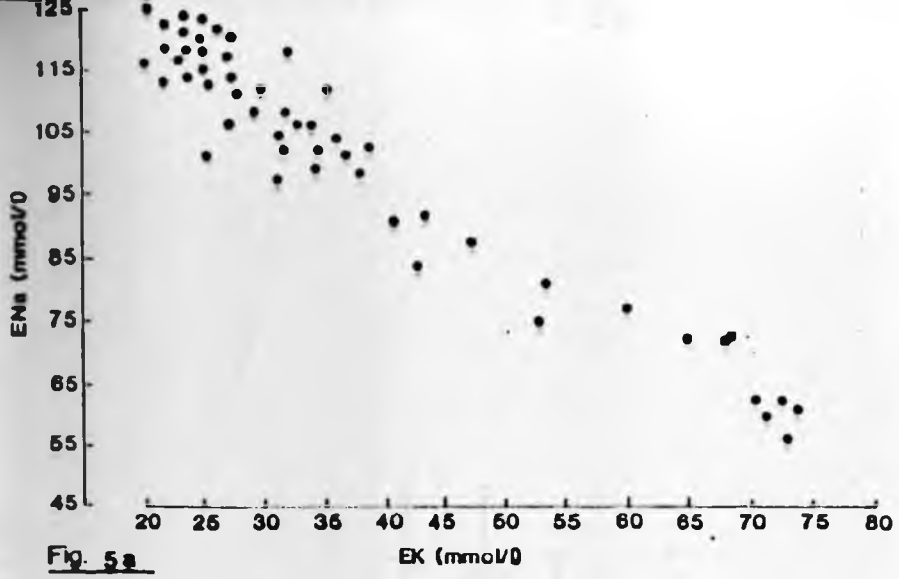

Fig. 5a

Figure 5a. The relationship between erythrocyte Na and K concentrations (n = 54)

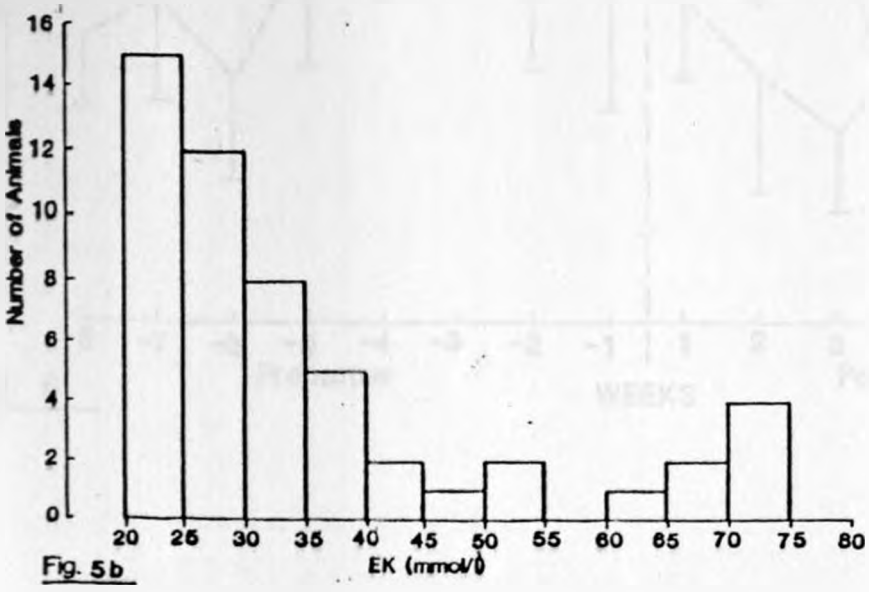

Fig. 5b

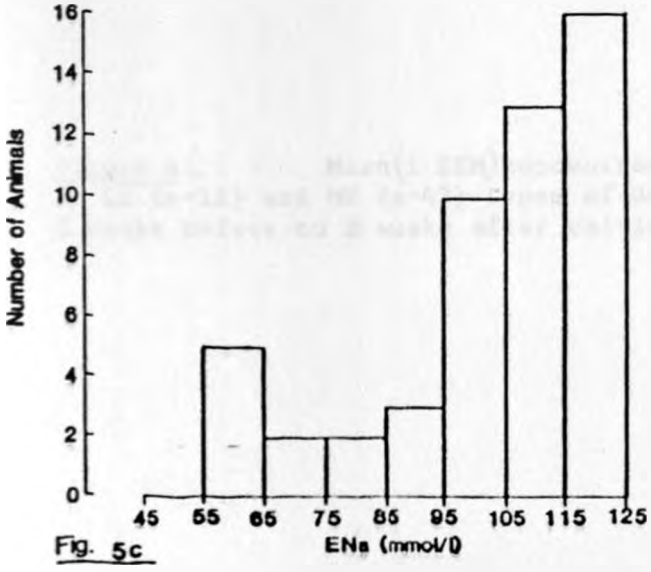

Fig. 5c

Figures 5b-5c. Frequency distribution of animals according to erythrocyte Na and K concentrations (n=54)

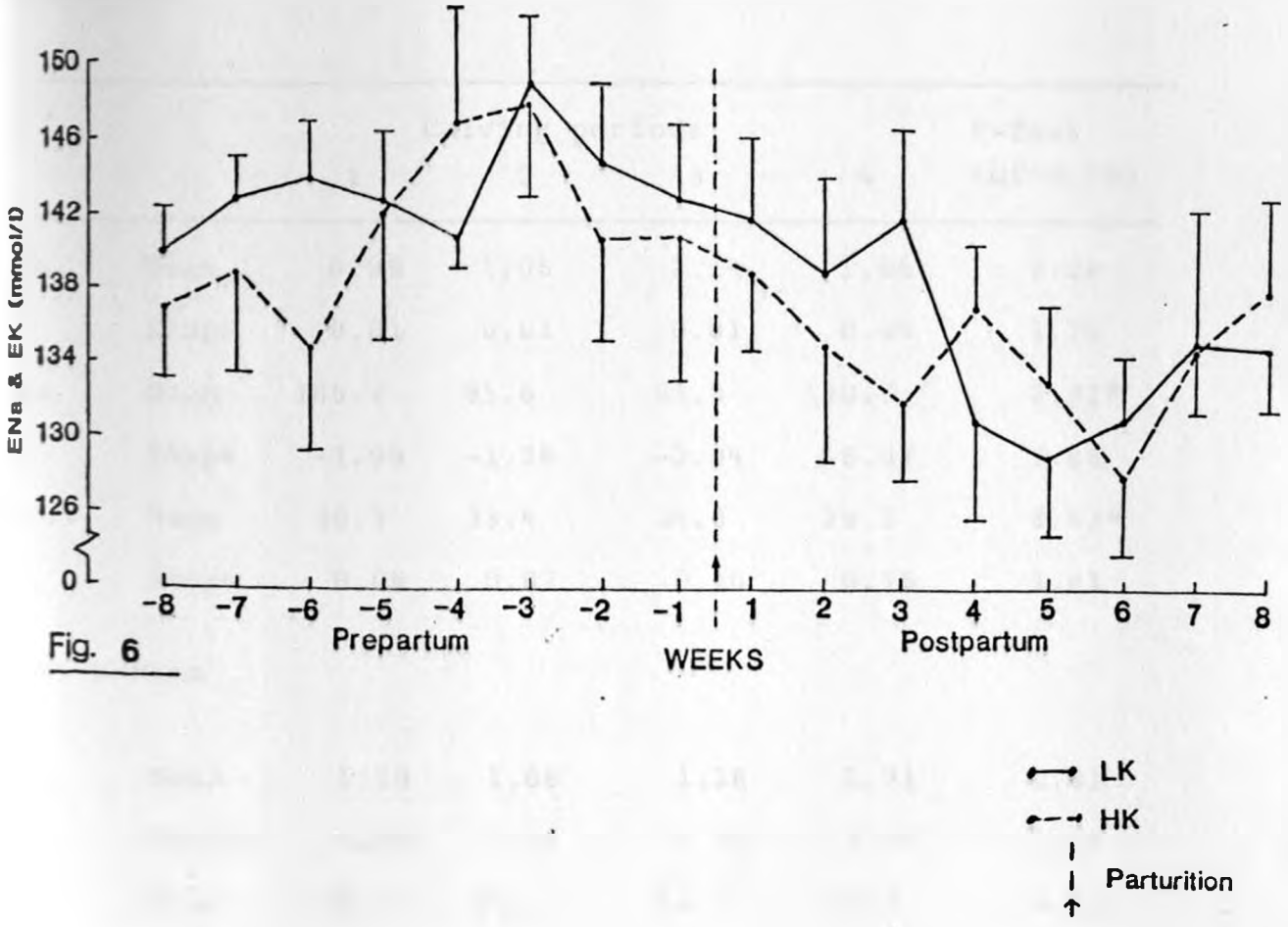

Fig. 6

Figure 6 . Mean (± SEM) concentration of ENa + EK (mmol/l) according to LK (n=11) and HK (n=43) types of dairy cows recorded weekly from 8 weeks before to 8 weeks after calving

Table 10: The mean concentrations (mmol/l) and slopes (on time) of EMg, ENa and EK according to calving periods 8 weeks before and 8 weeks after calving.

		Calving periods 1	2	3	4	F-Test (df=3,50)
Pre-partum						
EMg	Mean	0.99	1.05	1.14	1.06	2.28
	Slope	0.01	0.02	0.01	0.04	1.74
ENa	Mean	109.2	95.6	93.8	110.6	3.31*
	Slope	-1.99	-1.38	-3.04	-5.37	2.88
EK	Mean	30.3	33.4	34.8	29.1	3.53*
	Slope	0.06	0.07	0.10	0.15	1.81
Post-partum						
EMg	Mean	1.29	1.08	1.16	1.21	2.81
	Slope	0.09	0.05	0.04	0.06	1.44
ENa	Mean	88.4	87.7	86.9	83.9	0.54
	Slope	-10.6	-5.00	-1.43	-7.11	2.70
EK	Mean	34.5	35.5	36.4	34.8	1.38
	Slope	0.63	0.21	0.06	0.63	2.78

Table 11: The mean (± SEM) concentrations of EMg, ENa and EK in female dairy cattle of varying ages in Southern Queensland.

Age (Years)	Number of animals	EMg(mmol/l) Mean	ENa(mmol/l) Mean	EK(mmol/l Mean
1 week	11	1.70 ± 0.21	80.1 ± 8.5	115.8 ± 2.71
+(Dams	11	1.27 ± 0.11	105.0 ± 5.8	32.7 ± 2.58)
0.5	10	1.26 ± 0.11	96.8 ± 7.6	35.8 ± 2.20
1.0	13	1.22 ± 0.12	97.1 ± 7.2	34.2 ± 1.31
2.0	10	1.28 ± 0.11	96.2 ± 6.9	34.0 ± 0.54
3.0	10	1.05 ± 0.11	102.7 ± 7.0	33.1 ± 1.30
4.0	12	1.01 ± 0.12	107.0 ± 5.8	33.7 ± 2.02
5.0	13	1.30 ± 0.09	108.8 ± 5.2	32.0 ± 2.00
6.0	12	1.26 ± 0.13	105.8 ± 5.9	32.3 ± 2.45
7.0	10	1.40 ± 0.15	103.4 ± 6.3	32.9 ± 2.14
>8.0	10	1.24 ± 0.10	101.1 ± 6.0	30.0 ± 200
F-Test on differences between means.		6.82**	5.91**	6.08**

+ Values for the dams of the 1 week-old calves.

concentrations and the slopes did not show any significant differences between calving periods (Table 10). The mean EMg concentrations differed significantly ($P<0.01$) between different age groups (ranging from 1 week old to over 8 years)(Table 11). The one-week-old calves had higher ($P<0.01$) EMg concentrations than their dams and the other groups. The young cows (3-4 years old) had the lowest ($P<0.05$) mean EMg concentrations. There were significant ($P<0.01$) differences between whole blood Mg (BMg), PMg and EMg concentrations (Table 12).

The Mg concentrations were higher in the erythrocytes than in the plasma while BMg levels were intermediate. The EMg levels were significantly correlated with RCC ($r=-0.618^{**}$) and with BMg (as was expected) ($r=0.851^{**}$) (Table 13).

The mean ENa concentrations showed significant ($P<0.05$) weekly variation and between cow variation ($P<0.01$) in both the pre-partum and post-partum periods (Table 14). They showed no pattern in any group before calving and decreased significantly ($P<0.05$) in each group after calving (Figs. 2b, 3b, 4b). The pre-partum mean ENa concentrations differed significantly ($P<0.05$) between calving periods, they were higher in calving periods 1 and 4 than in calving periods 2 and 3 (Table 10). The pre-partum and post-partum slopes did not differ significantly between the calving periods (Table 10). The mean ENa concentrations differed significantly ($P<0.01$) between the age groups and were lowest in the young calves (1 week old) (Table 11). There were significant ($P<0.01$) differences between PNa,

Table 12: Whole blood, plasma and erythrocyte concentrations of Mg, Na and K (mmol/l) in dairy cows in Southern Queensland (n=62).

	Whole blood Mean ± SEM	Plasma Mean ± SEM	Erythrocyte Mean ± SEM	F-Test
Mg	0.98 ± 0.02	0.88 ± 0.02	1.16 ± 0.04	30.6***
Na	126.6 ± 1.42	138.8 ± 0.64	105.0 ± 3.03	75.8***
K	14.0 ± 0.83	4.40 ± 0.06	32.9 ± 3.00	81.4***

Table 13. *Correlation coefficients between plasma, blood and erythrocyte concentrations of Mg, Na and K, PCV, Hb, RCC, MCV, MCH and MCHC in dairy cows (n=52)*

	PCV	PMg	PNa	PK	RCC	BMg	EMg	ENa	EK	MCV	MCH	MCHC	BNa	BK
Hb	0.802***	0 113	-0.105	0.106	0 272	-0.196	-0 277	0 294	-0.396*	0 232	0 533**	0 555**	-0 157	-0 009
PCV	-	-0 389	-0 241	0 193	0 145	-0 195	-0 292	0 280	-0 464**	0 464**	0 442*	0 001	-0 152	-0 192
PMg	-	-	0 072	0.121	0 078	0 376*	0.254	-0 203	0.292	-0 170	-0 007	0 292*	-0.106	0 214
PNa	-	-	-	0 037	-0 394*	0 059	-0 249	-0 127	0 055	0 166	0 221	0 003	0 168	0 191
PK	-	-	-	-	0 051	0 210	0 093	-0.396**	0 242	0 005	-0 005	-0.147	-0.231*	0 140
RCC	-	-	-	-	-	-0 222	-0 618**	0 350*	-0 388*	-0 766***	-0 587**	0 308	-0 298*	0 102
BMg	-	-	-	-	-	-	0 651**	0.083	0 217	0 003	-0 114	-0 321	0 006	-0 002
EMg	-	-	-	-	-	-	-	0 009	0 088	0 350*	-0 144	-0 255*	-0 007	-0 001
ENa	-	-	-	-	-	-	-	-	-0.844***	-0 345*	-0 468*	-0 380*	0.423*	-0 618**
EK	-	-	-	-	-	-	-	-	-	0 543**	0 561**	0 372*	-0 375*	0 594**
MCV	-	-	-	-	-	-	-	-	-	-	0.829***	-0 248*	0 172	-0 230
MCH	-	-	-	-	-	-	-	-	-	-	-	0 297*	0 152	-0 154
MCHC	-	-	-	-	-	-	-	-	-	-	-	-	0.007	0 008
BNa	-	-	-	-	-	-	-	-	-	-	-	-	-	-0 658**

Table 14: Mean squares (M.S) from the analysis of variance of pre- and post-partum ENa and EK concentrations in dairy cows in Southern Queensland (n = 54).

	Weekly concentration M.S.	Cow M.S.	Error M.S.
Pre-partum			
ENa (mmol/l)	141.2*	1617.1**	46.4
EK (mmol/l)	1.17	1695.1**	26.1
Post-partum			
ENa (mmol/l)	133.5*	638.3**	43.9
EK (mmol/l)	117.0*	1547.9**	38.5

blood Na (BNa) and ENa values (Table 12), with that in plasma being higher than that in erythrocytes. Correlations between ENa and other parameters are shown in Table 13.

The mean EK concentration showed significant ($P<0.01$) between cow variation in both pre-partum and post-partum periods and the weekly variations were significant ($P<0.05$) only in the post-partum period (Table 14). The mean EK concentrations did not show any significant change before calving, however they increased after calving ($P<0.06$) (from week 1 to week 8) (Figs. 2c, 3c, 4c). The pre-partum mean EK concentrations also showed significant ($P<0.05$) differences between calving periods, they were higher in calving periods 2 and 3 than in calving periods 1 and 4 (Table 10). The pre-partum and post-partum slopes did not differ significantly between the calving periods (Table 10). The mean concentrations of EK in different age groups differed significantly ($P<0.01$) with the young calves (1 week old) having the highest mean levels (Table 11). There were significant differences ($P<0.01$) between blood K (BK), PK and EK levels; they were lower in plasma than in erythrocytes (Table 12). The correlations between EK and other blood parameters are shown in Table 13.

(b) Frequency distribution of animals according to ENa and EK concentrations

ENa and EK concentrations have been plotted against each

other (Fig. 5a) with each point representing one animal. Two groups of animals are evident from the figure, i.e. animals with low (LK) and animals with high EK (HK) concentrations. The animals with low EK concentrations had significantly ($P<0.05$) higher ENa concentrations than the animals with high EK concentrations, and the effect of this was that the total ENa + EK concentrations were not significantly different between the LK and HK animals (Fig. 6).

The frequency distribution for ENa and EK concentrations are shown separately (Fig. 5b and 5c) and both show wide ranges. The values for ENa and EK varied between 55.0 and 125.0 mmol/l cells and 20.0 and 75.0 mmol/l cells respectively. The EK distribution shows a clustering at 20-25 mmol/l and a smaller peak at 70-75 mmol/l, while ENa distribution shows a small peak at 55-65 mmol/l and a larger peak at 115-125 mmol/l.

The range of values over the eight week pre-partum period was 0.50 to 2.20 mmol/l for EMg concentrations, 52.7 to 135.0 mmol/l for ENa concentrations and 19.6 to 84.2 mmol/l for EK concentrations. For the eight week post-partum period the erythrocyte values were EMg=0.60 - 2.33 mmol/l, ENa=45.7 - 70.2 mmol/l and EK = 18.9 - 72.0 mmol/l.

DISCUSSION

The results of this study show that EMg concentrations were usually higher than PMg levels (Table 12) as previously

reported in cattle (Green and MaCaskill 1928, Wise et al. (1948). However, it differed from the findings of Greenberg et al. (1933), Eveleth (1937) and Salt (1950) who reported the reverse. It has been suggested that greater proportions of reticulocytes and young erythrocytes (which contain higher EMg levels) and old erythrocytes could be responsible for the discrepancies in the reported normal EMg levels in both man and animals (Seelig 1980). The differences could also be partly due to differences in analytical techniques, the earlier techniques involved many large dilutions which could have resulted in a summation of errors. In the present study only one dilution (i.e. 0.39 dilution factor) was used and hence there was less chance of dilution errors.

It has been reported that EMg and PMg concentrations are independent of each other i.e. Mg ions do not exchange across the erythrocyte membrane (Greenberg, Lucia, Mackey and Tufts 1933; Salt 1950). Salt (1959) concluded that cellular Mg concentration once determined remained the same until the cell died. However, Bernstein (1959) and Ginsberg et al. (1962) found that EMg concentrations decreased with the age of the erythrocytes, and that reticulocytes and young erythrocytes had higher EMg concentrations than older erythrocytes.

The decrease in EMg concentrations in older cows (<4 years old) from 8 weeks to calving (Fig. 3a) could have been due to the formation of erythrocytes with lower EMg values during

the period of lower PMg concentrations observed before calving (Fig. 1g). Erythrocytes formed during such periods have been shown to have lower EMg levels (Tufts and Greenberg 1937, Salt 1950, Elin et al. 1971). The concentrations of EMg and PMg were found to be significantly lower in normal pregnant women in the last trimester of pregnancy than in normal non-pregnant women (Lim et al. 1969). These workers suggested that this could have been due to a marginal Mg deficiency, especially due to an increased Mg demand made by the foetus, at this stage of pregnancy.

The increase in EMg concentrations after calving (Fig. 3a) could have occurred partly in association with an increase in PMg levels and increased production of reticulocytes and young erythrocytes as a result of decreased PCV, Hb and RCC levels (Figs. 1a, 1b, and 1q). Decreases in PCV, Hb and RCC levels (erythrocyte pool) would lead to production of reticulocytes and young erythrocytes with higher EMg concentrations if PMg is adequate (Bernstein 1959, Ginsburg et al. 1962). The older cows (>4 years) had a greater decrease in the erythrocyte pool than the young cows (Fig. A1a, A1b, A11). This could have led to a higher production of reticulocytes and young erythrocytes thus accounting for the greater increase in EMg concentrations after calving, and possibly contributed to the higher overall EMg concentration in older cows (> 4 years) compared to the young cows ($\leq$ 4 years) (Fig. 3a). This contention may be supported by the observation that EMg concentration was negatively correlated with RCC (r=-0.618)

(Table 13) i.e. cows with low RCC's (hence greater stimulation for erythrocyte production) had higher EMg concentration than those with high RCC's. The positive correlation between EMg and MCV (Table 13) also suggests that the increase in EMg concentration after calving could have been due to an increased number of circulating reticulocytes and young erythrocytes which have higher MCV's (Elin et al. 1971).

The higher EMg concentration in young calves (1 week old) (Table 11) than in the other age groups suggests that young calves have either higher numbers of circulating reticulocytes and young erythrocytes than older cows or foetal erythrocytes contain higher EMg levels than adult erythrocytes. Young calves contain HbF which is replaced by HbA (the more common adult type) at 2-3 months of age (Grimes, Duncan and Lassiter 1958). Therefore, since most of the EMg is associated with Hb (Rose 1968) it could be that HbF of young calves has a higher affinity for Mg than adult Hb hence resulting in higher Mg levels in foetal erythrocytes. It could also be that foetal erythrocytes have a higher metabolic rate than adult erythrocytes which would enable them to maintain higher EMg concentrations than adult erythrocytes (Bernstein 1959).

The range of ENa values (55.0 - 125.0 mmol/l) and of EK (20.0 - 75.0 mmol/l) were similar to those reported by Christnaz and Schatamann (1972) in thirty-three breeds of cattle.

The pre-partum and post-partum ENa concentrations varied significantly between weeks ($P<0.05$) and cows ($P<0.01$) (Table 14). The variations in ENa concentrations from week to week have also been reported previously in cattle (Evans and Phillipson 1957, Christinaz and Schatamann 1972) and in sheep (Koch and Turner 1961). These weekly variations in ENa concentrations are difficult to explain. The decrease in ENa and the increase in EK concentrations after calving could have been due to increased production of reticulocytes and young erythrocytes (as discussed above) which contain low ENa and high EK levels (Evans and Blunt 1963, Timms and Murphy 1980, Albers and Jalambre 1983). The differences in proportions of reticulocytes, young erythrocytes and older erythrocytes in the circulation (which could have occurred due to the significant differences in mean PCV's and Hb mean concentrations observed between the calving periods -Table 2 and 4) may also be responsible for the differences observed between the mean concentrations and slopes of ENa and EK in each calving period (Table 10). The negative and positive correlations between MCV, MCH and MCHC and ENa and EK concentrations respectively observed (Table 13) and also reported by Elin et al. (1971) supports this contention.

The observation of lower ENa concentrations in the young calves (1 week old) compared to older cows was similar to the reports of Blechner (1960, 1961) in sheep and goats. The erythrocytes of young calves contain low ENa levels and high EK levels a reverse situation to that in adult cows (Ellory and Tucker 1970) i.e. ENa and EK levels are negatively

correlated (r= -0.84**) (Table 13). The positive relationship between ENa and RCC levels probably indicates that cows with high RCC's do not have high rates of erythrocyte production and hence have smaller numbers of circulating reticulocytes and young erythrocytes.

The pre-partum and post-partum EK concentrations showed significant ($P<0.01$) between cow variation and the weekly variations were significant only in the post-partum period (Table 14). These results show that EK concentrations vary between cows and they do not show any significant weekly variation in any individual cow during the pre-partum period. Similar findings were reported by Evans (1963a) in ewes, he found that EK concentrations varied significantly between lactating ewes and non significantly in dry ones. The between cow differences in EK concentrations may be due to the inheritance of factors that control the actual EK concentrations in any animal (Evans and Mounib 1957) (see introduction). The significant ($P<0.05$) weekly variation in post-partum EK concentrations suggests that their increase after calving may vary from week to week depending on the rate of production of reticulocytes and/or young erythrocytes (see EMg discussion).

EK concentrations were higher in calves (1 week old) than in the other age groups. The same observation was made by Wise *et al.* (1948) and Rasmussen *et al.* (1974). These workers also found that the mean adult EK concentrations were not reached until approximately four months after birth.

Similar results were reported in goats and sheep by Blenchner (1960, 1961). Erythrocytes of sheep have a L antigen on their membrane which controls active K transport into the cells (Ellory and Tucker 1969). This L antigen is poorly developed at birth and its activity increases with increasing age (Tucker and Ellory 1970). Since this L antigen acts as an inhibitor of active K transport and is not fully developed in young animals (Tucker and Ellory 1970) it might help to explain why young animals have higher EK concentrations than older animals. Ellory and Tucker (1970) suggested that the mechanism that controls erythrocyte K concentration in cattle could be similar to that in sheep.

There were two peaks in the distribution of individual animal EK and ENa concentrations, those with low EK concentrations had higher ENa concentrations and vice versa (Fig. 5a). The total ENa and EK concentrations in both HK and LK types of animals were not different and similar observations have been reported (Ellory and Tucker 1970, Christinaz and Schatamann 1972).

The inheritance of EK type in cattle is simple, and similar to that in sheep, the gene for LK being dominant to that of the HK type (Evans and Mounib 1957). In sheep the heterozygous LK red cells have significantly higher EK levels than the homozygous LK cells (Evans et al. 1956). It has been suggested that in cattle there is a pair of codominant alleles controlling the overall EK concentrations, in that homozygous

low cells have low EK concentrations and homozygous high cells have high EK values (Rasmussen et al. (1974). Thus according to this hypothesis it seems possible that the higher values in LK groups of animals represented the heterozygous type.

The results of this study show that EMg and EK concentrations can increase and those of ENa decrease after calving in dairy cows. The results also show that EMg and EK levels are higher and ENa levels lower in young calves (1 week old) than their dams and the other cows (0.5 to > 8 years old).

Since mean concentrations of EMg, ENa and EK may reflect situations in which there is a depression of the erythrocyte pool (and could also be related to the cows ability to adapt to that depression - Evans 1963a), they should be included in metabolic profile testing of dairy cattle and their relationship with production parameters would also be worthwhile investigating. The potential use of EMg to determine long term latent Mg deficiency in cattle should also be investigated and examined to determine if it has advantages over PMg estimations.

CHAPTER 5

THE RELATIONSHIP BETWEEN BLOOD COMPOSITION CHANGES IN LATE PREGNANCY (EIGHT WEEKS PRE-PARTUM) AND EARLY LACTATION (EIGHT WEEKS POST-PARTUM) AND REPRODUCTIVE PERFORMANCE AND MILK PRODUCTION IN DAIRY COWS

INTRODUCTION

In the absence of obvious organic irregularities in cows (Delange 1950) farmers face the problem of how to recognise functional infertility early i.e. before the projected management system is thrown out of phase (Hewett 1974). This is especially important if the replacement cow requirements and production schedules are to be met. Heavy emphasis is placed on the detection of irregularities of oestrus (Failure of cows to cycle, failure to show oestrus) and multiple services/conception which results in uneconomically long calving to conception, and calving to calving intervals (Amiel 1970).

The basic causes of reproductive or productive problems in a herd are not always apparent and herd management, nutritional and pathological factors can be involved. Blood profiles might be a potential aid in predicting these problems and could also be of value in diagnosing mineral deficiencies or other conditions that may affect both fertility and milk production. However, blood analyses have not demonstrated a consistent relationship to fertility (Parker and Blowey 1976, Lee *et al.* 1978, Rowlands 1980) nor has providing supplemental minerals always been reflected in higher concentrations of the particular mineral in

the blood (Adams et al. 1978, Manston and Allen 1981). In a study of 15 commercial dairy herds, blood components showed no consistent relationship to fertility (Parker and Blowey 1976). In another study of cows from 21 herds, concentrations of plasma albumin, PK and PCV's were inversely related while the concentration of plasma globulin was directly related to the number of services per conception (Rowlands 1980, Rowlands et al. 1980). Thus additional studies are needed to determine the value of blood analysis in defining the fertility and production status of cows in dairy herds. The objective of this study was to assess the changes in blood components that occur in late pregnancy and early lactation and the relationships of the changes and mean values to reproductive and production performance of dairy cows.

MATERIALS AND METHODS

The number and management of the animals, blood components measured and methods of analyses have been described earlier in the section on general materials and methods. The production indices studied were (Table 15 shows their means);

(a) Number of services/conception

(b) Interval from calving to conception or days open

(c) Milk production rank (described in the section of general materials and methods) was used as an index of productive performance.

Table 15 Mean (± SEM) of the production indices studied.

	Herd I (n=23)	Herd II (n=62)
Services per conception	3.00 ± 0.34	1.97 ± 0.19
Days open	139.1 ± 10.6	91.6 ± 4.9
Milk production rank	110.2 ± 2.54	117.1 ± 4.44

Statistical analyses

The blood component data (pooled) was arranged into 4 groups as follows:

(i) Means and (ii) "Slopes" for the period eight weeks pre-partum. (iii) Means and (iv) "Slopes" for the period eight weeks post-partum ("Slope" is the regression coefficient for the respective variable regressed on time in weeks.)

These values plus the age variable gave a combination of 81 variables that could be used in the prediction of (a) services/conception (b) days open and (c) production rank. The data from each of the two herds used in this study was analysed separately to eliminate herd effects.

Multiple regressions

Initially all the variables were correlated with the three dependent variables (services/conception, days open and production rank) and the individual independent variables that were significantly correlated ($P<0.05$) with the dependent variables were used in selecting the best multivariate model. For the most part, this was a step-wise procedure, but occasionally ad hoc variables (which approached significance - $P<0.1>0.05$) were included because of the suspected value to the equation. Usually the selection criterion was that any model that reduced the error(or residual) mean squares was accepted as a better model than the previous one. This method was used to select the best combination of the independent variables (usually 5 or 6) for the pre-partum and post-partum periods separately

and for both periods combined which could be used for predicting fertility and production rank. These were then refined further by looking for those combinations that resulted in the lowest standard error of the estimate (from the residual M.S.). This method took into account both (i) the different number of predictors and (ii) the different number of missing values present in the alternative models.

RESULTS

(a) Correlations between blood components and services/ conception, days open and production rank

The blood components which had significant ($P<0.05$ or $P<0.01$) correlations with services/conception, days open and production rank are shown in Tables 16 and 17 and their mean concentrations and slopes on time are shown in Tables 18 and 19.

(b) Multiple linear regression analyses of the effects of blood composition on fertility indices and production rank

These were carried out to relate fertility indices (services/ conception and days open) and production rank with blood components (means and slopes) to assess whether blood components measured in late pregnancy and/or early lactation could be used to predict fertility and production. The separate regression analyses for the pre-partum and post-partum periods were carried out to find out if changes or mean concentrations of blood

Table 16 Significant simple correlation coefficients (r) between pre-partum blood components (individual means "$\bar{x}$" and slopes "b")+ and services/conception, days open and production rank

Herd I (n=23)		Herd II (n=62)	
Variable	r	Variable	r
(a) Services/conception			
Albumin (b)	0.436*	Albumin ($\bar{x}$)	-0.686**
WCC (b)	-0.780**	Albumin (b)	0.335*
Globulin (b)	-0.614**	Globulin (b)	-0.420*
		Pi (b)	-0.333*
(b) Days open			
Albumin (b)	0.410*	Albumin ($\bar{x}$)	-0.551**
WCC (b)	-0.679*	Globulin (b)	-0.506**
Globulin (b)	-0.585**	Pi (b)	-0.436**
(c) Production rank			
Glucose (b)	-0.417*	EMg ($\bar{x}$)	0.533**
Albumin ($\bar{x}$)	-0.617**	EK ($\bar{x}$)	-0.424*
EMg ($\bar{x}$)	-0.617**	RCC ($\bar{x}$)	-0.500*
RCC ($\bar{x}$)	-0.513*	Hb ($\bar{x}$)	-0.375*

\+ Individual means are the mean of the eight weekly observations for each cow and the slopes are the individual cows slopes on time.

* $P<0.05$

** $P<0.01$

Table 17 Significant simple correlation coefficients (r) between post-partum blood components (individual means "$\bar{x}$" and mean slopes "b")+ and services/conception, days open and production rank

Herd I (n=23)		Herd II (n=62)	
Variable	r	Variable	r
(a) Services/conception			
Hb ($\bar{x}$)	-0.776**	Hb($\bar{x}$)	-0.597**
Glucose ($\bar{x}$)	-0.887**	Glucose ($\bar{x}$)	-0.681**
Globulin ($\bar{x}$)	0.447*	Albumin ($\bar{x}$)	-0.421*
Ca ($\bar{x}$)	-0.519*	Globulin ($\bar{x}$)	0.730**
Ca (b)	0.534*	Ca ($\bar{x}$)	-0.408*
RCC (b)	0.638**	EMg ($\bar{x}$)	-0.349*
(b) Days open			
Hb ($\bar{x}$)	-0.665**	Hb ($\bar{x}$)	-0.548**
Glucose ($\bar{x}$)	-0.667**	Glucose ($\bar{x}$)	-0.584**
Globulin ($\bar{x}$)	0.480*	Albumin (b)	0.332*
Ca ($\bar{x}$)	-0.440*	Globulin ($\bar{x}$)	0.666**
RCC (b)	0.610**	Ca ($\bar{x}$)	-0.576**
		EMg ($\bar{x}$)	-0.458**
(c) Production rank			
EK ($\bar{x}$)	-0.531*	Hb ($\bar{x}$)	-0.447**
WCC (b)	0.465*	EK ($\bar{x}$)	-0.690**
EMg ($\bar{x}$)	0.830**	B.wt (b)	0.413*

+ See footnote Table 16

Table 18 The overall pre-partum means ($\bar{x}$)* and mean slopes (b)+ of blood components which gave significant ($P<0.05$) correlation coefficients (r) with services/conception, days open and production rank

Blood component	Herd I (n=23) Mean ± SEM	Herd II (n=62) Mean ± SEM
Albumin (b)	-0.08 ± 0.02	-0.04 ± 0.01
WCC (b)	-0.26 ± 0.33	- -
Globulin (b)	-0.15 ± 0.03	-0.14 ± 0.03
Glucose (b)	0.008 ± 0.02	- -
Albumin ($\bar{x}$)(g/l)	37.0 ± 0.70	35.1 ± 0.60
EMg ($\bar{x}$)(mmol/l)	1.17 ± 0.19	1.10 ± 0.02
RCC($\bar{x}$)(x10^{12}cells/l)	6.23 ± 0.15	6.55 ± 0.10
Pi (b)	-	-0.02 ± 0.01
EK($\bar{x}$)(mmol/l)	26.1 ± 1.27	28.4 ± 0.31
Hb ($\bar{x}$) (g/l)	-	106 ± 1.3

* Mean of all the cows observations pooled

\+ Mean of all the individual cow slopes

Table 19 The overall post-partum means ($\bar{x}$)' and mean slopes (b)' of blood components which gave significant ($P<0.05$) correlation coefficients (r) with services/conception, days open and production rank

Blood component	Herd I (n=23) Mean ± SEM	Herd II (n=62) Mean ± SEM
Hb ($\bar{x}$) (g/l)	95 ± 2.7	94 ± 0.7
Glucose ($\bar{x}$)(mmol/l)	2.99 ± 0.06	2.82 ± 0.03
Globulin ($\bar{x}$) (g/l)	37.3 ± 0.12	36.7 ± 0.03
Ca ($\bar{x}$) (mmol/l)	2.26 ± 0.05	2.21 ± 0.03
Ca (b)	-0.04 ± 0.10	-
RCC (b)	-0.20 ± 0.02	-0.09 ± 0.03
EK ($\bar{x}$) (mmol/l)	29.2 ± 1.27	30.3 ± 0.04
WCC (b)	-0.05 ± 0.33	1.17 ± 0.01
EMg ($\bar{x}$) (mmol/l)	1.24 ± 0.19	1.17 ± 0.01
Albumin ($\bar{x}$) (g/l)	-	37.3 ± 0.06
Albumin (b)	-	0.03 ± 0.01

*, + See footnote Table 18

components for each period could be used to predict fertility and production rank in dairy cows. The pre-partum relationships would be of greater importance since they would alert the farmer to correct the potential problem before it was manifested in either low fertility or poor milk production.

Attached to each multiple regression equation are multiple coefficients of determination (R^2), multiple correlation coefficients (R), standard errors of estimate (SEE) and the standard errors of the partial regression coefficients (SEB) for each independent variable, "Student's t" values for the partial regression coefficients (T values), and F values for the multiple regression (F value) are also shown. The standardized regression coefficients (B) for each regression equation are shown in Table 20. The B values provide the best way of comparing the relative effect on the dependent variable of each independent variable in the multiple regression equation when the latter are measured in different units (Nie et al. 1975).

(a) Services/conception

(1) Pre-partum independent variables

The best equation for the pre-partum independent variables found for predicting services/conception was;

Table 20 The standardized regression coefficients (B) for the multiple regression equations (for predicting fertility and production rank in dairy cows).

(c) Services/conception

(i) Pre-partum independent variables

Herd	Variable	B	Regression Equation
I	Globulin (b)	-0.643	(1)
	WCC (b)	-0.332	
II	Albumin ($\bar{x}$)	-0.583	(2)
	Albumin (b)	0.335	
	Globulin (b)	0.300	

(ii) Post-partum independent variables

I	Glucose ($\bar{x}$)	-0.538	(3)
	Globulin ($\bar{x}$)	0.295	
	RCC (b)	-0.240	
II	Glucose ($\bar{x}$)	-0.384	(4)
	Albumin ($\bar{x}$)	-0.251	
	Globulin ($\bar{x}$)	0.484	

(iii) Combined pre- and post-partum independent variables

I	b Glucose ($\bar{x}$)	-0.626	(5)
	a Globulin ($\bar{x}$)	-0.135	
	b Globulin ($\bar{x}$)	0.247	
II	b Glucose (x)	-0.251	(6)
	b Albumin (b)	0.319	
	a Globulin (b)	-0.264	
	b Globulin ($\bar{x}$)	0.547	

Table 20 continued

(b)	Days open		
(i)	Pre-partum independent variables		
II	Albumin ($\bar{x}$)	-0.446	(7)
	Globulin (b)	-0.383	
(ii)	Post-partum independent variables		
I	Globulin ($\bar{x}$)	0.502	(8)
	RCC (b)	-0.400	
II	Glucose ($\bar{x}$)	-0.226	(9)
	Albumin (b)	-0.188	
	Globulin ($\bar{x}$)	0.436	
	Ca ($\bar{x}$)	-0.296	
(iii)	Combined pre- and post-partum independent variables		
II	a Globulin (b)	-0.390	(10)
	b Globulin ($\bar{x}$)	-0.582	
(c)	Production rank		
(i)	Pre-partum independent variables		
I	Albumin ($\bar{x}$)	-0.277	(11)
	EMg ($\bar{x}$)	0.707	
II	EMg ($\bar{x}$)	-0.545	(12)
	RCC ($\bar{x}$)	0.546	
(ii)	Post-partum independent variables		
I	EMg ($\bar{x}$)	0.653	(13)
	EK ($\bar{x}$)	-0.902	

Table 20 continued

II	Hb ($\bar{x}$)	-0.246	(14)
	EK ($\bar{x}$)	-0.610	

(iii) Combined pre- and post-partum independent variables

I	a Albumin ($\bar{x}$)	-0.296	(15)
	b EMg ($\bar{x}$)	0.598	
	b EK ($\bar{x}$)	-0.242	
II	b Hb ($\bar{x}$)	-0.235	(16)
	a EMg ($\bar{x}$)	0.260	
	b EK ($\bar{x}$)	-0.384	
	a RCC ($\bar{x}$)	0.466	

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a = Pre-partum b = Post-partum.

($\bar{x}$) = Mean value b = mean slope (regression on weeks)

(i) Herd I

Services/conception = 1.90+(Glob. x -6.31)+(WCC x -0.41) (1)

(R^2=0.822, R=0.907, SEE=0.59, Seb1=2.36, Seb2=0.29, T value b1=2.68*, T value b2=1.38, F value =1.38**)

where Glob is slope of plasma globulin concentration over 8 weeks pre-partum and

WCC is slope of WCC over 8 weeks pre-partum.

The slope of globulin for the 8 weeks pre-partum contributed to most of the variation in services/conception on the basis of the B values (Table 20).

(ii) Herd II

Services/conception = 9.21+(Alb.1 x -0.21)+(Alb.2 x 6.85)+ (Glob. x -2.45) (2)

(R^2=0.638, R=0.799, SEE.=0.82, Seb1=0.40, Seb2=2.21,Seb3=0.91, T value b1=5.24**, T value b2=3.11**, T value b3=2.68*F value =18.8***).

where Alb.1 is the mean plasma albumin concentration over 8 weeks pre-partum;

Alb2 is slope of plasma albumin concentration over 8 weeks pre-partum;

Glob. is slope of plasma globulin concentration over 8 weeks pre-partum;

Although all the independent variables in the equation contributed significantly (see T values) to the variation in services/conception, the mean albumin concentration accounted for most of the variations in services/conception in this herd on the basis

of the standardized regressions (Table 20).

(2) Post-partum independent variables

The best equations for the post-partum independent variables found for predicting services/conception were;

(i) Herd I

Services/conception = 8.33+(Gluco. x -3.43)+(Glob. x 0.123)+ (RCC x -1.59) (3)

(R^2=0.868, R=0.92, SEE.=0.63, Seb1=0.54, Seb2=0.29, Seb3=0.24, T value b1=3.59**, T value b2=2.08*, T value b3=2.27*, F value= 35.2***).

where Gluco. is the mean plasma glucose concentration over 8 weeks post-partum;

Glob. is the mean plasma globulin concentration over 8 weeks post-partum;

RCC is the mean slope of RCC over 8 weeks post-partum.

All the independent variables in the equation gave significant T values for the partial regressions (b's), thus all contributed significantly to the variation in services/conception. However, the mean glucose concentration accounted for most of the variation in services/conception on the basis of the B values (Table 20).

(ii) Herd II

Services/conception =6.42+(Gluco. x -2.31)+(Alb. x -0.161)+ (Glob. x 2.17) (4)

(R^2=0.719, R=0.848, SEE=0.719, Seb1=0.66, Seb2=0.62, Seb3=0.49, T value b1=3.48**, T value b2=2.61*, T value b3=4.40**, F value= 27.3***).

Where Gluco. is the mean plasma glucose concentration over 8 weeks post-partum;

Alb. is the mean plasma albumin concentration over the 8 weeks post-partum;

Glob. is the mean plasma globulin level over 8 weeks post-partum;

The regression equation accounted for 71.9% of the variation in services/conception, with the mean globulin concentration for the 8 weeks post-partum accounting for most of it followed by the mean concentration of glucose on the basis of B values (Table 20).

(3) Combined pre-partum and post-partum variables

The best equations found for predicting services/conception were;

(i) Herd I

Services/conception = 10.84+(Gluco x -3.99)+(Glob1 x -1.54)+ (Glob2 x 0.103) (5)

(R^2=0.837, R=0.915, SEE=0.699, Seb1=1.01, Seb2=1.45, Seb3=0.704, T value b1=3.94**, T value b2=1.06, T value b3=1.47, F value= 27.4***).

Where Gluco is the mean plasma glucose concentration over 8 weeks post-partum;

Glob1 is the slope of plasma globulin concentration over 8 weeks pre-partum;

Glob2 is the mean plasma globulin concentration over 8 weeks post-partum.

The regression equation accounted for 83.7% of the variation in services/conception. The mean plasma glucose concentration for the post-partum period accounted for most of this variation and the other variables did not have significant T values for their partial regressions and did not contribute significantly to the services/conception variation on the basis of the standard regression coefficients (Table 20).

(ii)

Services/conception = -2.67+(Gluc x -1.61)+(Alb x 0.34)+
(Glob1 x 2.16)+(Glob2 x 0.258) (6)

(R^2=0.788, R=0.888, SEE=0.635, Seb1=0.63, Seb2=1.77, Seb3=0.73, Seb4=0.43, T value b1=2.41**, T value b2=3.7**, T value b3=2.9*, T value b4=5.65**, F value 28.8***).

Where Gluc. is the mean plasma glucose concentration over 8 weeks post-partum;

Alb. is the slope of plasma albumin over 8 weeks pre-partum;

Glob1 is the slope of plasma globulin over 8 weeks pre-partum;

Glob2 is the mean plasma globulin concentration over 8 weeks post-partum.

All independent variables contributed significantly (i.e. had significant T values for the partial regression) to the variation in services/conception. The post-partum globulin concentration accounted for most of this variation followed by the pre-partum slope of albumin on the basis of the B values (Table 20).

(b) Days open

(1) Pre-partum independent variables

(i) Herd I

In herd I no combinationof the pre-partum independent variables had any significant relationship with days open.

(ii) Herd II

The following equation was found to be the best for predicting days open.

Days open = 224.5+(alb x -4.11)+(Glob x 79.9) (7)

(R^2=0.440, R=0.663, SEE =25.56, Seb1=12.47, Seb2=28.30, T value b1=3.30**, T value b2=2.82*, F value=12.97***).

Where Alb. is the mean plasma albumin concentration over 8 weeks pre-partum and

Glob is the slope of plasma globulin 8 weeks pre-partum.

Both the independent variables had significant T values for the partial regressions i.e. contributed significantly to days open variation, pre-partum albumin mean concentration accounting for most of the variation on the basis of the B values (Table 20).

(2) Post-partum independent variables

The best equations to predict days open using the post-partum independent variables were;

(i) Herd I

Days open = -121.7+(Glob x 6.56)+(RCC x -82.7) (8)

(R^2=0.579, R=0.761, SEE =34.02, Seb1=22.62, Seb2=35.75, T value b1=2.90, T value b2=2.31*, F value = 11.73**).

Where Glob is the mean plasma globulin concentration over 8 weeks post-partum and

RCC is the slope of RCC over 8 weeks post-partum.

Both the independent variables contributed significantly (had significant partial regressions) to the variation in days open, although most of the variation was due to the mean post-partum globulin concentration on the basis of the B values (Table 20).

(ii) Herd II

Days open = 98.6+(Gluco x -34.8)+(Alb x -42.7)+(Glob x 5.06)+ (Ca x -42.0) (9)

(R^2=0.652, R=0.808, SEE =20.77, Seb1=19.7, Seb2=25.2, Seb3=14.3, Seb4=17.1, T value b1=1.76, T value b2=1.70, T value b3=3.50**, T value b4=2.45*, F value =14.6***).

Where Gluco is the mean plasma glucose concentration over 8 weeks post-partum;

Alb is the slope of plasma albumin over 8 weeks post-partum;

Glob is the mean plasma globulin concentration over 8 weeks post-partum;

Ca is the mean plasma Ca concentration over 8 weeks post-partum..

Mean globulin concentration accounted for most of the variation in days open followed by mean Ca concentration (see B values in Table 20).

(3) Combined pre-partum and post-partum independent variables

In herd I no combination of pre-partum and post-partum independent variables gave any significant relationship with days open. However in herd II the following equation was the best for predicting days open.

(i) Herd II

Days open = -164.4+(Glob1 x -81.5)+(Glob2 x 6.66) (10)

(R^2=0.582, R=0.763, SEE=22.9, Seb1=13.5, Seb2=16.8, T value b1=4.49**, T value b2=3.45**, F value=22.9***).

Where Glob1 is the slope of plasma globulin over 8 weeks pre-partu

Glob2 is the mean plasma globulin concentration over 8 weeks post-partum.

Both independent variables contributed significantly (had significant partial regressions) to variation in days open, however the post-partum mean globulin concentration accounted for most of the variation on the basis of the B values (Table 20).

(c) Production rank

(1) Pre-partum independent variables

The best equations for predicting production rank found were;

(i) Herd I

Production Rank = 42.4+(Alb x -1.03)+(EMg x 90.9) (11)

(R^2=0.765, R=0.875, SEE=6.11, Seb1=4.87, Seb2=17.21, T value b1=2.06, T value b2=5.28**, F value = 27.7***).

Where Alb is mean plasma albumin concentration over 8 weeks pre-partum;

EMg is mean EMg concentration over 8 weeks pre-partum.

The mean EMg concentration accounted for most of the variation in production rank on the basis of B values (Table 20).

(ii) Herd II

Production Rank = 151.3+(EMg x 116.1)+(RCC x 21.5) (12)

(R^2=0.545, R=0.739, SEE.=15.4, Seb1=27.7, Seb2=5.1, T value b1=4.19**, T value b2=44.20**, F value=16.2***).

Where EMg is the mean concentration of EMg over 8 weeks pre-partum;

RCC is the mean RCC over 8 weeks pre-partum.

Both the independent variables (EMg and RCC) contributed equally to the variation in the production rank (Table 20).

(2) Post-partum independent variables

The best equations found for predicting production rank were;

(i) Herd I

Production Rank = 162.9+(EMg x 105.2)+(EK x -6.29) (13)

(R^2=0.572, R=0.756, SEE=8.26, Seb1=33.42, Seb2=1.34, T value b1=3.39**, T value b2=4.67**, F value 11.34**).

Where EMg is the mean EMg concentration over 8 weeks post-partum.

EK is the mean EK concentration over 8 weeks post-partum. Although both EMg and EK contributed significantly to the variation in production rank, EK accounted for most of it on the basis of the B values (Table 20).

(ii) Herd II

Production Rank = 351.8+(Hb x -6.14)+(EK x -5.84) (14)

(R^2=0.531, R=0.729, SEE=15.5, Seb1=0.36, Seb2=1.29, T value b1=1.83, T value b2=4.53**, F value=16.4***).

Where Hb is the mean Hb concentration over 8 weeks post-partum;

EK is the mean EK concentration over 8 weeks post-partum.

The partial regression for post-partum mean EK concentration was highly significant (P<0.01) and explained most of the variation in production rank on the basis of the standardized regressions (Table 20).

(3) Combined pre-partum and post-partum independent variables

The best equations found for predicting production rank were;

(i) Herd I

Production Rank = 102.9+(Alb x -10.7)+(EMg x 76.8)+(EK x -1.48) (

(R^2=0.814, R=0.902, SEE=5.61, seb1=4.48, Seb2=17.24, Seb3= 0.829, T value b1=2.39*, T value b2=4.45**, T value b3=2.04*, F value = 23.30***).

Where Alb is the mean plasma albumin concentration over 8 weeks pre-partum;

EMg is the mean EMg concentration over 8 weeks pre-partum;

EK is the mean EK concentration over 8 weeks post-partum;

This regression equation accounted for 81.4% of the variation in milk production rank and the pre-partum mean EMg concentration accounted for most of this variation on the basis of the B values (Table 20).

(ii) Herd 11

Production Rank=102.6+(Hb x -5.95)+(EMg x 55.2)+(EK x -3.66)+ (RCC x 18.4) (16

(R^2=0.753, R=0.868, SEE.=11.76, Seb1=2.98, Seb2=25.02, Seb3= 1.26, Seb4=4.02, T value b1=1.99, T value b2=2.21*, T value b3=3.09*, T value b4 = 4.57**, F value = 19.1**)

Where Hb is the mean Hb concentration over 8 weeks post-partum;

EMg is the mean EMg concentration over 8 weeks pre-partum;

EK is the mean EK concentration over 8 weeks post-partum;

RCC is the mean RCC over 8 weeks pre-partum;

This regression equation accounted for 75.3% of the variation in milk production rank, and most of this variation was accounted for by the pre-partum RCC levels followed by the post-partum EK mean concentrations on the basis of the B values (Table 20).

DISCUSSION

(a) Relationship between blood components and fertility

There is probably no single satisfactory index of fertility (Bane 1964). Both the conception rate at first service and services/conception may be affected by calving to first service interval and the latter may even be affected by the stock owners opinion as to how long it is economically worthwhile persisting in trying to get a cow pregnant. This in turn may be influenced by yield and replacement resources (Hewett 1974).

Days open are likely to be strongly influenced by the calving to first service intervals and the effectiveneness of heat detection. Herds with better heat detection methods show higher conception rates and shorter calving to conception intervals (Hewett 1974). An assessment of the effects of variations in the blood profile on fertility must be made with knowledge that it is probably impossible to eliminate all the effects of background factors (heat detection methods, herd management and nutrition) from the field data.

Variation in certain constituents of blood have been used as aids to diagnose diseases and poor performance in dairy cows under field conditions (Kronfeld 1972). Abnormal metabolic profiles have been associated with a high incidence of such disorders as infertility, parturient paresis as well as with decreases in milk production and quality (Payne et al 1970,

Payne et al. 1973a). An examination of the individual high producing cow prior (8 weeks) to calving has proved to be an important aid in identifying animals likely to succumb to the production disease complex in West Germany (Sommer 1975).

Thus the importance of the observed relationships of various blood components to fertility and production rank in the present study is assessed in the light of the above mentioned potential sources of error, the biological feasibility of the relationships and the relevant information from the literature.

The services/conception, days open and production rank differed between the two herds studied. Herd I had higher services/conception and days open and lower production rank than herd II (Table 15). The differences in the fertility indices probably reflect efficiency of heat detection between the two herds since their blood chemistry was generally similar for most of the blood components (Figs. A2a to A2q). In herd I some cows had very long days open even when they conceived to first service (see large SEM in Table 15). However despite the differences in the dependent variables (services/conception, days open and production rank) between the two herds, some relationships between blood components and fertility and production rank were consistent in both herds (Tables 16 and 17).

Post-partum mean glucose concentration was negatively correlated to services/conception and days open in both herds, that is, cows with higher plasma glucose concentrations in the post-partum period had better fertility than those which had low

plasma glucose concentrations. The relationship between plasma glucose concentration and fertility has been previously reported by many workers (McClure 1965, McClure 1966, McClure 1968, Payne et al. 1970, Allen, Manston and Rowlands 1978). In a survey of 75 dairy herds, the mean concentrations of blood glucose were found to be low in 6 out of 20 herds with poor fertility (Payne et al. 1973a). They found that herds with low blood glucose concentrations suffered from anoestrus. Conception rate has been reported to be better when blood glucose concentrations were increasing at time of mating (McClure 1966, Mcclure 1968, Hunter 1977) and more recently Andersson and Pehrson (1986) reported a trend to lower fertility in cows with lower blood glucose concentrations at the time of insemination. However not all workers have reported a significant relationship between blood glucose and fertility. Blowey et al. 1973) and Parker and Blowey (1976) did not find any significant relationship between fertility and blood glucose concentration. This might mean that there is a "threshold" for blood or plasma glucose values below which fertility is impaired or it may only be important when combined with other clinico-physiological deviations in the cows or with management abnormalities.

The mean post-partum Hb concentration was negatively correlated with services/conception and days open in both herds, while the post-partum slopes of RCC (Herd I = -0.06 and herd II = -0.08) were positively correlated to services/conception and days open in herd I. Thus cows with low Hb concentrations and greater decrease (slope) in RCC levels post-partum required more services to conceive and had longer days open. Reduced fertility

(long days open and multiple services/conception) has been associated with anaemia (Hansel 1965, Payne et al 1970, Rowlands 1980). However, Parker and Blowey (1976) did not find any association between Hb concentrations and conception rate.

The pre-partum slope of albumin concentration (Herd I = -0.08 and Herd II = -0.04) was positively related to services/conception in both herds and to days open in herd I only The pre-partum mean albumin concentration was negatively correlated with both services/conception and days open in herd II only. The post-partum slope (0.03) of albumin concentration was positively correlated to days open while the mean was negatively correlated to services/conception in herd II. The implications of these relationships are that cows which were able to maintain albumin concentrations had better fertility. This supports the hypothesis of Rowlands et al (1977) and Rowlands et al (1980), that cows which are able to maintain higher stable albumin concentrations are likely to have better fertility and agrees with trends of lower fertility in cows with lower plasma albumin concentrations at insemination reported by Andersson and Pehrson (1986). However, Rowlands et al (1980) suggested that interactions with other herd factors could affect the relationship between albumin concentration and fertility.

The pre-partum slopes (Table 6 and 7) of globulin concentrations (-0.14 for both herds) were negatively correlated with services/conception and days open, whereas the post-partum mean globulin concentration was positively correlated to services/conception and days open in both herds (Table 16 and 17).

The negative relationship between the pre-partum globulin slope and fertility indices indicates that cows with a large slope (i.e. rate of decrease, Fig. A2f) had better fertility i.e. required less services/conception and had a shorter number of days open than the cows which had smaller slopes. Similar observations were reported by Rowlands et al. (1980) who found that the rate of decrease in globulin concentrations 4 weeks before parturition was related to fertility. They found that cows requiring more than 3 services/conception had smaller decreases in globulin concentration than those which required less than 3 services/ conception.

The positive relationship between post-partum mean globulin concentration and fertility suggests that high post-partum globulin concentrations are associated with multiple services/ conception and days open. High globulin concentrations have been associated with low fertility (multiple services/conception) (Amiel 1970, Payne et al. 1977, Rowlands et al. 1977, Rowlands et al. 1980). Payne et al. (1977) found that cows with raised globulin concentrations had poor fertility and suggested that this relationship could be due to an association with endometritis, mastitis or other diseases which could lead to increased plasma globulin concentrations. Amiel (1970) found that cows requiring more than one service/conception had higher plasma globulin concentrations than those which conceived to the first service. He also found that endometritis and salpingitis ranging from mild to severe were a common feature in cows culled due to infertility. Thus this suggests that higher than normal post-partum globulin concentrations may indicate underlying inflammatory processes which could impair fertility.

The post-partum mean plasma Ca concentration was negatively correlated with both services/conception and days open in both herds while its slope (-0.04, Fig. 1.3h) for herd I was significantly ($P<0.05$) and positively correlated with services/conception. Thus the results suggest that high plasma Ca concentrations and maintenance of plasma Ca concentrations post-partum could be associated with better fertility. An increase in dietary Ca has been found to result in more rapid uterine involution (Ward, Campbell and Dunham 1971). Thus it could be that cows with high plasma Ca concentrations have a more rapid involution of the uterus and hence a higher chance of becoming pregnant earlier than cows with low plasma Ca concentrations. Amiel (1970) found that cows which came into oestrus and conceived within 45 days after calving had relatively higher levels of plasma Ca than those which conceived later. The positive relationship between the post-partum plasma Ca slope (rate of decrease) and days open could mean that cows which show a decrease in plasma Ca levels (Fig. A2h) are not able to maintain their normal physiological Ca concentrations which could then affect their uterine involution process.

The pre-partum slope (-0.02) of Pi plasma concentrations was negatively correlated to services/conception and days open in herd II. This negative relationship suggests that cows which had the greatest slope (i.e. rate of decrease) in Pi concentration required less services to conceive and had shorter days open than cows which had a smaller slope. Plasma Pi concentrations decrease towards calving (Hackett *et al.* 1957, Rao *et al.* 1981) and this has

been suggested to be due to increased phosphorus utilization with the enhanced carbohydrate metabolism of late pregnancy (Hackett et al. 1957). Thus it could be that cows with high phosphorus utilization (i.e. large slopes) during this period of high energy demand are maintaining a higher than average energy balance (and approaching a positive energy balance) which if maintained post-partum could lead to better fertility. In a group of high-producing dairy cows, Butler, Everett and Coppock (1981) found that ovulation was delayed in animals with a high average energy deficit.

The pre-partum slope of WCC (0.02) was negatively correlated to services/conception and days open in herd I, thus cows which had large increases in WCC's had better fertility. This relationship is difficult to explain, however it could be that cows with a small increase in WCC's pre-partum are not able to adapt to the stress of early lactation after the stress of parturition. The level of WCC rises in stressful conditions and in sustained stress the levels fall especially in animals which are not able to withstand prolonged stress (Paape et al. 1974a). Cows not able to adapt to sustained stress have been shown to have lowered milk production and reduced fertility (Thatcher 1974). Herd I had significantly ($P<0.01$) lower WCC's than herd II (Fig. A2K) and this might explain why the slope of WCC's on time (pre-partum) was significantly ($P<0.05$) related to fertility (services/conception) in herd I only, i.e. the slope of WCC's on time (pre-partum) may be important in animals with low WCC's.

The post-partum mean EMg concentration was negatively correlated to services/conception and days open in herd II, that is, high EMg concentrations were associated with better fertility. This relationship between EMg concentration and fertility has not been reported before and its physiological role in fertility is not easy to explain. However, it could be due to the close relationship between tissue Mg and EMg concentrations especially in long-term subclinical Mg deficiency (MacIntyre and Davidson 1958, MacIntyre, Hanna, Booth and Read 1961, Lim et al. 1969). Rats with low body tissue Mg as a result of feeding a Mg deficit diet were found to have lower fertility than the control rats (Hurley, Cosens and Theriult 1976). Seeling et al. (1980) suggested that prolonged Mg deficiency which is generally accompanied by low EMg concentrations (Tufts and Greenberg 1937, Elin et al 1971) may be a contributory factor to damage to the products of conception and hence low fertility. This relationship of EMg concentration and fertility in dairy cows requires further investigation.

Multiple regression analyses were carried out to relate means and slopes of blood components for both pre-partum and post-partum periods with fertility indices. Although many blood components gave significant simple correlation coefficients with both services/conception and days open (Tables 16 and 17) only a few yielded significant partial regression coefficients when they were included in the stepwise multiple regression analysis (multiple regression equations 1 to 16).

This indicates that most of the significant simple correlation coefficients of the blood components with fertility

indices were due either to correlation with the other blood components which showed up in the multiple regressions or were due to chance (Snedecor and Cochran 1971). Partial regression coefficients should be free from any bias due to the other independent variables in the multiple regression equation but are subject to bias from omitted variables which are related to the independent variable (Snedecor and Cochran 1971). Thus this could explain why some blood components although not having a significant partial regression coefficient still helped to reduce the residuals (i.e. when omitted from the multiple regression equation the residual was higher).

The combinations of blood components which gave significant multiple regressions with fertility indices differed between the herds. However some blood components which occurred in the regression equations for each herd accounted for most of the variations of either services/conception or days open (Table 20). The blood components which featured most consistently in the multiple regression relationships with fertility indices were glucose, albumin and globulin. The post-partum mean glucose concentration was important in multiple regression relationships with both services/conception and days open in both herds. The cows with high mean glucose concentration had better fertility i.e. fewer services/conception and shorter days open than cows with lower mean glucose concentrations. However, since the mean post-partum glucose concentration alone did not give a significant F value for the regression equation with fertility indices, blood glucose level alone can not be used to predict fertility in dairy cows.

The slopes and mean concentrations of albumin and globulin

contributed significantly to the multiple regression relationships with both services/conception and days open in both herds (multiple regression equations 1 to 10, Table 20). The relationships were that cows which were either able to maintain stable albumin concentrations (i.e. small albumin decrease in pre-partum and post-partum periods) or had high albumin levels in both pre-partum and post-partum periods had better fertility. Similar observations were reported by Rowlands et al. (1977) and Rowlands et al. 1980). The physiological relationship between albumin concentration and fertility is not known but, it could suggest adequate protein intake and balance. Since hypoalbuminaemia in dairy cows can be brought about by elevated plasma globulin levels (due to the negative correlation between albumin and globulin levels- Kitchenham et al. 1975), Payne 1977), the relationship between albumin concentration and fertility could well be an indirect one.

The relationships between the slope (pre-partum) and mean globulin concentrations (post-partum) and fertility suggested that cows which either showed a greater decrease in globulin concentrations pre-partum or had low globulin concentrations post-partum had better fertility than cows which showed a smaller decrease in globulin concentration or which had high post-partum globulin concentration. These observations are similar to those of Rowlands et al. (1980) who found that cows with a greater decrease in globulin concentration pre-partum required less services/conception than those which showed little or no decrease in globulin concentration towards calving. The physiological relationship between the globulin concentration slope towards calving and fertility is not clear and hence further investigations may be required. However the relationship of globulin

concentration and fertility could be due to the association of the plasma immunoglobulins and reproductive tract diseases (e.g. subclinical endometritis, metritis or salpingitis) which have been shown to impair fertility (Amiel 1970, Payne 1977, Rowlands et al. 1977).

The other blood components which contributed significantly to the variation in fertility indices though not in both herds were the pre-partum WCC slope, post-partum RCC slope and the post-partum mean concentration of Ca. The relationship of the WCC slope and fertility could be due to the reported association of WCC changes and adaptation to prolonged stress in animals as discussed earlier.

The RCC's decreased with weeks of lactation (Fig. 1q) and this decrease (slope) contributed significantly to the variations in services/conception and days open (multiple regressions equations 3 and 8). This could be due to the reported association of anaemia and poor fertility i.e. long days open and multiple services/conception (Hansel 1965, Payne et al. 1970). Thus it could be that cows with a large post-partum RCC slope (i.e. greater decrease in RCC levels) are prone to developing anaemia and are more likely to have poor fertility. The post-partum mean concentration of Ca contributed significantly ($P<0.05$) to the variation in days open (multiple regression equation 9). Calcium is important in the uterine involution process (Ward et al. 1971, Silva and Noakes 1984) and hence cows with high post-partum Ca levels could have faster uterine involution and therefore likely to conceive earlier than those with low Ca levels.

The fact that a given blood component was not significantly related to any of the fertility indices employed in the present study does not of course mean that deviations in that component could not influence fertility. The results of this study show that concentrations as well as slopes of blood components (especially glucose, albumin, globulin, WCC, RCC and Ca) of dairy cows in late pregnancy and early lactation could be useful in prediction equations for fertility in herds composed of cows of different age groups (age did not show any significant relationship with fertility).

(b) Relationship between blood components and production rank

The pre-partum slope (-0.04) of plasma glucose concentration was negatively correlated to production rank in herd I (Table 16). This suggests that cows which show a greater pre-partum decrease in plasma glucose concentration may not be able to meet the high glucose demand for milk production in early lactation. Schwalm and Schultz (1976) found that a pre-partum decrease in plasma glucose concentration in dairy cows was associated with sub-clinical ketosis and low milk production in early lactation.

The mean pre-partum Hb concentration and RCC's were negatively correlated with milk production rank (Table 16). This suggests that high yielding cows had lower Hb concentrations and RCC's than the low yielding cows. Hb concentrations and RCC's decrease towards calving (Fig. 1b and 1q) due to an increased protein demand for foetal development and colostrum formation (Cornelius and Kaneko 1963, Williams and Miler 1979). Thus it could be that high yielding cows have a higher protein demand for

foetal development and colostrum formation than low yielding cows and consequently lower pre-partum Hb and RCC levels. During lactation high yielding cows have lower Hb and RCC levels than low yielding cows (Kossila 1970, Hewett 1974). Thus according to this study it seems that this relationship is also apparent in the last two months of gestation when there is a very high protein and energy demand for foetal development and formation of colostrum (Moe and Tyrrell 1972, Williams and Miller 1979).

The negative correlation between mean pre-partum albumin concentration and milk production (Table 16), suggests that high yielding cows have lower pre-partum mean albumin concentrations. Albumin concentration decreased towards calving (Fig. A. 2e) and this would lead to low albumin concentrations at parturition. The decrease in albumin concentration towards calving occurs only in some cows and has been shown to be a characteristic of these animals (Payne et al. 1977, Rowlands et al. 1983). Possibly it is also a characteristic of high yielding cows.

The post-partum slope (-0.48) of WCC level was negatively correlated to production rank (Table 17) i.e. low yielding cows had large WCC slopes (decreases) after calving. This relationship suggests that cows with large decreases in WCC's after calving were not able to adapt to the stress of early lactation after the stress of parturition. Cows not able to adapt to sustained stress have been shown to have lowered milk production (Thatcher 1974).

The mean pre-partum EK concentrations were negatively correlated to production ranks in herd II while the mean post-partum EK concentration was negatively correlated with milk

production rank in both herds. This suggests that high yielding cows had or maintained lower EK concentration than the low yielding cows. In a herd composed of Jersey, Friesians and Herefords, Rasmussen et al. (1974) found that high yielding cows had lower EK concentrations than low yielding cows.

The mean pre-partum and post-partum EMg concentrations were positively correlated to milk production rank. This relationship is not easy to explain, however it could be that a low EMg concentration impairs milk production in cows as in rats. Rats depleted of Mg during gestation exhibited impaired lactation compared to control rats (Wang, Wang, Khairalla and Schwartz 1971).

Multiple regression analysis carried out to relate means and slopes of blood components with milk production rank showed that only mean EMg and EK concentrations contributed significantly to the milk production rank variation in both herds (multiple regression equations 11 to 16). High milk production was associated with high EMg levels and low EK levels.

The relationship of EMg concentration and milk production rank has not been observed before in dairy cows, and its physiological association with milk production requires clarification. The EMg levels do not fall as quickly or as much as do PMg levels in acute Mg deficiency (Tuft and Greenberg 1937, Salt 1950, Elin et al. 1971). However, lesser degrees of Mg deficiency if prolonged can cause prolonged drops in EMg levels to half of control values (Elin et al. 1971). Thus low EMg concentrations may reflect a prolonged low grade Mg deficiency. Prolonged Mg deficiency can lead to a decrease in the

mitochondrial number in the soft tissue cells and hence disruption of their function (Ghazi and Heaton 1975). Thus if the function of mammary glands is impaired milk synthesis could be impaired leading to low milk production.

The negative relationship between EK concentration and milk production was also observed by Rasmussen et al. (1974), who found that high yielding cows had lower EK concentrations than the low yielding cows. However the biological relationship between EK concentrations and milk production is not apparent. It could be that the gene(s) that control(s) EK levels (Ellory and Tucker 1970, Tucker and Ellory 1970, Rasmussen et al. 1974) also influence milk production.

CHAPTER 6

(a). CHANGES IN BLOOD AND PLASMA CONSTITUENTS DURING A SHORT STARVATION (40 HRS) PERIOD AND THE RELATIONSHIP OF SUCH CHANGES WITH GROWTH RATE IN YEARLING HEIFERS.

INTRODUCTION

The variation in many blood biochemical and haematological components among animals have been shown to be under genetic control (Rowlands et al. 1974, Freeman et al. 1978). The most important implication of this knowledge is the possibility of its application to predict genetic potential for growth rate or milk production potential in both male and female animals relatively early in life. The availability of such a facility would enable more rapid progress in genetic improvement of growth rate or milk production. Recently interest has been increasing in physiological factors that relate to performance of cattle under different environments (Tilakaratne et al. 1980; Osmond, Carr, Hinks, Land and Hill, 1981; Land, Carr, Hart, Osmond, Thompson and Tilakaratne 1983).

The adaptability of cattle to nutritional and environmental stress is thought to be related to certain blood constituents in cattle (Erwin 1969). The purpose of this study was to investigate the variation in blood components in yearling replacement heifers during a short starvation period and the possible relationships of the responses with growth rate.

MATERIALS AND METHODS

Experimental animals

Ten replacement dairy heifers aged 11 ± 0.3 months (mean ± SEM), with body weight of 225 ± 4.4 kg (mean ± SEM) and mean growth rate of 14.8 ± 0.7 kg/month (mean ± SEM) were used. They were running at pasture (see General Materials and Methods) and had free access to water. They were weighed and put into a concrete yard, where they were deprived of food for forty (40) hours but provided with water ad libitum. After the end of the starvation period they were weighed and fed lucerne hay ad libitum and left for 8 hours before the first post-starvation blood sample was taken.

Sample collection

Blood samples were taken from the jugular vein at -24, 0 hours (pre-starvation), 8, 16, 24, 32 and 40 hours (during starvation) and at 8 and 16 hours (post-starvation).

Sample preparation and analytical techniques

These have been described earlier in the section on general materials and methods. The blood samples were analysed for PCV, Hb, TPP, albumin, globulin, glucose, Mg, Ca, Pi, BUN, creatinine, PNa, PK, WCC, RCC, EMg, ENa and EK.

Statistical analysis

The data was subjected to analysis of variance and multiple regression analysis. The regression procedure was based on the maximum R^2 improvement technique (Draper and Smith 1966). In this technique the procedure begins by identifying the one variable model producing the highest R^2 statistic. It then adds another variable which would produce the greatest increase in R^2. Once the two variable model is obtained, the variables are compared to each variable not in the equation and the variable that would produce the greatest improvement in R^2 is added in the next equation and the procedure is repeated. The independent variables were expressed as the arithmetic difference between Time 0 and Time 40 hrs and their means and standard errors are shown in Table 21.

RESULTS

The results of the changes that occurred during starvation are shown in Figure 7.

PCV's and Hb concentrations increased significantly ($P<0.05$) by 7.8% and 8.0% respectively and then fell to levels slightly lower than the pre-starvation period after introduction of feed (Figs. 7a and 7b). The plasma glucose concentration showed a significant ($P<0.01$) transient increase of 24.2% after 16 hours of starvation and then

Table 21: Mean changes in blood components during starvation and their simple correlation coefficients (r) with growth rate (n=10)

Blood components	Mean ± SEM	r
PCV (l/l)	+0.26 ± 0.033	-0.472
Hb (g/l)	+6.00 ± 1.50	0.330
Glucose (mmol/l)	-0.40 ± 0.06	0.069
TPP (g/l)	+12.0 ± 2.50	0.038
Albumin (g/l)	+1.30 ± 0.10	0.371
Globulin (g/l)	+10.0 ± 2.40	0.260
PMg (mmol/l)	-0.11 ± 0.03	0.626*
Ca (mmol/l)	-0.44 ± 0.09	0.252
Pi (mmol/l)	+1.00 ± 0.26	0.367
PNa (mmol/l)	+2.00 ± 1.45	-0.193
PK (mmol/l)	-0.80 ± 0.10	-0.037
Urea (mmol/l)	+2.01 ± 0.39	-0.609*
Creatinine (umol/l)	+1.51 ± 2.74	0.187
RCC ($x10^{12}$cells/l)	+0.60 ± 0.14	0.135
WCC ($x10^{9}$cells/l)	+2.22 ± 0.43	0.584 ($p<0.06$)
ENa (mmol/l)	+6.00 ± 0.29	0.055
EMg (mmol/l)	-0.07 ± 0.05	0.604*
EK (mmol/l)	-4.00 ± 0.02	0.619*

* $P<0.05$

\+ increase

\- decrease

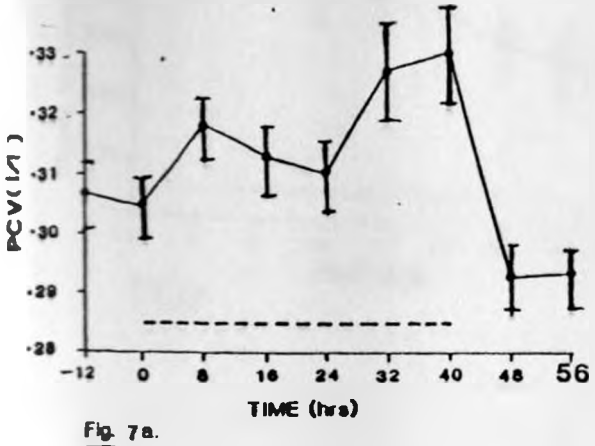

Fig. 7a.

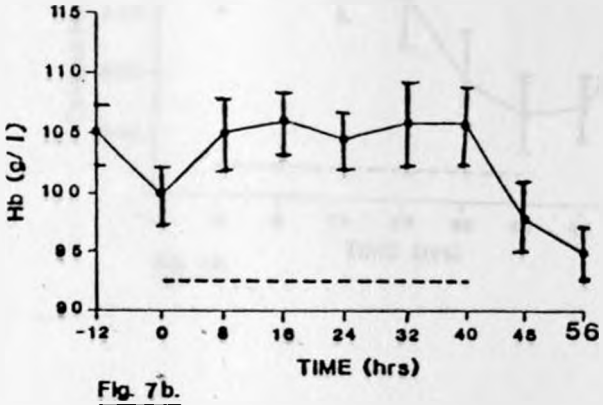

Fig. 7b.

Fasting Period

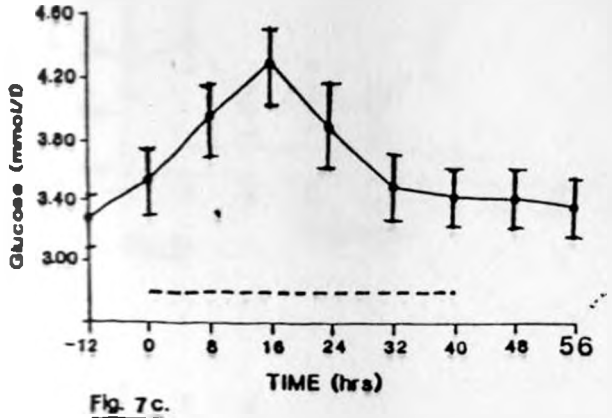

Fig. 7c.

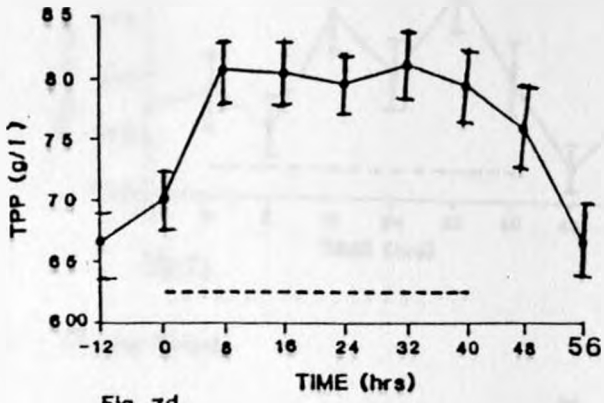

Fig. 7d.

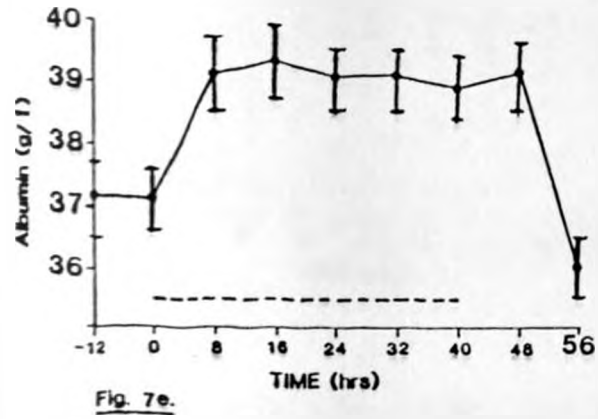

Fig. 7e.

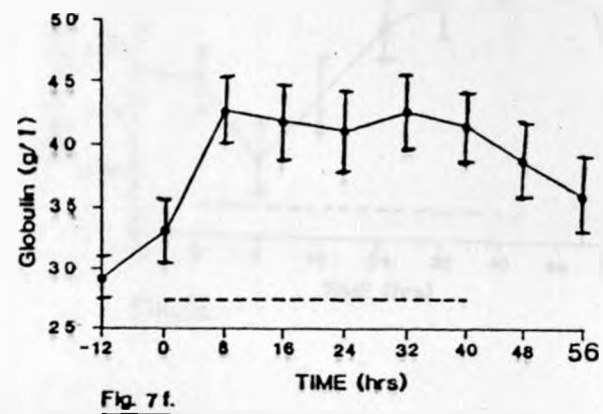

Fig. 7f.

Figures 7a-7f. Changes in PCV's, Hb, plasma glucose, TPP, albumin and globulin (mean ± SEM) values in 10 heifers (11 month old) during a 40 hour starvation period

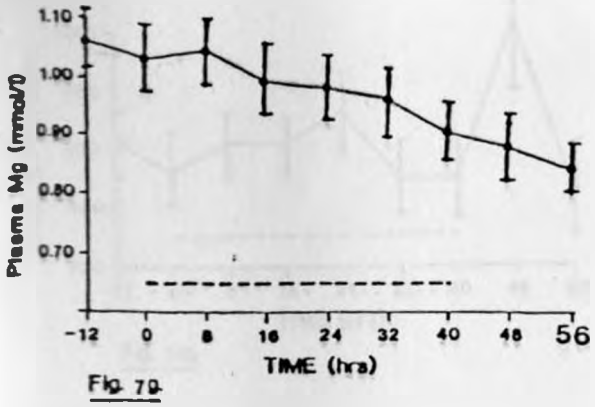

Fig. 7g

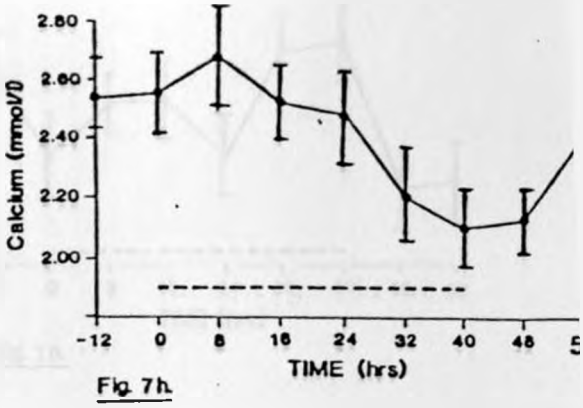

Fig. 7h

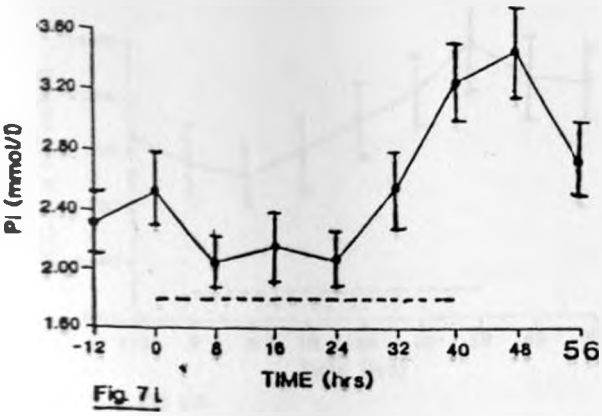

Fig. 7i

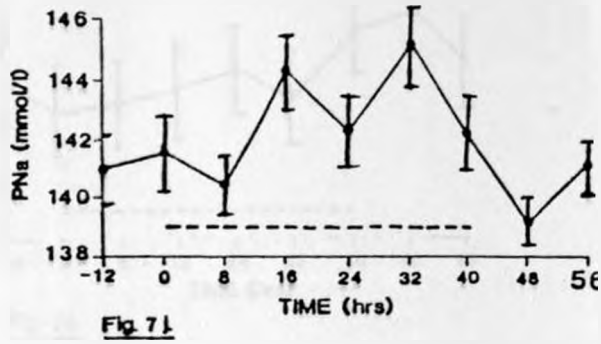

Fig. 7j

-------- Fasting Period

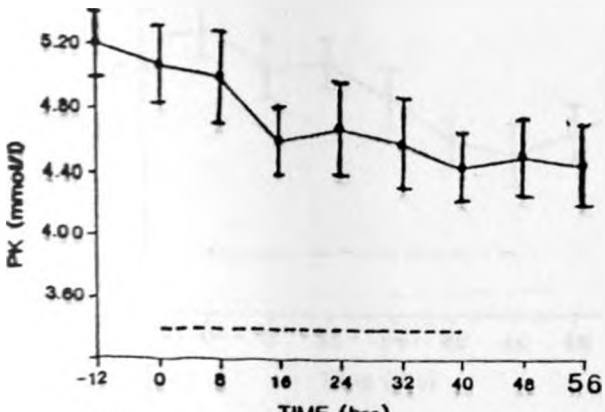

Fig. 7k

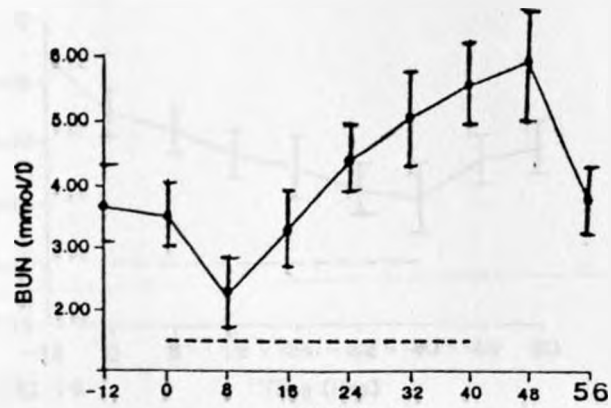

Fig. 7l

Figures 7g - 7l. Changes in plasma Mg, Ca, Pi, Na, K and BUN (mean ±SEM) concentrations in 10 heifers (11 months old) during a 40 hour starvation period

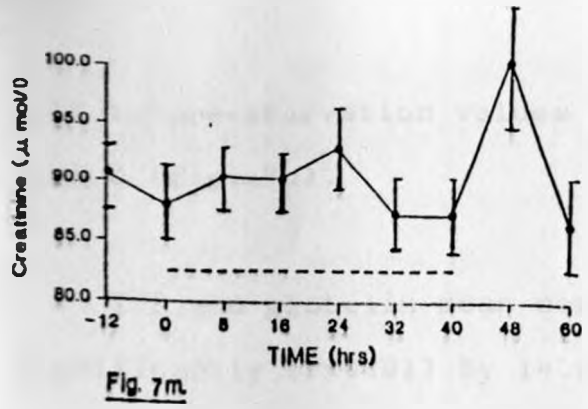

Fig. 7m.

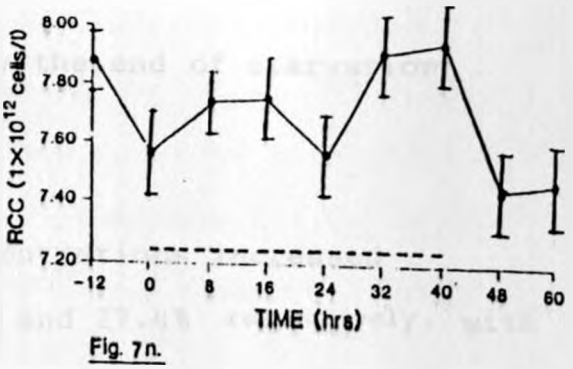

Fig. 7n.

---- Fasting Period

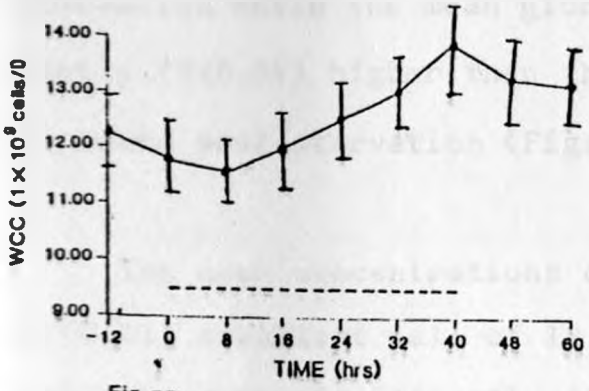

Fig. 7p.

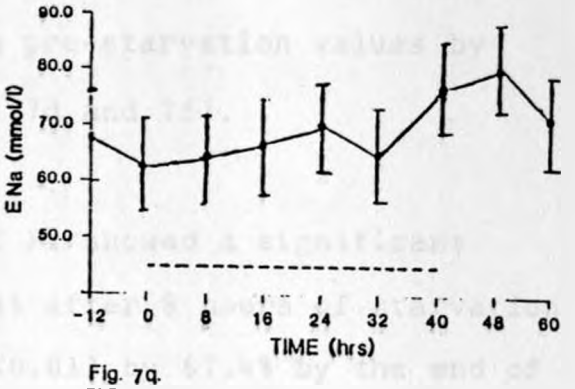

Fig. 7q.

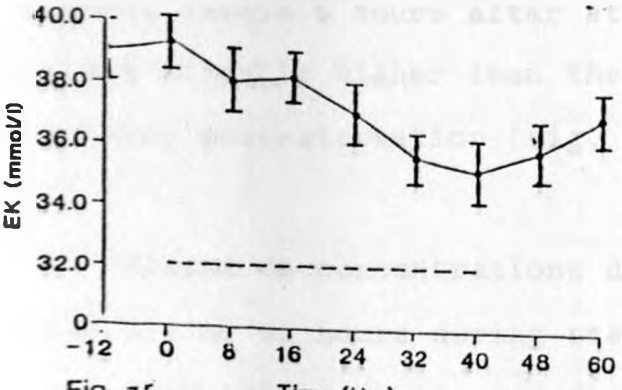

Fig. 7r

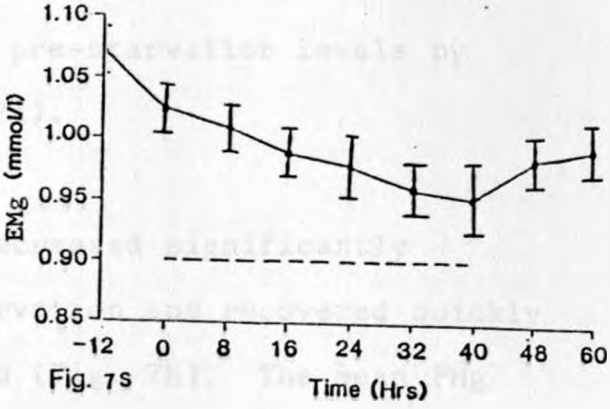

Fig. 7s

Figures 7m-7s. Changes in creatinine, RCC, WCC, ENa, EK and EMg (mean ± SEM) values in 10 heifers (11 months old) during a 40 hour starvation period

fell to pre-starvation values by the end of starvation period (Fig. 7c).

TPP and globulin mean concentrations increased significantly ($P<0.01$) by 14.0% and 27.4% respectively with starvation and fell after introduction of feed. The mean TPP levels fell to pre-starvation values 24 hours post-starvation while the mean globulin values remained significantly ($P<0.05$) higher than the pre-starvation values by 16 hours post-starvation (Figs. 7d and 7f).

The mean concentrations of Pi showed a significant ($P<0.01$) transient fall of 18.4% after 8 hours of starvation and then rose significantly ($P<0.01$) by 57.4% by the end of the starvation period. They continued rising, reaching the highest levels 8 hours after starvation and then fell to values slightly higher than the pre-starvation levels by 16 hours post-starvation (Fig. 7i).

Plasma Ca concentrations decreased significantly ($P<0.05$) by 32 hours during starvation and recovered quickly after the reintroduction of feed (Fig. 7h). The mean PMg concentrations decreased significantly ($P<0.05$) by 20.0% by the end of starvation, and the lowest levels which were significantly ($P<0.05$) lower than the pre-starvation levels were observed 16 hours after reintroduction of feed (Fig. 7g).

The mean PNa concentrations increased significantly ($P<0.05$) by 4.8 mmol/l with starvation while the mean PK

concentrations showed a significant ($P<0.05$) decrease of 0.1 mmol/l with starvation (Figs. 7j and 7k). The mean plasma creatinine concentration did not show any significant change with starvation (Fig. 7m). The mean BUN concentrations increased significantly ($P<0.01$) by 57.8% during starvation, and reached the highest level 8 hours after reintroduction of feed and then fell to prestarvation levels by 16 hours post-starvation (Fig. 7l).

The mean RCC's increased significantly ($P<0.05$) by 9.8% with starvation and fell to pre-starvation levels after introduction of feed (Fig. 7n). The mean WCC's increased significantly ($P<0.05$) by 17.1% by the end of starvation and remained significantly ($P<0.05$) higher than the pre-starvation levels in the post-starvation period (Fig. 7p).

The mean EMg concentrations decreased nearly significantly ($P<0.06$) by 9.7% by the end of starvation (Fig. 7q). The mean ENa concentrations increased significantly ($P<0.05$) with starvation by 11.6%, the levels rose significantly ($P<0.01$) 8 hrs after reintroduction of feed and then fell to pre-starvation levels by 16 hrs post-starvation (Fig. 7q). The mean EK concentrations decreased significantly ($P<0.05$) by the end of starvation (Fig. 9r).

Most of the blood parameter responses showed significant variations among animals including plasma albumin, glucose,

Pi, Ca, Mg, plasma creatinine, BUN, RCC and WCC ($P<0.05$) (Table 22).

Correlations between blood chemistry responses during starvation and growth rate

The simple correlation coefficients between growth rate (G.R.) and changes in blood components during starvation are shown in Table 22. Growth rate was most closely correlated with change in PMg followed by changes in EK, BUN, EMg and WCC's.

The changes in PMg, EK, EMg and WCC were positively correlated with growth rate while the change in BUN was negatively correlated with growth rate.

Multiple linear regressions of growth rate on blood chemistry changes during starvation.

The best regression equations were:-

G.R. = 11.42 + (14.9 x PMg) (1)

(SEB = 8.29, r = 0.532, SEE = 1.66).

G.R. = 14.23 + (20.9 x PMg)+(-12.1 x PCV) (2)

(SEB1=5.85, SEB2=0.41, Multiple R=0.667, SEE=1.15)

G.R. = 12.97+(19.4 x PMg)+(-9.80 x PCV)+(6.07 x EMg) (3)

Table 22. Mean squares from analysis of variance of blood component responses to starvation

Blood	Heifers		Times	
Component	M.S	Error M.S	M.S	Error M.S
PCV (l/l)	40.9**	1.12	16.9**	1.21
TPP (g/l)	2.46**	0.13	3.98**	0.14
Albumin (g/l)	4.19**	0.20	0.39	0.29
Globulin (g/l)	1.84*	0.20	3.80**	0.22
Hb (g/l)	1.23**	0.09	0.37*	0.05
Glucose (mmol/l)	2.10*	0.21	0.35**	0.03
Pi (mmol/l)	0.21	0.06	2.06**	0.06
RCC ($x10^{12}$cells/l)	0.56**	0.03	0.60	0.13
WCC ($x10^{9}$cells/l)	0.80**	0.04	1.43*	0.21
Ca (mmol/l)	0.12*	0.02	0.68*	0.06
Urea (mmol/l)	0.31*	0.05	4.06**	0.12
Creatinine (µmol/l)	4.70**	0.15	7.53	2.13
PMg (mmol/l)	0.46*	0.07	0.88	0.05
PNa (mmol/l)	0.23	0.15	0.18	0.04
PK (mmol/l)	0.09	0.05	0.42*	0.05
EMg (mmol/l)	0.27*	0.001	0.20	0.04
ENa (mmol/l)	3.70**	0.06	3.36*	0.61
EK (mmol/l)	0.72**	0.013	0.47*	0.09

* $P<0.05$

** $P<0.01$

(SEB1=2.91, SEB2=0.20, SEB3=1.26, Multiple R=0.778, SEE=0.53).

G.R. =9.80+(16.2 x PMg)+(-6.40 x PCV)+(7.80 x EMg)+

(1.19 x Albumin) (4)

(SEB1=2.12, SEB2=0.18, SEB3=11.04, SEB4=4.36, Multiple R=0.790, SEE=0.35).

(Where b1 to b4 are the partial regression coefficients in the same order as in equation 4). (GR = growth rate in kg/ month)

The intercepts for equations 2, 3 and 4 were significant as follows; equation 2 ($P<0.05$) and equation 3 and 4 ($P<0.01$).

Thus the "best" equation producing the largest R was that containing mean changes of PMg, PCV, EMg and albumin which accounted for 62.4% of the variation in the growth rate. The F ratios for this regression equation based on partial mean squares divided by residual mean squares are shown in Table 23. They provide a measure of the relative importance of the four independent variables with respect to variation in growth rate. The changes in PMg and EMg concentrations were the most important variables in predicting growth rate. The next in order of importance were mean changes of PCV and albumin and all of the partial regression coefficients shown in equation 4 were significant ($P<0.05$).

Table 23. Multiple regression analysis of growth rate on blood chemistry changes during starvation (equation 4 with the highest R^2)

Source	df	F value
Total	9	
Due to regression	8	64.8**
PMg	1	58.4**
PCV	1	12.56*
EMg	1	56.2**
Albumin	1	7.54*
Residual	1	

These multiple regression equations show that calves with greatest change in PCV had the lowest growth rate while those with highest changes in PMg, EMg, and albumin concentrations had the highest growth rate.

DISCUSSION

(a) Changes in blood chemistry during starvation

The increase in PCV, Hb and RCC levels during starvation was probably due to dehydration that can occur during starvation (Rumsey and Bond 1976). Restricted feed intake decreases water intake and vice versa (McFarland and Wright 1969). Thus the fall in these parameters observed post-starvation was probably due to increased water intake as a result of feed reintroduction.

The increases in TPP and globulin with starvation has also been observed by Pehrson (1966) and Tilakaratne *et al.* (1980) and was probably also due to dehydration. The post-starvation fall in TPP and globulin concentrations was attributed to increased water intake following re-introduction of feed. The transient rise in plasma glucose levels was also observed by Baird *et al.* (1972), Robertson and Thin (1953) and Robertson *et al.* (1980). This has been attributed to release of hormone possibly noradrenaline in response to the stimulus of starvation (Bergman 1971).

The continued high level of plasma glucose has been associated with the increase in glycogenolysis that occurs during starvation (Kronfeld, Simesen and Dungworth 1960). The fall at 32 hours of starvation may be explained by depletion of liver glycogen reserves, since plasma glucose values are directly related to liver glycogen concentration and both decrease during starvation (Kronfeld et al. 1960, Pehrson 1966, Baird et al. 1972). The rate of glycogenolysis and gluconeogenesis falls in the presence of increased amino acids and other nutrients (Kertz, Prewitt, Lane and Campbell 1969) and this might explain the fall in plasma glucose levels 8 hours after reintroduction of feed.

The fall in plasma Pi values in the first 24 hours of starvation could have been due to decreased phosphate absorption from the gut. The rise thereafter to the end of starvation period might reflect increased tissue (especially protein) catabolism and Pi release that occurs during periods of inadequate energy supply (Dale et al. 1954, White et al. 1956). The continued rise 8 hours after feeding was probably due to cessation of Pi excretion in urine together with increased phosphate absorption from the gut (Dale et al. 1954). The subsequent fall after 8 hours post-starvation may have been due to increased Pi utilization for formation of high energy phosphate bonds and other organic phosphorus containing compounds (Robertson et al. 1960).

The gradual decrease in plasma Ca levels with starvation was similar to the observations of Halse (1958) and Herd (1966) and could be due to reduced Ca absorption from the gut. The quick recovery of Ca levels after introduction of feed reflected the ability of animals to rectify Ca levels by both Ca mobilization of bone reserves and absorption from the gut (Herd 1966).

The decrease in PMg concentrations during starvation may have been partly due to the absence of Mg absorption from the gut into circulation (Herd 1966) and partly due to chelation of Mg by free fatty acids (FFA) (Flink, Flink, Shane, Jones and Steffes 1973). During starvation there is increased production of FFA as a result of catabolism of body fat reserves (Rayssiguer 1977; Tilakaratne et al. 1980; Sinnet-Smith, Slee and Wolliams 1987) which would lead to a decrease in PMg concentrations following tissue uptake of FFA-Mg complex (McAleese et al. 1961; Rayssiguer 1977). Thus the decrease in PMg concentrations could reflect the animals ability to utilize body fat during periods of energy deficiency. Animals of good genetic merit (for milk production) have been shown to have higher FFA levels than those of poor genetic merit during periods of energy deficiency (starvation) (Tilakaratne et al. 1980). The implication of this is that (calves) which utilize body fat reserves in preference to body proteins obtain more energy since the metabolism of fat is more

energy yielding than an equivalent amount of body proteins (Webster 1978). Therefore such animals should show better production (growth rates).

The increase in PNa concentrations may have been due to the reduction in plasma volume that occurs during starvation (Pehrson 1966, Rumsey and Bond 1976). The PK levels should have increased with starvation along with PNa levels. However, this did not occur and the observed decrease in PK levels may have been due to the stress of starvation. This could have led to an increase in plasma corticosteroids levels with subsequent increase in urinary K excretion (Trape 1984) and hence cause a decrease in PK levels. Similar decreases in PK levels have also been reported in stressful conditions such as forced recumbency in cows (Daniel 1977). The decrease in PK levels during starvation may thus reflect the animal's (calf's) ability to respond and possibly adapt to stressful condition(s) which could otherwise limit its performance. PK concentration is not always a meaningful indicator of the body K status (Rose 1981). This is because most of the K is intracellular and is not consistently related to the plasma K concentration (Muylle, Van den Hende, Nuytten, Deprez, Vlaminck and Oyaert 1984).

The rise in BUN concentration could have been due to the probable increase in protein catabolism occuring during starvation (periods of energy deficiency)(Dale et al. 1954,

Dalton 1967). Similar observations have been reported in young calves by others (Tilakaratne et al. 1980, Sinnet-Smith, Slee & Woolliams, 1987). These workers also found that calves of low genetic merit had higher BUN levels than calves of good genetic merit. Freeman et al. (1978) also observed similar differences in BUN concentrations between high and low yielding dairy cows. As pointed out when discussing PMg changes, animals which utilize body proteins (i.e. high BUN levels) more than body fat will have less available energy for production (growth) than those which utilize body fat more than body proteins during periods of energy deficiency. Thus changes in BUN levels during starvation could be used to identify animals which do (or do not) preferentially utilize body protein and hence have a reduced level of energy available for production during periods of energy deficiency.

The increase in WCC's may have been due to the stress of starvation. Starvation could have led to an increase in plasma corticosteroid levels which promote an increase in WCC's (Smith and Merrill 1954, Wegner and Stott 1972). Thus changes (increase) in WCC during starvation may reflect an animal's ability to respond and possibly adapt to stressful conditions that could limit their performance.

The decrease in EMg concentration during starvation could have been due to diffusion of Mg from the erythrocytes into the extracellular fluid. During starvation there is a decrease in erythrocyte ATP level (Baird et al. 1972) and in PMg concentration (Herd 1966), both of which could result in an increased cell membrane permeability (Elin et al. 1973) and an increase in the labile (diffusable) EMg concentration (Rose 1968, Bunn et al. 1971). Reduction in erythrocyte ATP and PMg concentrations has been reported to reduce the activity of Na-K-Mg ATPase and also to lead to increased cell membrane permeability (Elin 1973, Cantley and Josephson 1976). Most (70-80%) of the EMg is bound in an indiffusable form to ATP (Rose 1968, Bunn et al. 1971), hence reduction in erythrocyte ATP could increase diffusible (unbound) EMg levels and lead to some loss of EMg into the plasma. This would be assisted by the changes in cell membrane permeability.

The decrease in EMg concentration during starvation could also be related to utilization of body fat reserves through decrease in PMg (see PMg discussion). Thus the decrease in EMg concentration might be related to an animal's ability to mobilize sufficient energy for production during periods of energy deficiency.

The increase in ENa and the decrease in EK levels could have been due to increased influx of Na into the

cells and increased outflux of K out of the cells as a result of reduced activity of Na+K+Mg ATPase (as suggested above). The low PMg concentration observed during starvation could have reduced the erythrocyte electrolyte pump activity and hence increased Na influx into cells and K outflux out of the cells. Low PMg concentrations have been reported to reduce erythrocyte electrolyte pump activity in rats (Welt 1964, Wang et al. 1971). Changes in EK concentrations also occur in animals as a result of either metabolic or respiratory acidosis (Frape 1984). The increase in hydrogen ions induces release of K^+ ions out of the cells into extracellular components with subsequent urinary excretion (Flink 1975, Flink and Luttgau 1976, Frape 1984). To maintain the intracellular cation-anion balance a decrease in intracellular K concentrations is accompanied by an increase in the intracellular Na concentrations (Hays and Swenson 1977). Therefore the increase in ENa and the decrease in EK concentrations could also have been as a result of metabolic acidosis which is known to occur during starvation periods (Baird et al. 1972). The changes in both ENa and EK concentrations could be related to the animal's ability to mobilize sufficient energy for production during periods of energy deficiency through changes in PMg concentration.

(b) Individuality of response

The changes in most of the blood components during starvation showed significant differences among calves (Table 21). These among calves differences in response to starvation suggest that individual calves respond differently to the stress of starvation. Since some of the responses were significantly correlated with growth rate (Table 20), it suggests that calves might be selected for growth rate according to their blood chemistry response to stress of starvation (multiple regression equations 2, 3 and 4).

(c) Correlation of changes in blood components during starvation with growth rate

An association between growth rate and concentrations of blood components has been shown by several workers (Payne et al. 1973a, Rowlands et al. 1974, Little et al. 1977). However, there is no data on the correlations between growth rate and blood components changes that occur during the challenge of starvation.

In the present study changes in PMg, EK, BUN, and EMg concentrations and WCC's were significantly correlated with growth rate. The relative responses of BUN and FFA concentrations to fasting in Friesian calves of differing merits (Tilakaratne et al. 1980; Sejrsen, Larsen

and Anderson 1984; Sinnett-Smith et al. 1987) suggest that animals of high dairy merit derive energy preferentially from fat stores. The present study, indicates that during fasting circulating BUN concentrations are lower in calves with low growth rate. This implies that fasting circulating BUN concentrations could be a useful predictor of growth rate in young calves.

The relationship between changes in PMg, EMg and EK concentrations during starvation and growth rate could have been related to increased plasma FFA levels (as suggested earlier). That is, calves with higher FFA levels than BUN levels would have a greater decrease in PMg, EMg and EK concentrations during periods of energy deficiency. Thus the significant correlation between rates of decrease in PMg, EMg and EK concentrations with growth rate would be in accordance with the results of Tilakaratne et al. (1980), and such changes could possibly be used to identify calves of good genetic merit in terms of growth rate.

The positive correlation ($P<0.06$) between the changes in WCC's and growth rate suggest that the changes in WCC levels could be used to determine an animals response and possibly its ability to adapt to a stressful condition without compromising energy requirements for weight gain.

Regression analyses were carried out to relate growth rate and changes in blood components during starvation. Examination of equation 4 indicates which variables accounted for most of the variation in growth rate. This equation accounted for 62.4% of the variation in growth rate, thus most of the important blood factors that can be used to predict growth rate were included.

The change in albumin concentration during starvation gave a significant regression coefficient with growth rate. This could indicate that calves which are able to increase plasma albumin levels during periods of reduced feed intake have a higher growth rate. Rowlands et al. (1974) and Rowlands and Manston (1976) reported a significant relationship between albumin concentrations and growth rate in young calves. The significant negative regression coefficients between PCV change and growth rate would indicate that calves which are not able to maintain their PCV levels during periods which can cause dehydration have a low growth rate. The significant positive partial regression coefficients for PMg and EMg changes during starvation on growth rate indicate that calves which showed greatest changes in PMg and EMg concentrations during starvation have higher growth rates.

The results of the multiple regression analyses indicate that changes in PCV, albumin, PMg and EMg levels during starvation are worthy of further investigation as a method

of predicting growth rate of calves. The relationships derived from this study should be tested on a larger number of calves under differing environmental conditions before a reliable predictive formula could be developed. Because the change in plasma albumin concentration only increased the multiple R from 0.778 to 0.790 a predictive formula based on changes in PCV and PMg and EMg concentrations might be adequate (i.e. equation 3).

(b) CHANGES IN BLOOD COMPONENTS AND LEUCOCYTE COUNTS IN DAIRY HEIFERS AFTER INJECTION OF ACTH AND THE RELATIONSHIP OF SUCH CHANGES TO GROWTH RATE

INTRODUCTION

The normal response to a wide variety of physical and psychological stresses is the adrenocorticotropic hormone (ACTH) mediated synthesis and release into the blood of large quantities of adrenocorticosteroid hormones (Cope 1972, Paape et al. 1977, Gwazadanskas et al. 1980). In cattle and sheep, circulating corticosteroid concentrations increase following stressors such as transport, confinement (Reid and Millers 1962) heat, cold (Reid 1962, Paape et al. 1974) and diseases (Dvorak 1971, Robertson, Mixner, Barley and Lennon 1958).

The adrenal corticoids, mainly cortisol in the cow (Venkataseshu and Estergreen 1970) cause or permit physiological adjustments, enabling the animal to tolerate stress (Cope 1972). An impaired ability to increase and/or to maintain elevated plasma corticoids during exposure to a stressor stimulus to which an animal is not adapated would reduce its tolerance to the stress. In dairy cows, Trimberger et al. (1972) reported an abnormally high mortality rate for three consecutive lactations in animals with impaired ability to tolerate stress at parturition and during early lactation.

The response to exogenous ACTH provides a measure of the animals ability to withstand stress. Therefore the degree of change in blood chemistry induced by exogenous ACTH could differ between animals even though the dose is similar for all individuals. If individuality of response occurs, this response could be an index of the animals adaptability to adverse conditions. If such individuality of response were associated with physiological function, then the indices may serve as a criterion for the selection of animals for breeding purposes. The ability to adapt to stress with minimum expenditure of energy should enable agricultural animals to divert maximum amount of energy towards agricultural production (Schultze 1959).

To explore further the use of ACTH stimulated stress response, the present study was undertaken to evaluate the changes in blood chemistry of replacement dairy heifers after exogenous ACTH administration and to determine if the rate of change was characteristic of the individuals, if it differed between individuals and if it was related to growth rate.

MATERIALS AND METHODS

Experimental animals

Ten replacement dairy heifers, five Friesians and five Jerseys with mean (± SEM) age of 14 ± 0.4 months and weighing 260 ± 7.8 kg (mean ± SEM) and with mean (± SEM) growth rate of 15.1 ± 0.9 kg/month were used. Five heifers selected at random were each given one intramuscular injection of 100 iμ of porcine ACTH diluted to 5 ml in physiological saline (Corticotrophin)[1]. The other group of five heifers was given one intramuscular injection of 5.0 ml of sterile physiological saline solution as control. The saline treatment in addition to acting as control also provided an opportunity to study the effects of repeated sampling stress on blood components of young cattle.

Blood collection

Blood samples were taken at time 0 (pre-injection), 1, 2, 4, 6, 8 and 24 hours (post-injection) from the jugular vein. During the blood collection period the heifers were given lucerne hay and water ad libitum. After one week the experiment was repeated with the group which had been given ACTH now receiving saline solution and the control group receiving ACTH. Thus responses in 10 animals were available for each treatment. The same design of the experiment was repeated again after 8 weeks.

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[1] Arnolds Coy, Reading, England

Sample preparations and analytical techniques

These have been described earlier in the section on general materials and methods. The samples were analysed for PCV, Hb, plasma glucose, total proteins, albumin, globulin, Mg, Pi, Ca, Na, K, BUN and creatinine, erythrocytes Mg, Na and EK, WCC's, RCC's and differential leucocyte counts.

Statistical analysis

The mean levels of each component at each sampling time were found and compared by student's T-test. Correlations were calculated between blood composition changes after ACTH injection and monthly growth rates. The independent variables used in the correlations and later in multiple linear regression analyses were expressed as the arithmetic difference between time 0 and time 4 hrs or 6 hrs (depending on which of these times the mean response was maximal for each component - most of the components reached their peak or lowest level at one or the other of these times) according to the method of Stevenson and Britt (1980). The variables used in the multiple linear regression analyses were those which showed significant ($P<0.05$) correlation coefficients with growth rate. The variations in blood chemistry and leucocyte responses between animals and between each set of experiments (8 weeks apart) following ACTH administration were compared by analysis of variance.

RESULTS

The results of the changes that occurred after exogenous ACTH injection in heifers in the first experiment are shown in figures 8a to 8u. Table 24 shows variations in responses due to ACTH treatment in the 10 heifers for each of the two occasions. There were no significant differences between mean responses on each occasion but there were between animal differences in some responses.

PCV and Hb concentrations did not show any significant changes after ACTH or saline treatment (Figs. 8a and 8e). TPP, plasma albumin and globulin concentrations and RCC's showed a tendency to increase with time in both ACTH and saline-treated groups (Figs. 8b, 8c, 8d and 8h).

Plasma glucose concentration remained unchanged after saline injection but increased by 28.5% ($P<0.01$) by 6 hours after ACTH injection, and its values remained higher ($P<0.05$), than the base line at 24 hours post ACTH injection (Fig.8i).

Plasma Pi and creatinine concentrations did not show any significant change in either ACTH or saline-treated groups (Fig. 8g). Plasma Ca and BUN concentrations increases reached significant ($P<0.05$) levels only in the ACTH-treated group 4 hours after ACTH injection (Figs. 8j and 8k).

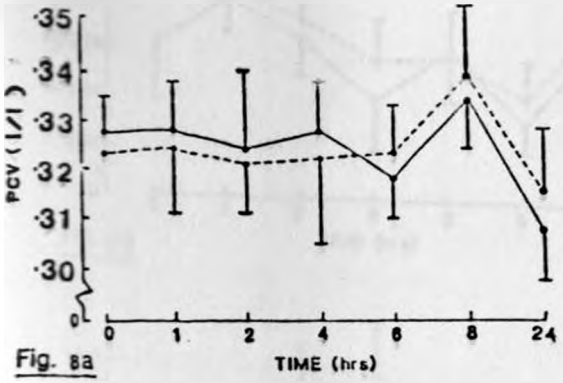


Fig. 8a

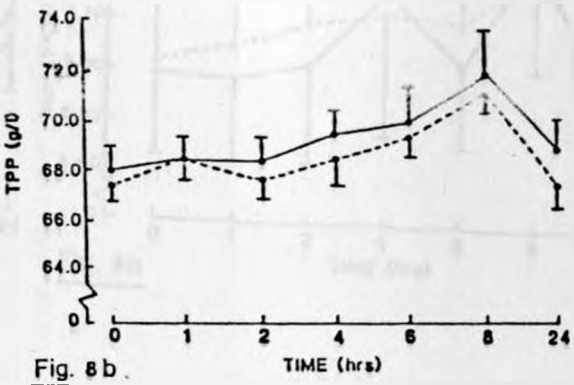


Fig. 8b

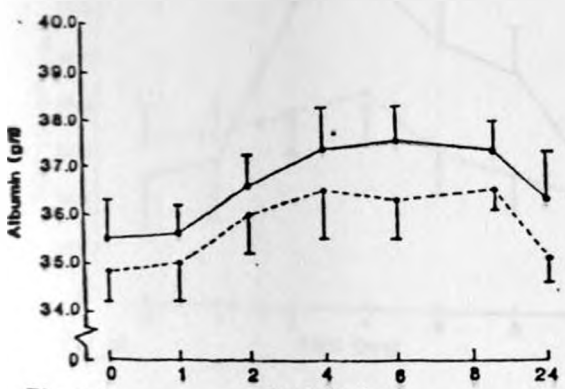


Fig. 8c

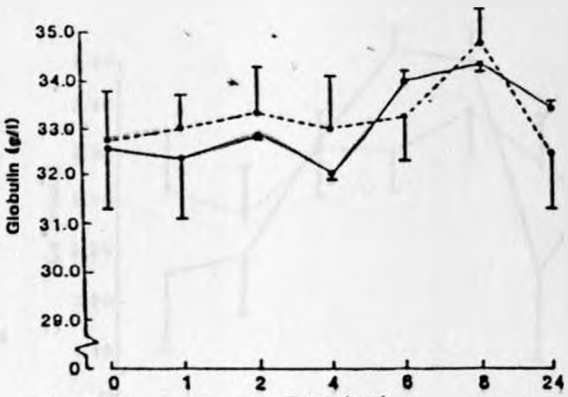


Fig. 8d

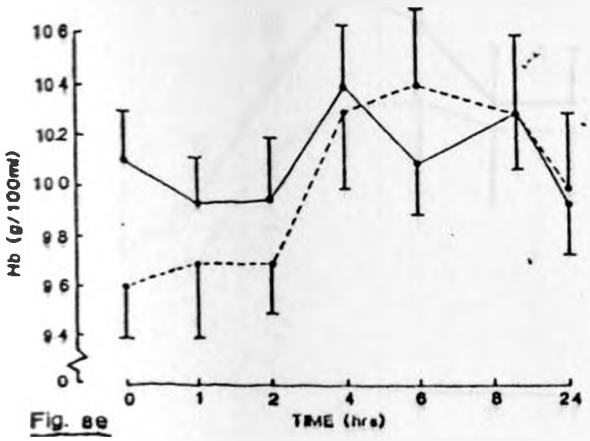


Fig. 8e

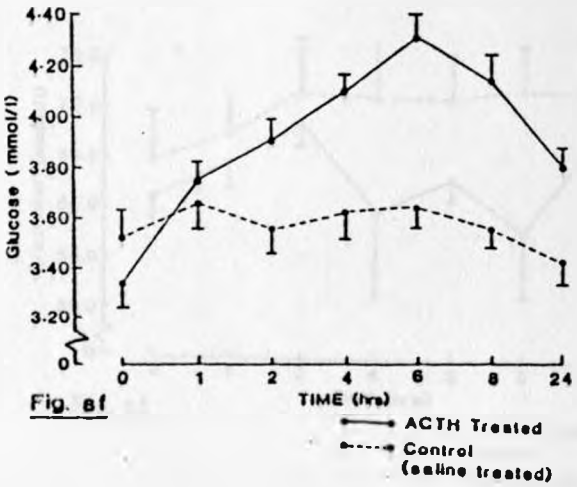


Fig. 8f

Figures 8a-8f. Changes in PCV's, TPP, albumin, globulin, Hb and plasma glucose (mean ± SEM) concentrations in 10 heifers (14 months old) after ACTH and 0.9% saline solution administration (first experiment)

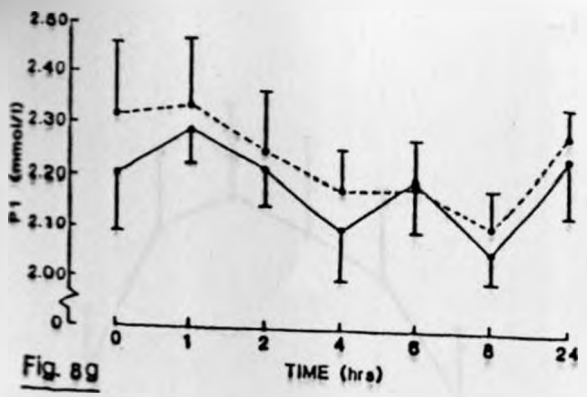


Fig. 8g

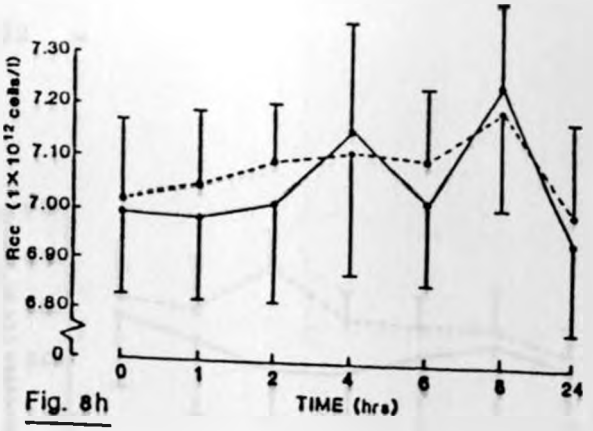


Fig. 8h

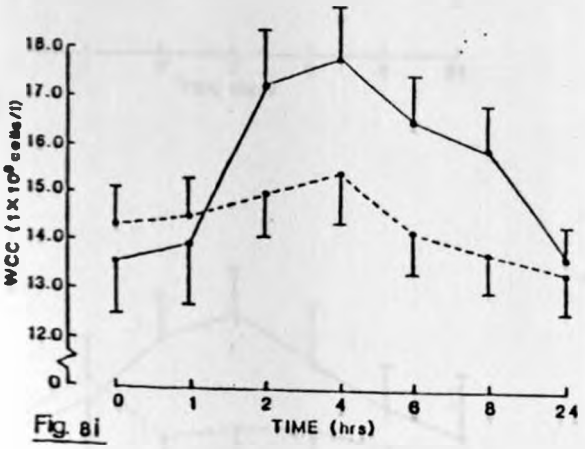


Fig. 8i

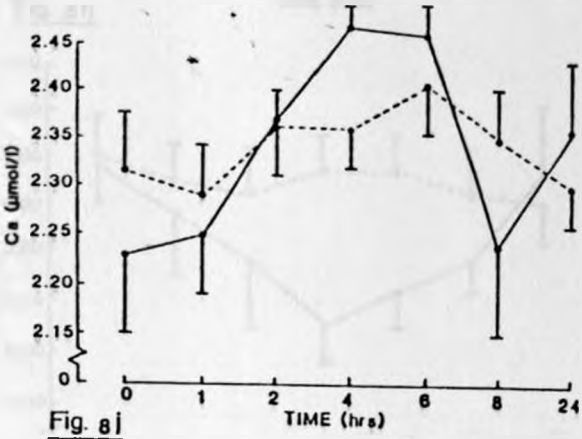


Fig. 8j

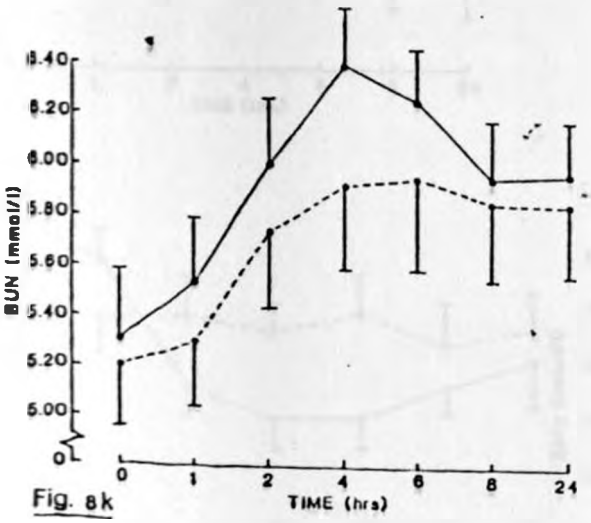


Fig. 8k

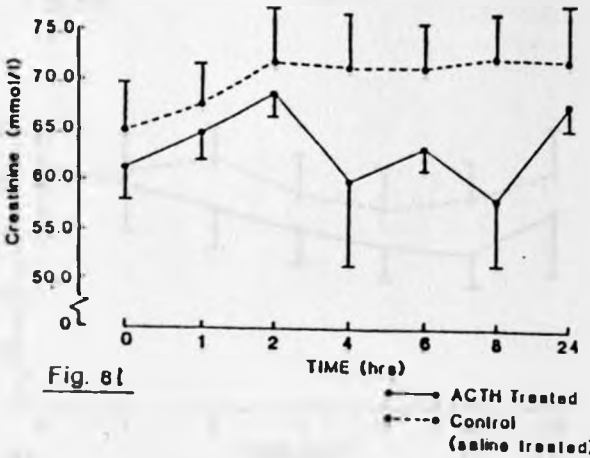


Fig. 8l

ACTH Treated
Control
(saline treated)

Figures 8g-8l. Changes in Pi, RCC, WCC, Ca, BUN and creatinine (mean ± SEM) values in 10 heifers (14 months old) after ACTH and 0.9% saline solution administration (first experiment)

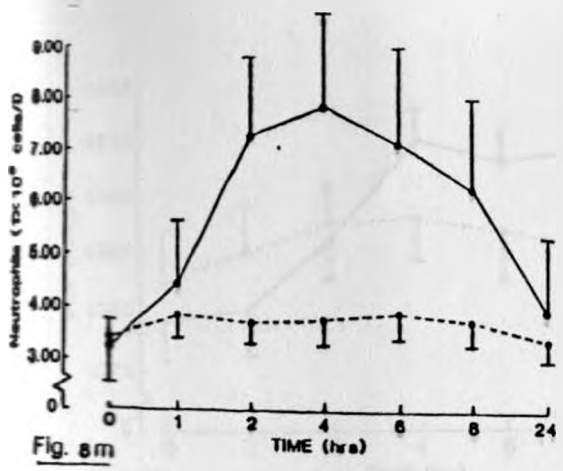


Fig. 8m

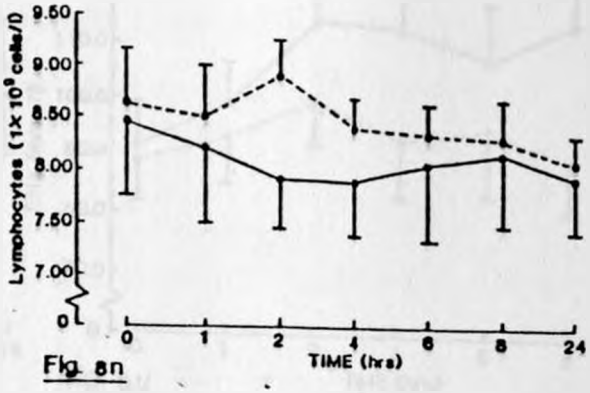


Fig. 8n

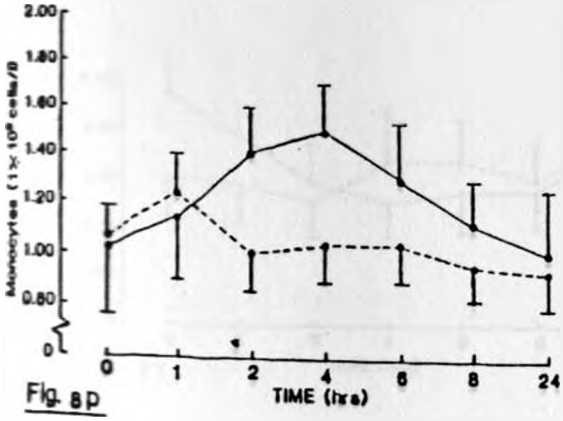


Fig. 8p

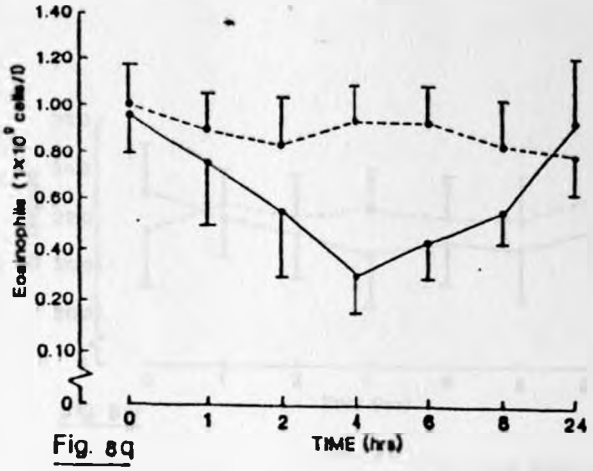


Fig. 8q

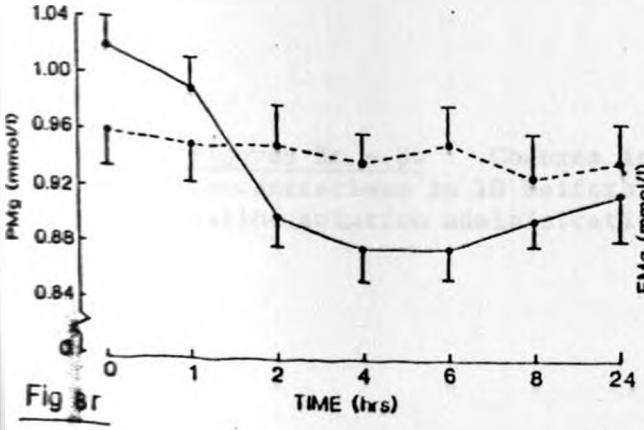


Fig. 8r

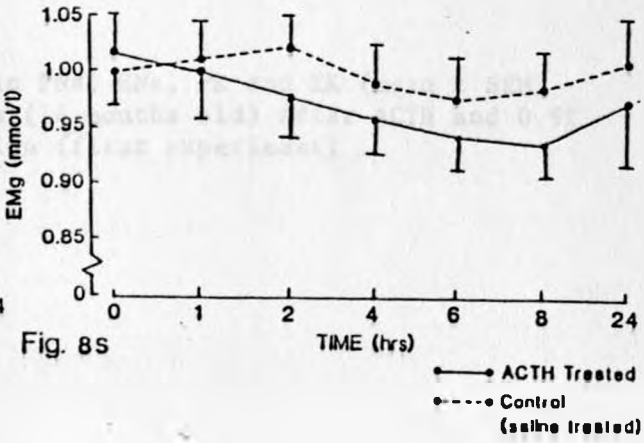


Fig. 8s

Figures 8m-8s. Changes in PNa, ENa, PK and EK (mean ± SEM) concentrations in 10 heifers (14 months old) after ACTH and 0.9% saline solution administrati (first experiment).

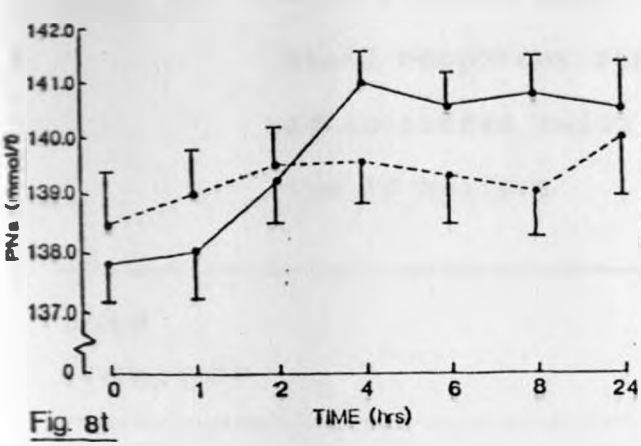


Fig. 8t

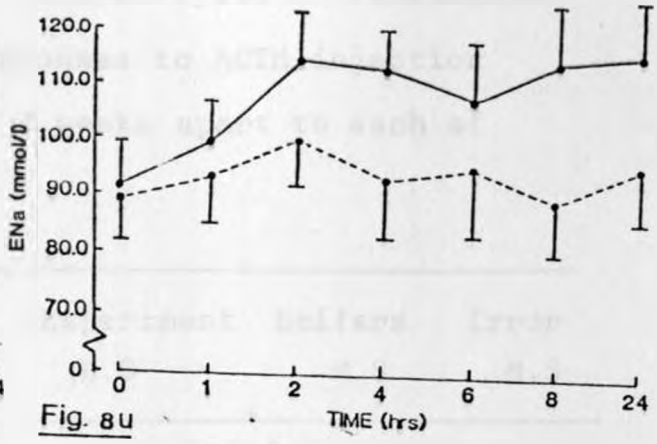


Fig. 8u

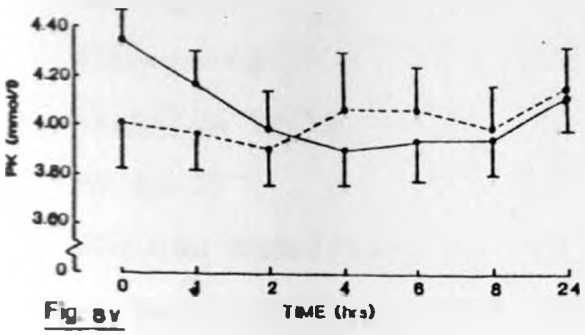


Fig. 8v

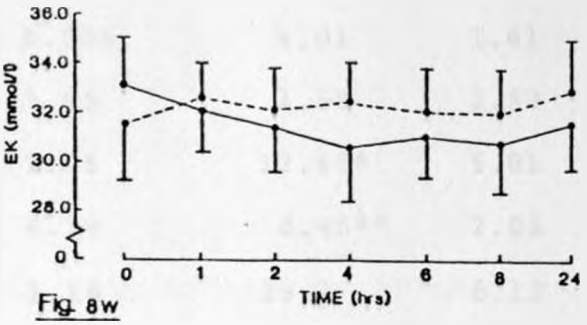
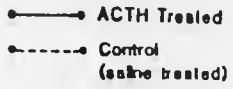


Fig. 8w



Figures 8t - 8w Changes in PNa, ENa, PK and EK (mean ± SEM) concentrations in 10 heifers (14 months old) after ACTH and 0.9% saline solution administration (first experiment)

Table 24. Mean squares (M.S) from analysis of variance of blood component responses to ACTH injection administered twice 8 weeks apart to each of the 10 heifers

Blood components	+Experiment M.S	Heifers M.S	Error M.S
PCV (l/l)	0.05	0.250	0.161
TPP (g/l)	2.45	7.12	3.56
Albumin (g/l)	0.005	4.01	1.61
Globulin (g/l)	2.45	1.69	2.89
Hb (g/l)	2.45	12.69*	5.01
Glucose (mmol/l)	4.14	8.46**	2.09
Pi (mmol/l)	1.28	19.1	6.13
WCC ($x10^9$cells/l)	0.42	2.68*	0.99
Neutrophils ($x10^9$cells/l)++	0.145	3.59**	0.66
Lymphocytes ($x10^9$cells/l)++	0.28	12.5**	0.69
Monocytes ($x10^9$cells/l)++	6.73	27.96**	3.29
Eosinophils ($x10^9$cells/l)++	0.72	23.07**	3.29
Ca (mmol/l)	0.113	1.29	1.27
BUN (mmol/l)	0.20	21.42**	2.64
Creatinine (umol/l)	2.45	3.45**	0.78
EMg (mmol/l)	0.18	30.2**	5.80
ENa (mmol/l)	0.50	45.00	27.0
EK (mmol/l)	0.50	42.7*	9.39
PMg (mmol/l)	0.08	2.38*	0.30
PNa (mmol/l)	1.25	7.05**	0.47
PK (mmol/l)	2.45	5.33**	0.89

* $p<0.05$, ** $p<0.01$

\+ Experiment refers to each of 2 ACTH injections experiments on the same 10 calves

++ Absolute counts

The mean circulating neutrophil counts remained unchanged in the saline-treated group but increased significantly ($P<0.01$) by 2 hours in the ACTH-treated group. The counts reached the highest values of 7.81×10^9 cells/l 4 hours post ACTH inoculation and returned to the baseline values 24 hours after ACTH administration (Fig. 8m). The percentage of neutrophils in the differential cell counts increased significantly ($P<0.01$) by 2 hours and increased further by 4 hours after ACTH injection. No significant change was observed in circulating lymphocyte counts in both ACTH- and saline-treated groups (Fig. 8n). However, the relative percentage of lymphocytes decreased by 30% ($P<0.01$) 4-6 hours post-ACTH injection and returned to values near the baseline levels 24 hours after ACTH treatment. Neither the percentage nor the concentration of circulating monocytes was affected by either ACTH or saline treatment (Fig. 8p). Both the percentage and concentration of circulating eosinophils decreased significantly ($P<0.001$) by 70% and 68.7% respectively 4 hours after ACTH administration and returned to baseline values 24 hours post-ACTH treatment (Fig. 8q).

The PMg concentrations decreased significantly ($P<0.01$) 6 hours after ACTH administration (Fig. 8r). The EMg concentrations showed a tendency to decrease ($P<0.07$) within 6 hours after the ACTH administration and then returned towards the original values 24 hours post-ACTH injection (Fig. 8s).

PNa concentrations increased significantly ($P<0.05$) by 4 hours post-ACTH treatment and remained slightly higher than the baseline values 24 hours after ACTH administration (Fig. 8t). ENa concentrations increased significantly ($P<0.05$) within 2 hours following ACTH administration and the values remained significantly ($P<0.05$) higher than the baseline values 24 hours after ACTH treatment (Fig. 8u). In heifers given saline solution ENa concentrations did not show any particular trend, however the values for individual animals in the 24 hour-period varied from 75-115 mmol/l.

The PK concentrations decreased significantly ($P<0.05$) 4 hours after ACTH administration and remained significantly ($P<0.05$) lower than the baseline levels 24 hours after ACTH injection (Fig. 8v). A decrease in EK concentrations approached significance ($P<0.06$) 4 hours after ACTH administration (Fig. 8w).

Correlations between blood components changes after ACTH injection and growth rate.

The simple correlation coefficients describing the relationships between growth rate and responses to ACTH administration are shown in Table 25.

Table 25. Correlation coefficients (r) between blood component changes after ACTH injection and growth rate in 10 dairy heifers

Blood parameter	r1	r2
PCV	0.106	0.150
TPP	0.096	0.226
Albumin	0.008	0.254
Globulin	0.025	0.025
Hb	0.008	0.302
Glucose +	0.779**	0.692**
Pi	0.152	0.170
WCC	0.584(P<0.06)	0.568(P<0.06)
Neutrophils ++	-0.133	-0.217
Lymphocytes ++	0.184	0.356
Monocytes ++	0.152	0.016
Eosinophils ++	0.775**	0.768**
Ca	-0.272	-0.502
BUN	0.584*	0.642*
Creatinine	0.399	0.441
EMg +	-0.413	-0.435
ENa	0.152	0.063
EK	0.087	0.311
PMg +	0.271	0.190
PNa	-0.543(P<0.07)	-0.565(P<0.06)
PK	-0.196	-0.211

* P<0.05, ** P<0.01

r1 = First ACTH treatment, r2 = Repeat ACTH treatment

\+ = Taken at 6 hours, others at 4 hours

++ = Absolute counts

Growth rate was most closely correlated with changes in glucose and eosinophils followed by changes in BUN, WCC and PNa. The changes in glucose, eosinophils, WCC and BUN were positively correlated with growth rate while that of PNa was negatively correlated with growth rate.

Multiple linear regression analyses

These were carried out to relate growth rate with mean changes in blood components and leucocytes after ACTH injection and to assess whether such changes could be used to predict growth rate.

Since there is no universal agreement among statisticians as to which multiple regression procedure is most advantageous in determining the best regression model (Zar 1974) only blood component changes with significant ($P<0.05$) correlation coefficients with growth rate (Table 25) whose means (± SEM) are shown in Table 26 were used in the multiple regression analyses. The multiple regression equations for each ACTH treatment are shown below. Attached to each equation are the multiple coefficient of determination (R^2), multiple correlation coefficient (R), standard error of estimate (SEE), standardized regression coefficients (B), standard error of the partial regression coefficients (SEB), "Students" T value of the partial regression coefficients (T value b) and F value for the multiple regression equation.

Table 26. Mean changes (± SEM) for variables in the multiple regression analysis (n=10). Dependent variable = growth rate

Blood components		Mean ± SEM
Glucose (mmol/l)	1	0.90 ± 0.060
	2	0.97 ± 0.080
WCC ($x10^9$cells/l)	1	4.85 ± 0.231
	2	5.06 ± 0.383
Eosinophils (x10 cells/l)	1	5.90 ± 0.658
(Absolute count)	2	6.20 ± 0.850
BUN (mmol/l)	1	0.85 ± 0.100
	2	0.84 ± 0.120
PNa (mmol/l)	1	4.30 ± 0.540
	2	4.80 ± 0.670

1 = First ACTH treatment experiment

2 = Repeat ACTH treatment experiment

Growth rate = 12.9+(.76 x Glu)+(.43 x WCC)+(.4 x Eo)+
(.78 x BU)+(-.63 x PNa) (1)

(R2=0.569, R=0.754, SEE=0.848, SEB1=2.92, SEB2 = 0.30, SEB3=0.24, SEB4=1.40, SEB5=0.235, T Value b1 = 1.02, T Value b2 = 0.96, T Value b3 = 2.04, T Value b4 = 0.56, T value b5 = 2.68*, F value = 8.18**)

Growth rate = 8.13+(2.29 x Glu)+(.66 x WCC)+(.31 x Eo)+
(1.08 x BU)+(-.3 x PNa) (2)

(R2=0.626), R=0.791, SEE=0.37, SEB1=0.85, SEB2=0.12, SEB3=0.11, SEB4=0.63, SEB5=0.01, T value b1 = 2.68*, T value b2 = 5.49**, T value b3 = 2.80*, T value b4 = 1.73, T value 5 = 5.11**, F value = 46.4**).

Where Glu is glucose (mmol/l)

WCC is WCC's (x10 cells/l)

Eo is absolute eosinophils counts (x10 cells/l)

BU is BUN (mmol/l)

and PNa is plasma Na (mmol/l) changes after ACTH injection.

In both experiments the changes in glucose, WCC's, absolute eosinophil counts, BUN and PNa concentrations gave significant multiple correlation coefficients with growth rate, however the F value was higher in the multiple regression analysis from experiment 2.

The changes in absolute eosinophil counts, PNa and WCC's were the most important variables in predicting growth rate. The partial regression analysis shows that in analysis one, only the change in PNa had a significant ($P<0.05$) T value while in analysis two all the variables except change in BUN gave significant T values. The results show that calves with the greatest absolute eosinophil counts, PNa, glucose & WCC's responses after ACTH administration had the higher growth rates.

DISCUSSION

(i) Changes in blood chemistry following ACTH administration in replacement dairy heifers

The adrenal cortex has been assigned a role in increasing the resistance of an animal to stress (Sayers 1950, Cope 1972). ACTH is the major hormone secreted by the adeno-hyphophysis in response to stress and this hormone stimulates the synthesis and secretion of corticosteroids (Sharyanrar et al. 1975, Paape et al. 1977, Gwazadanskas et al. 1980) and mineralocorticoids (Crabbe et al. 1959). The secretion of these hormones brings about changes in the blood components and the circulating leucocytes. The injections of exogenous ACTH have been used in the cows to assess adrenal function during physiological states such as high environmental temperature (Sharyanfar et al. 1975), long-term

feeding of imbalanced or deficient diets (Smith et al. 1975), social disruption, high cow density situations (Friend, Palan, Gwazadanskas and Heald 1977) and lactation (Gwazadanskas et al. 1980). The results from these studies show that cows under prolonged stress have reduced synthesis and release of adrenal corticosteroids after exogenous ACTH administration and their ability to withstand stress to which they are not adapted is reduced. This may contribute to a number of stress-associated diseases. The dose rate of 100 iu of ACTH per animal used in this experiment should have provoked maximum plasma corticosteroids response since according to Paape et al. (1977), this dose would produce the same plasma corticosteroids response as acute coliform mastitis (very severe stress) in cows. Thus the changes observed in the blood components and leucocytes should reflect the animal's ability to respond to stress.

The changes in the blood components and leucocytes were similar to those reported previously by other workers (Merrill and Smith 1954, Pehrson 1966, Braun et al. 1970, Paape et al. 1974a, Paape et al. 1977).

The increase in plasma glucose concentrations after ACTH administration was probably due to impairment of glucose utilization (Braun et al. 1970) and/or increased rate of gluconeogenesis associated with increased glucocorticoid

levels (Wilcke and Davis 1982). Glucocorticoids promote the process of gluconeogenesis in the liver by enhancing the synthesis of enzymes needed to process amino acids into glucose. They also play a part in the gluconeogenesis process itself (Wilcke and Davis 1982). Thus the degree of increase in plasma glucose concentration probably reflected the amount of glucocorticoids released by the adrenal cortex following ACTH administration. Heifers differed significantly (Table 24) in their plasma glucose responses and this agrees with other observations (Xing et al. 1988). The increase in BUN concentration following ACTH administration was probably due to an increase in protein catabolism that occurs as a result of increased blood glucocorticoid concentration (Wilcke and Davis 1982).

The increase in PNa and decrease in PMg and PK concentrations following ACTH administration is similar to that reported by Wegner and Stott (1972). Corticotrophin releases aldosterone as well as glucocorticoids from the adrenal cortex (Mulrow 1967, Willard, Refsal and Thacker 1987). Thus aldosterone released after ACTH administration would stimulate renal absorption of Na and excretion of K and Mg ions (Mulrow 1967), resulting in elevated PNa and decrease in PK and PMg concentrations. The elevated PNa levels persisted for up to 24 hours post-ACTH injection. This could have been due to a persisting renal Na absorption mechanism possibly involving de novo synthesis of enzyme systems as suggested by Falchuk and Sharp (1980). The PMg

concentrations remained low even after 24 hours post-ACTH inoculation. This probably indicates that in addition to active secretion of Mg ions due to the effects of aldosterone (Davanzo et al. 1958) the hypomagnesaemia could also have been due to a competitive ion effect in the kidney tubules (Samiy, Brown and Globus 1960).

The increase in plasma Ca after ACTH administration was similar to the reports of Westermarck (1953) who observed an increase in serum Ca of cows suffering from milk fever after ACTH treatment. However, it differed from the observations of Sayers (1960) and Wegner and Stott (1972) who observed a decrease in plasma Ca after ACTH administration into cows. The explanation of this discrepancy is not forthcoming.

The leucocytosis provoked by ACTH administration could be attributed to the neutrophilia. Corticosteroids reduce neutrophil stickiness and diapedesis (Paape et al. 1971) which then releases neutrophils from the marginal pool resulting in an increase in circulating neutrophils and consequently WCC's (Paape et al. 1974a). The absence of a decrease in total circulating lymphocytes concomitant with a reduction in the percentage of circulating lymphocytes (i.e. relative lymphopaenia) observed after ACTH administration could have been due to the marked increase in circulating neutrophils (Fig. 8m). Absolute lymphopaenia in cattle has been observed following injections of 50-1,000 mg of 9-

-fluoroprednisolone, a synthetic corticosteroid that is 67 times more potent than the naturally-occurring corticosteroid (hydrocortisone)(Schalm et al. 1965). It has also been suggested that a very high concentration of corticosteroids can result in a different leucogram to that produced by the low levels of corticosteroids induced by ACTH injection (Paape et al. 1977).

The increase in ENa and decrease in EK concentrations after ACTH administration may have been due to the shift of fluid and electrolytes between extracellular and intracellular spaces (Tosteson 1963). The increase in PNa concentration probably leads to an uptake of Na ions by the erythrocytes while the decrease in PK concentrations could have led to diffusion of K ions from the erythrocytes into plasma (from the intracellular to extracellular space).

The heifers given saline solution showed no significant changes in any of the blood components due to repeated bleeding. This probably indicated that the animals adjusted to the stress of repeated sampling or the stress was not sufficient to cause any significant changes. In sheep and goats significant changes in PCV, PNa, WCC, eosinophil count, EK and Pi have been reported by several workers (Block 1958, Gartner et al. 1970). Thus the lack of any significant changes in any of the blood components suggested that these heifers

were not as susceptible to handling stress as are sheep and goats. Moving of yearling steers from a pen to a squeeze chute was found not to affect their plasma cortisol levels (Ray, Hansen, Theurer and Stott 1972).

There are differing reports on the effects of repeated sampling, excitement and handling of cattle on the levels of their blood components. Excitement and exercise (chasing of cows around a yard, unusual noises and pricking of the hindquarters) of dairy cows had no effect on their plasma Pi, Ca and Mg concentrations (Moodie and Robertson 1962). Excitation and handling (noises and forced running of 100m) of beef animals before sampling was found to elevate PCV, Hb, Pi, PK, TPP, albumin and globulin concentrations (Gartner et al. 1965). However, Gartner et al. (1969) reported a marked fall in PCV., Hb and RCC levels in Australian Illawarra Shorthorn cattle (10-14 months old) bled for the first time during the first week of repetitive sampling in an unfamiliar environment. However, no significant change in Hb, RCC and WCC was noted in Holstein calves (2 to 4 months old) following repeated sampling or injection of adrenaline (1.2ml of 1:1000 solution of adrenaline per 100 lb body weight) (Schultze 1959). Therefore it appears that excitement, exercise and animal temperament do not have any consistent effect on blood component concentrations of cattle.

(ii) Relationship between changes in levels of blood components and leucocyte profiles produced by ACTH injection and growth rate.

Injection of exogenous ACTH stimulates corticosteroid (Gwazdauskas et al. 1980) and mineral corticoid production (Grabbe et al. 1959) which results in changes in blood chemistry and leucocyte profiles. The intensity of the change varies between animals (Anderson 1954, Pehrson and Wallin 1966) and reflects the responsiveness of adrenal glands to induced stress (Paape et al. 1971, Paape et al. 1974). Pigs susceptible to stress have been shown to have muted adrenal responsiveness to injection of exogenous ACTH (Marple et al. 1972, Sebranek, Marple, Cassens, Brisky and Kastenschmidt. 1973). It has also been shown that repeated injections of exogenous ACTH render the adrenal glands less responsive to further ACTH injection (Marple et al. 1969). Thus animals which are stress-susceptible could have reduced adrenal responsiveness to either exogenous ACTH injections or any environmental stress. As a consequence these animals will show less changes in blood chemistry after exogenous ACTH injection. Animals which are able to adjust to adverse situations with minimum expenditure of energy will therefore be able to expend more energy on desired agricultural production (e.g. gain in body weight, milk production, etc).

Thus the significant relationship between changes in plasma glucose, eosinophils, WCC, BUN and PNa concentrations with growth rate probably reflects the animal's ability to adapt to environmental stresses which otherwise would drain energy required for weight gains.

The results of this study show that heifers with the highest growth rate had the greatest changes in absolute eosinophil counts, plasma glucose, PNa and BUN concentrations in response to ACTH injection. This would reflect their ability to adapt to environmental stress that could have reduced their growth rate. Hopwood and Tibolla (1958) reported that the percentage decrease in eosinophil counts after ACTH administration in calves of the same age appeared to have some relationship with body weight, heavier calves had the largest decrease in eosinophil levels. In dairy calves (6-17 months of age) the highest eosinophil counts were in those animals with the highest body weight (Schultze 1957). These workers concluded that the heaviest calves had lower levels of circulating adrenocortical hormones since the levels of circulating eosinophils are inversely related to the levels of circulating adrenocortical hormones (Merrill and Smith 1954). Thus the decrease in both the levels of circulating eosinophils and eosinophil counts after ACTH injection could be used to assess an animal's adrenal cortical responsiveness in the cow as has been previously suggested (Hopwood and Tibolla 1958).

The negative relationship between the change in PNa and growth rate would indicate that heifers with highest growth rate were able to maintain their Na homeostasis after ACTH injection. Stress-resistant pigs were shown to maintain their blood electrolyte and base homeostasis in several stress conditions while stress susceptible ones were not able to maintain their homeostasis (Forrest, Will, Schmidt, Judge and Brisky 1968).

Regression analyses were carried out to relate growth rate and changes in the blood chemistry and leucocyte profile after ACTH injection. Examination of the regression analysis showed that changes in eosinophil counts, WCC's, glucose and PNa concentrations were the most consistent variables that explained most of the variation in growth rate.

The results of this study demonstrate that mean changes in blood chemistry and leucocyte profiles after ACTH administration and their relationship with growth rate appear to be repeatable (Table 26) and are worthy of further investigations with a view to their use for predicting weight gains in replacement dairy stock.

CHAPTER 7

THE RELATIONSHIP BETWEEN BLOOD CHEMISTRY AND GROWTH RATE AND MILK PRODUCTION RANK IN REPLACEMENT DAIRY CALVES AND HEIFERS

INTRODUCTION

There is evidence of the individuality of the blood composition of growing animals over short (Rowlands et al. 1974) and long periods of time (Payne et al. 1973a). This individuality has been shown to be inheritable for certain blood constituents (Rowlands et al. 1974) (Table 27). These workers studied 242 calves (9-12 weeks old) and found that blood chemistry differed significantly beween individual calves and for most blood components the between calf variation accounted for 45 to 55% of the relevant total variance. Heritability estimates of levels of some blood components differ (Plum and Schultze 1958, Wiener and Field 1971, Rowlands et al. 1973), and this could indicate that estimates of heritability may be influenced by the environment and plane of nutrition.

Investigations on the relationships between growth rate and blood chemistry conducted by several workers have shown some inconsistency (Arthaud et al. 1959, Rowlands, et al. 1974, Payne et al. 1974, Kitchenham et al. 1975a, Kitchenham et al. 1977). This suggests that relationships between growth rate and blood chemistry found in one environment may differ from those found in another area.

Table 27 Some heritability estimates of blood components reported in dairy cattle

Blood Components	Rowlands et al. (1974)		Kitchenham and Rowlands (1976)	
	h	s.e	h	s.e
Hb	0.93	0.36	-	0.32
PCV	0.76	0.33	0.41	0.32
Glucose	0.74	0.32	-	-
K	0.40	0.32	0.53	0.34
Ca	0.29	0.20	0.46	0.33
Albumin	0.28	0.19	0.47	0.33
Pi	0.26	0.18	-	-
Mg	0.21	0.16	0.52	0.34
Na	0.09	0.12	-	-
Globulin	0.01	0.08	0.63	0.36
TPP	-	-	-.52	0.34

The present study was undertaken within one herd to provide further evidence of the relationships between blood chemistry and growth rate in a group of dairy replacement calves (2-13 months of age) and with both growth rate and milk production ranking in another older group (heifers 13-24 months of age).

MATERIALS AND METHODS

Experimental animals

Ten replacement heifers, five Friesians and five Jerseys running at pasture were blood sampled monthly from the age of one year until first calving. Nine replacement (female) calves, five Friesian and four Jerseys also running at pasture were blood sampled monthly for a year from the age of 2 months to 13 months.

The heifers were bled from the coccygeal blood vessels and the calves were bled from the jugular vein. The heifers and the calves were weighed at each blood collection time and growth rate expressed as Kg/month. Production rank was obtained from the Department of Primary Industries Herd Recording Data and was based on the milk production of each heifer over the first three months of lactation.

Sample preparation and analytical techniques

These have been described in the section on general materials and methods. PCV's and concentrations of Hb, plasma glucose,

Na, K, Mg, Pi, Ca, albumin, globulin and total protein were determined in each blood sample.

Statistical analysis

The significance of the differences in monthly mean levels of each component was estimated by Student's T-test to determine if there were any trends with increasing age. The data for each animal was then arranged into 6 "parts" (in each group - i.e. calves and heifers) as follows:

	Calves	Heifers	"part"
Blood component levels at(months)	2	12	i
	7	18	ii
	13	24	iii
Change in component level between (months)	2-7	12-18	iv
	7-13	18-24	v
Mean of the 12 monthly values (Overall mean)	-	-	vi
(Growth rate-Kg/month ± SEM	14.7 ± 0.6	15.1 ± 0.5	
(Production Rank mean ± SEM		115.3 ± 2.25	

This arrangement of the data gave combinations of up to 66 independent variables which could be inserted into multiple regression equations to predict growth rate in the calves and heifers and production ranking in the heifers. The levels at

start, mid-point and end of the observation period were chosen for practical reasons (any practical predictive test would preferably be based on less than 3 samplings) and because inspection of Figs. 9a-K and 10a-K, suggested that where marked trends occurred they tended to be before or after the mid-period. (see Figs 9d, 9f, 9g, 9K, 10a, 10d and 10g).

Initially each independent variable was correlated with growth rate and with production rank (heifers only) and any that were significantly ($P<0.05$) correlated with the dependent variables (growth rate or production rank) were used in the multiple regression analysis to select the best multivariate model. The procedure for the latter is described in Chapter 5.

RESULTS

(a) Relationships between age and blood chemistry

The relationships between age and blood component concentrations (monthly means) are shown in Fig.9 for calves and Fig. 10 for heifers.

The mean blood glucose concentrations at 13 months of age in the calves were significantly ($P<0.05$) lower than those at 2 months of age (Fig. 9a). In the heifers the mean blood glucose concentrations remained steady up to 20 months of age and then increased significantly ($P<0.05$) by about 0.92 mmol/l in the next two months and remained relatively high up to the end of the study period (24 months) (Fig. 10a).

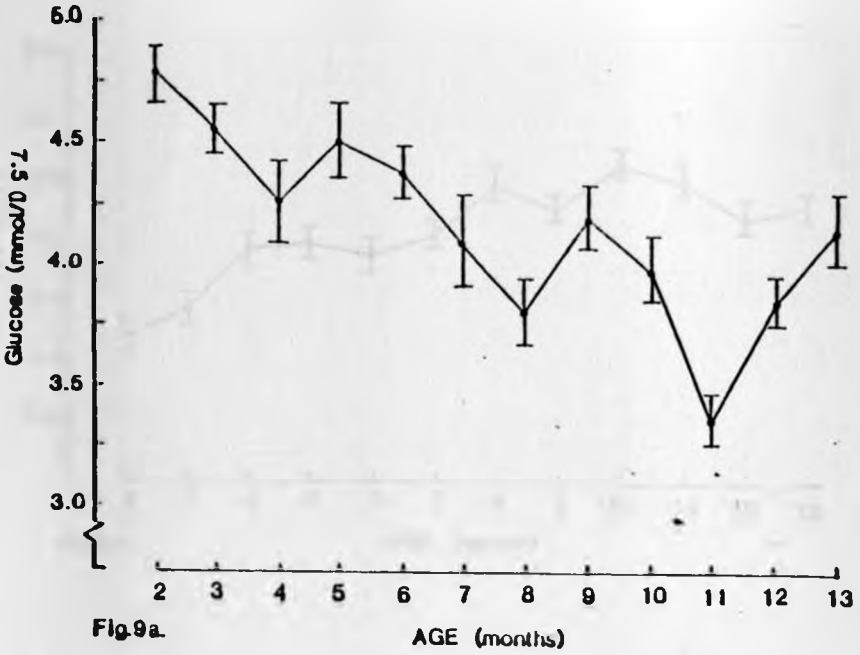


Fig.9a.

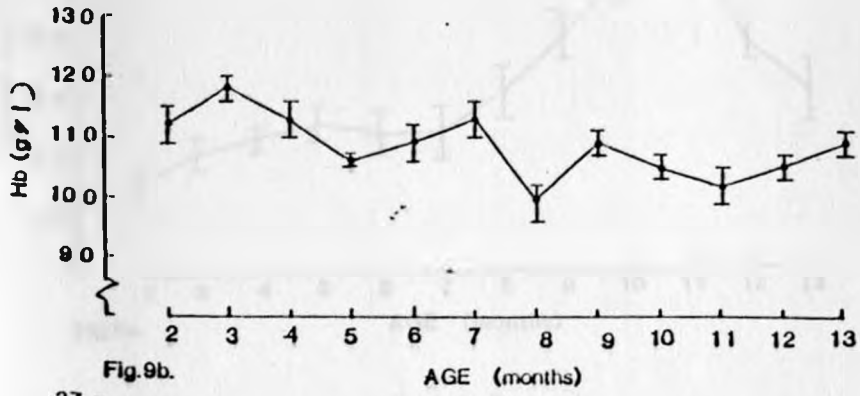


Fig.9b.

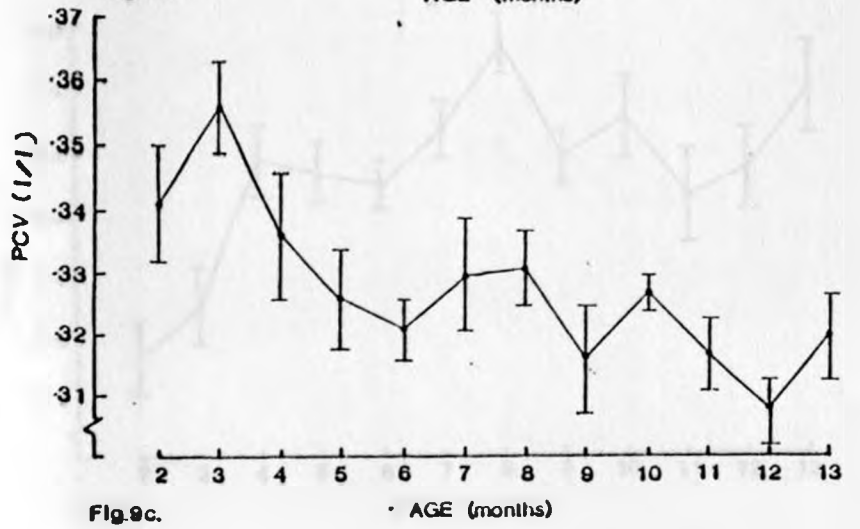


Fig.9c.

Figures 9a-9c. Changes in plasma glucose, Hb and PCV (monthly mean ± SEM) values in 10 calves (2-13 months)

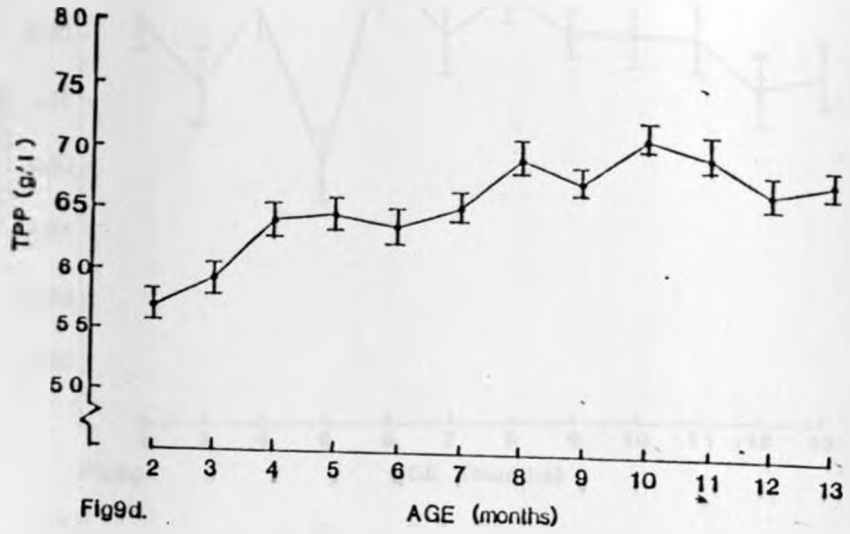


Fig9d.

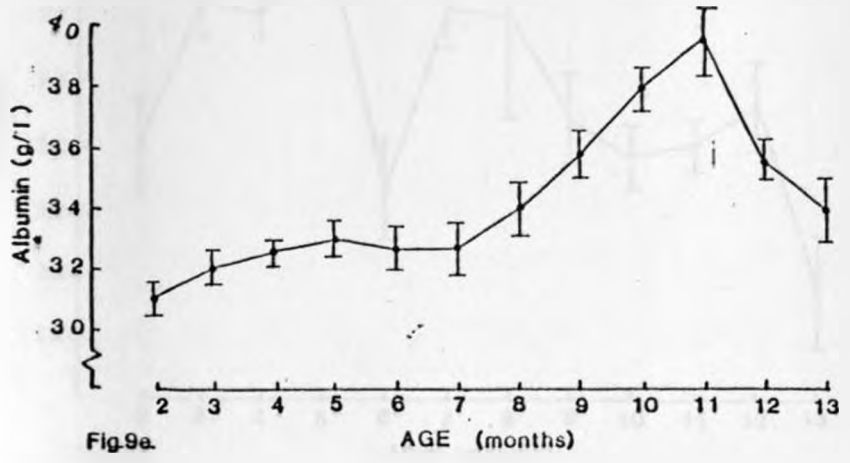


Fig.9e.

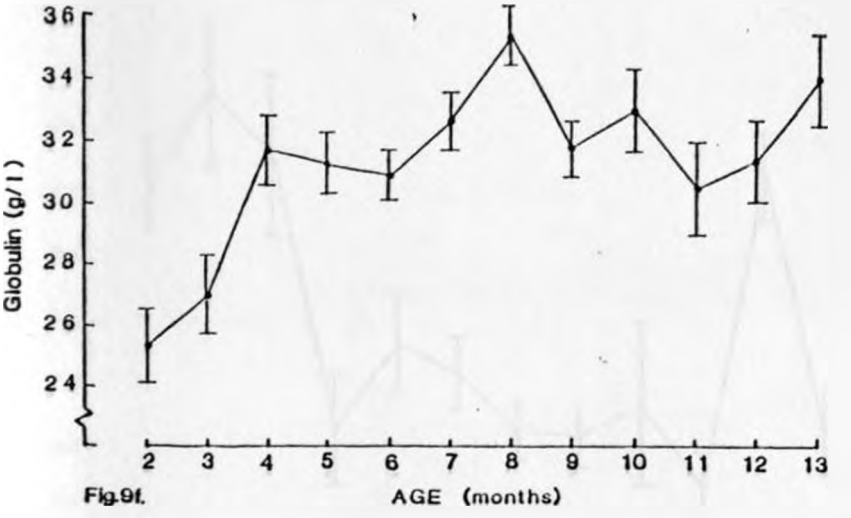


Fig.9f.

Figures 9d-df. Changes in TPP, albumin and globulin (monthly mean ± SEM) values in 10 calves (2-13 months)

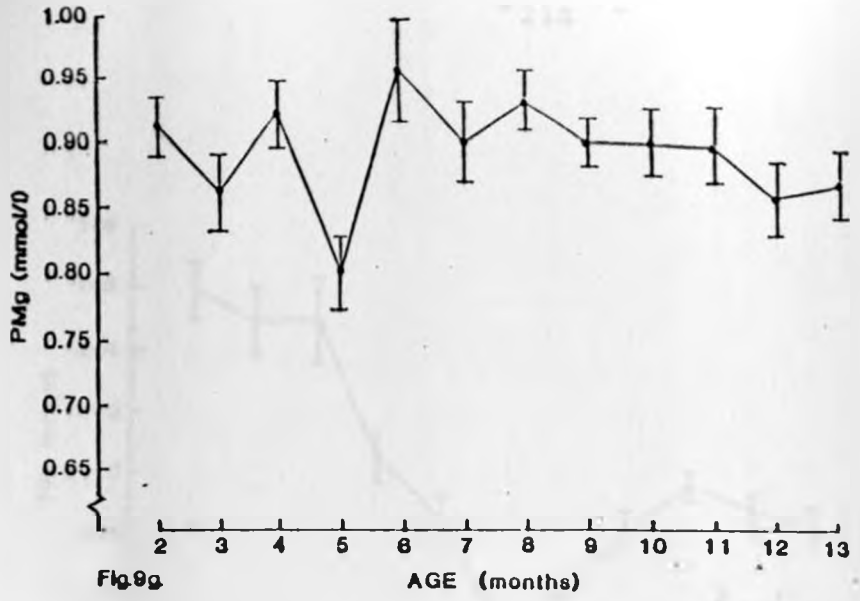


Fig.9g.

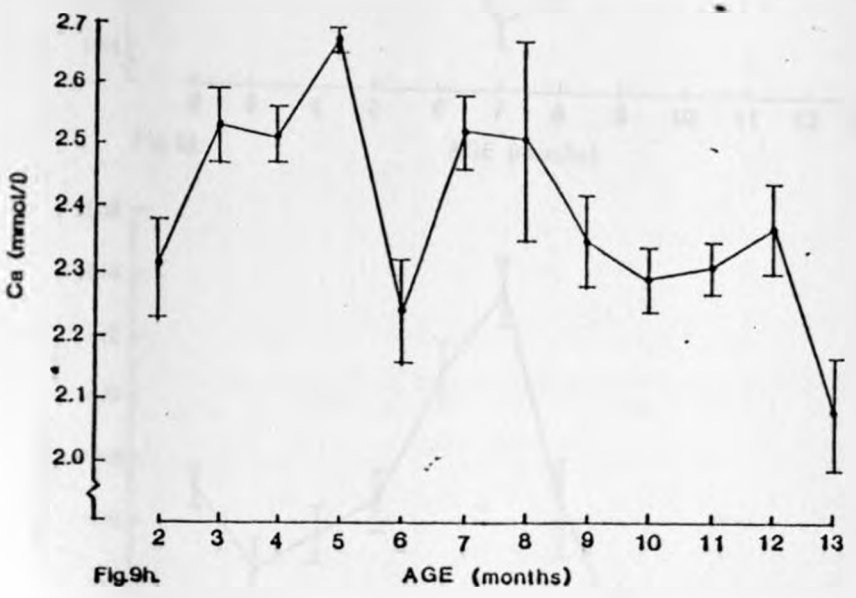


Fig.9h.

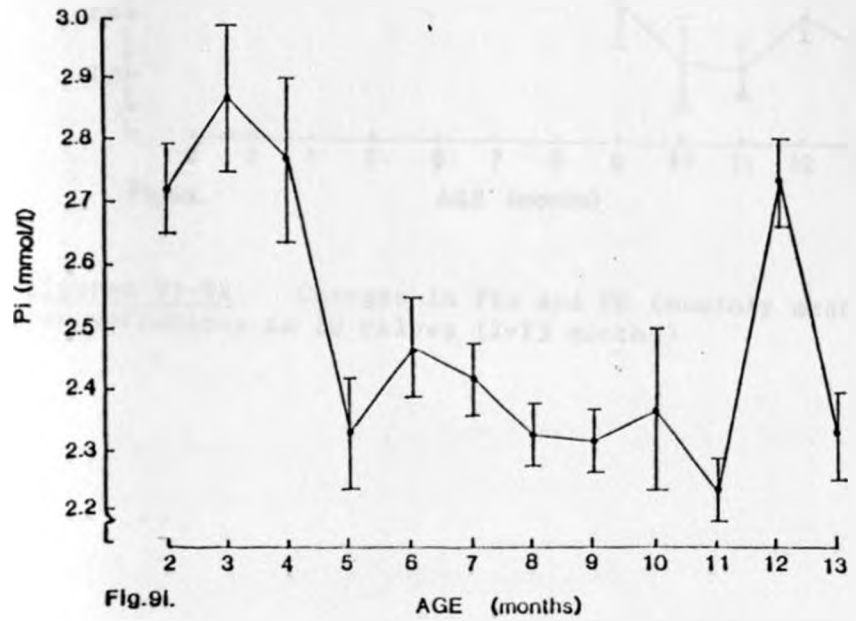


Fig.9i.

Figures 9g-9i. Changes in PMg, Ca and Pi (monthly mean ± SEM) concentrations in 10 calves (2-13 months)

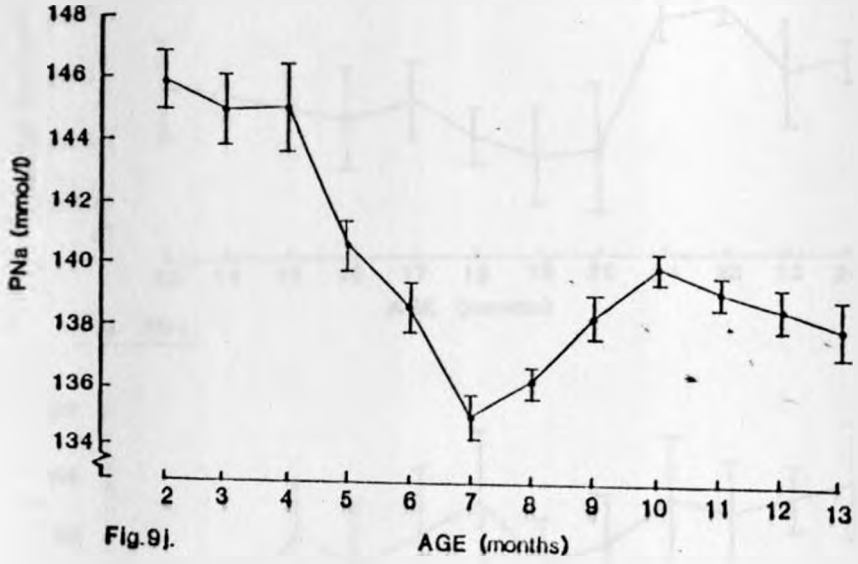


Fig.9j.

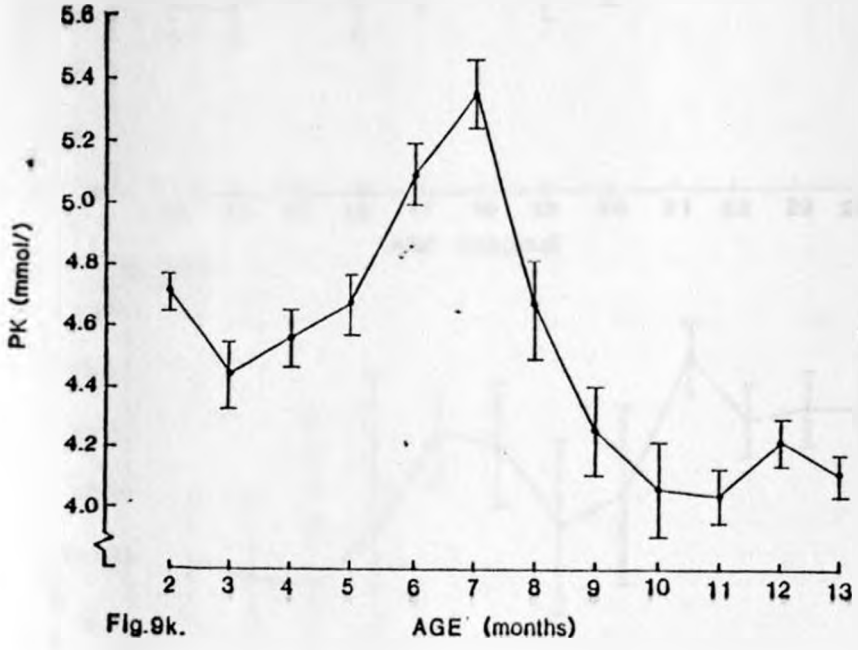


Fig.9k.

Figures 9j-9k. Changes in PNa and PK (monthly mean ± SEM) concentrations in 10 calves (2-13 months)

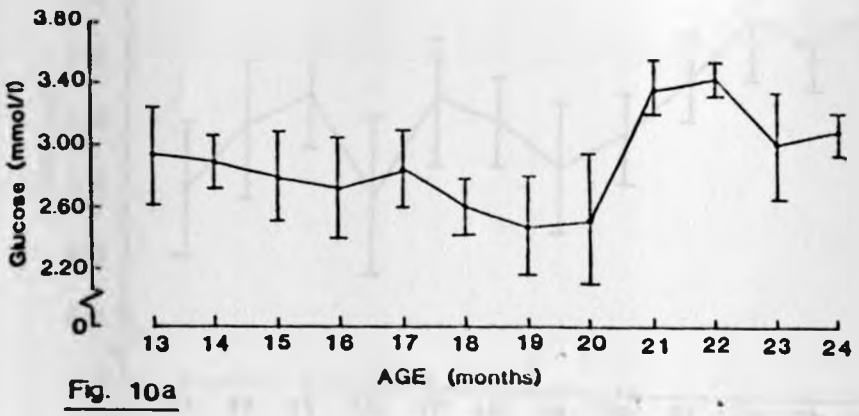


Fig. 10a

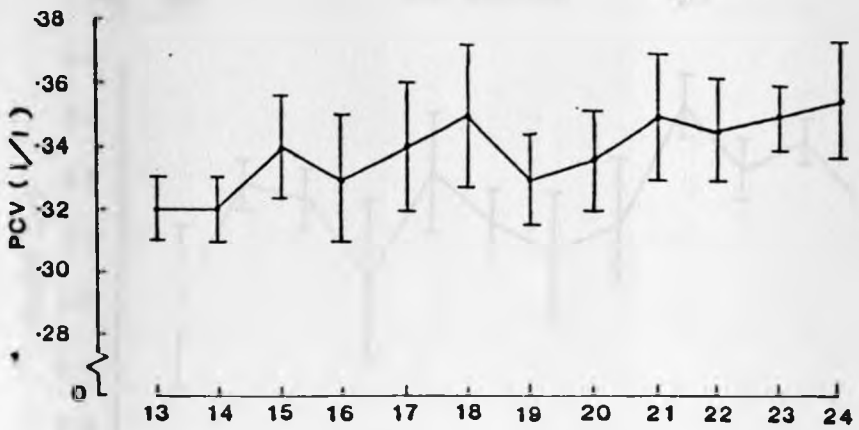


Fig. 10b

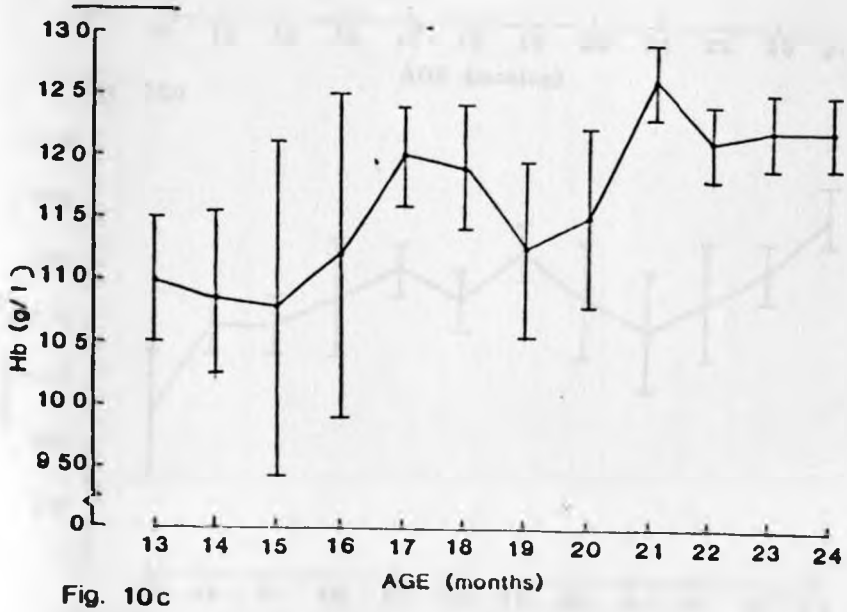


Fig. 10c

Figures 10a-10c. Changes in plasma glucose, PCV and Hb (monthly mean ± SEM) values in 10 heifers (12-24 months)

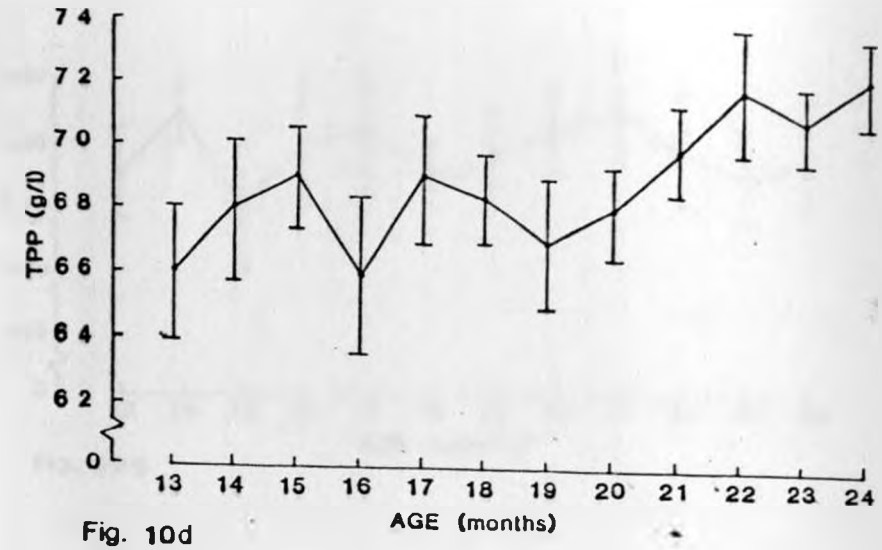


Fig. 10d

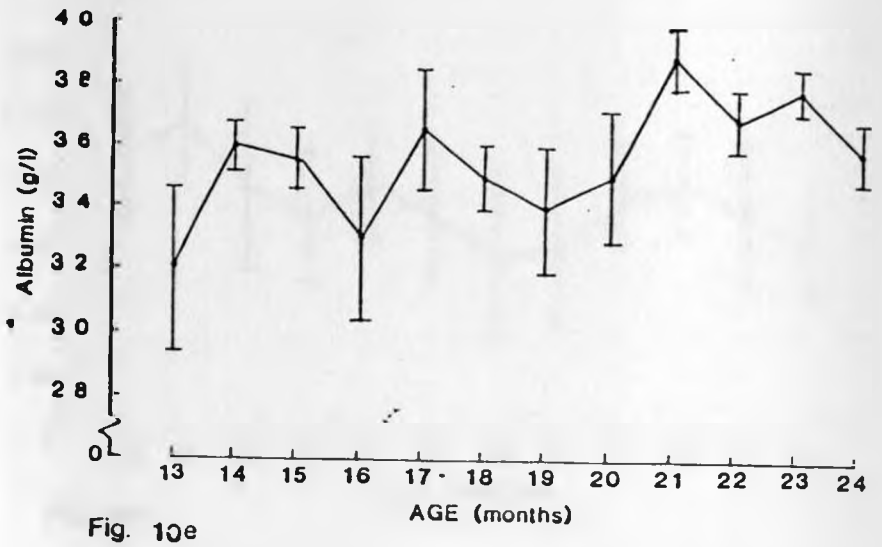


Fig. 10e

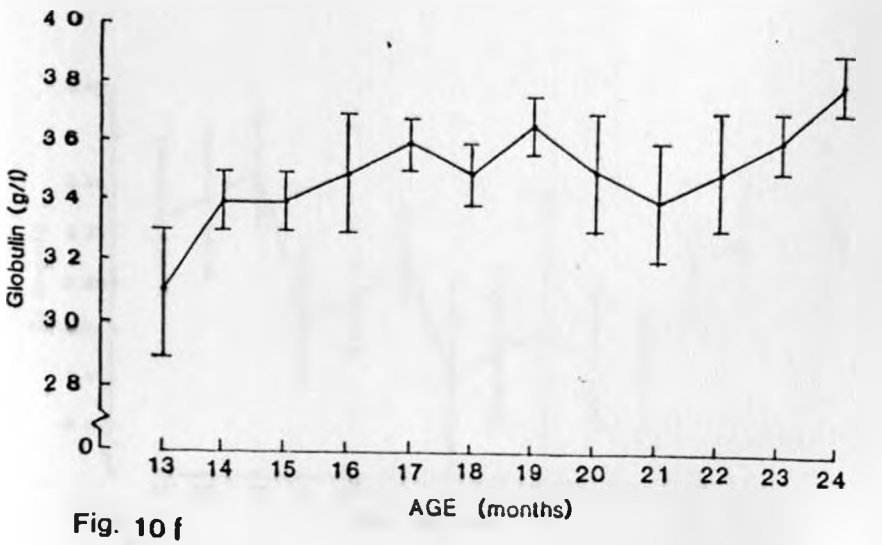


Fig. 10f

Figures 10d - 10f. Changes in TPP, albumin and globulin (monthly mean ± SEM) concentrations in 10 heifers (12-24 months)

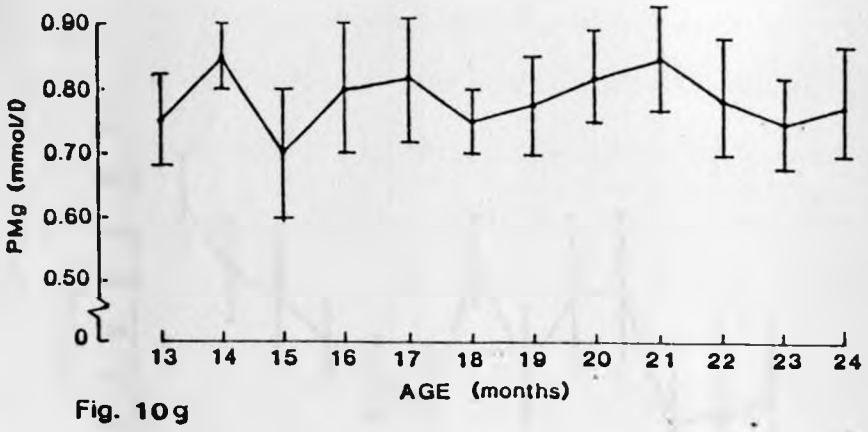


Fig. 10g

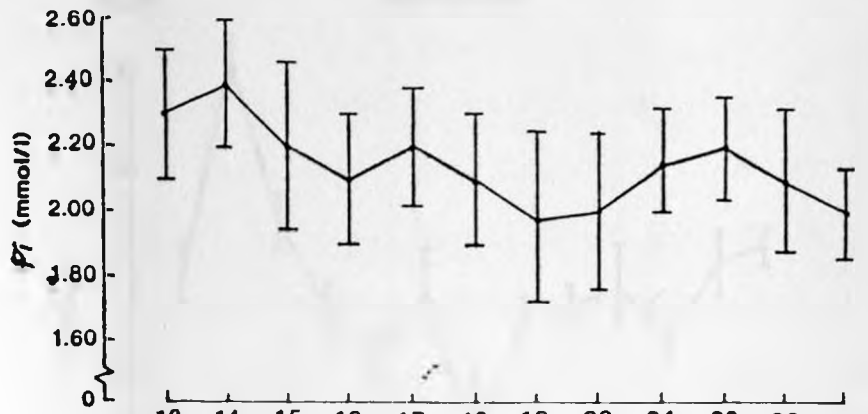


Fig. 10h

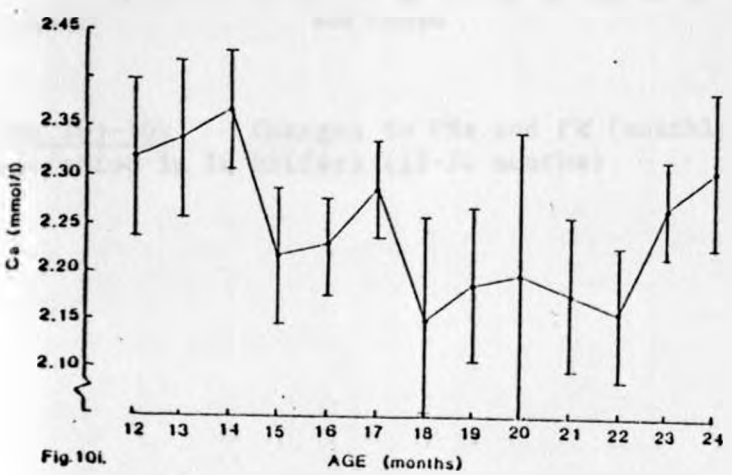


Fig. 10i.

Figures 10g - 10i. Changes in PMg, Pi and Ca (monthly mean ± SEM) concentrations in 10 heifers (12-24 months)

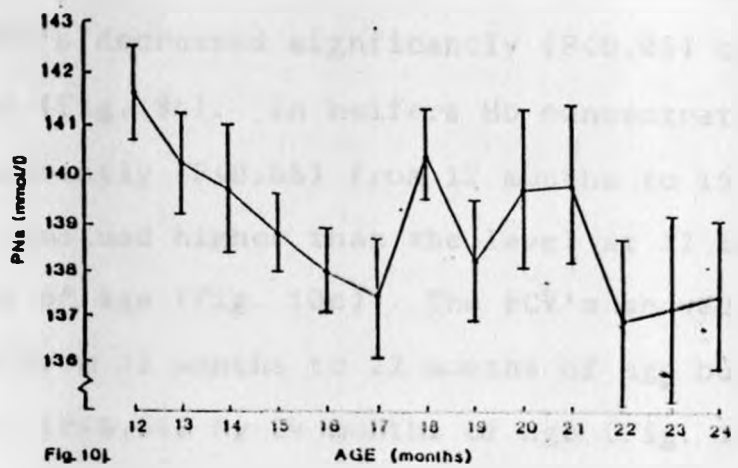


Fig. 10j

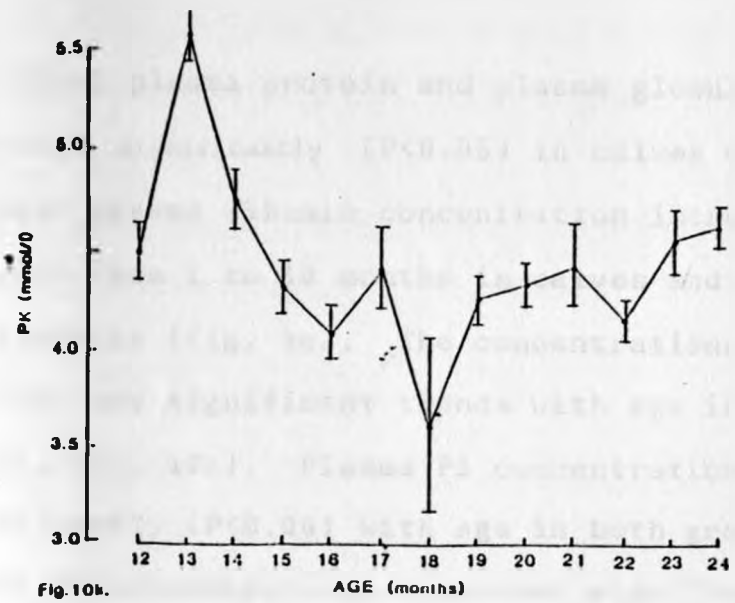


Fig. 10k.

Figures 10j-10k. Changes in PNa and PK (monthly mean ± SEM) concentration in 10 heifers (12-24 months)

In calves the mean Hb concentrations showed a tendency to decrease throughout the observation period (Fig. 9b) while the PCV's decreased signficantly ($P<0.05$) over the observation period (Fig. 9c). In heifers Hb concentrations increased significantly ($P<0.05$) from 12 months to 15 months of age and then remained higher than the level at 12 months up to 23 months of age (Fig. 10c). The PCV's showed no significant trend from 12 months to 22 months of age but were significantly higher ($P<0.05$) by 24 months of age (Fig. 10b).

Total plasma protein and plasma globulin concentrations increased significantly ($P<0.05$) in calves with age (Figs. 9d, 9f and mean plasma albumin concentration increased significantly ($P<0.05$) from 2 to 10 months in calves and then fell from 11-13 months (Fig. 9e). The concentrations of PMg and Ca did not show any significant trends with age in either group (Figs. 9g, 9h, 10g, 10h). Plasma Pi concentrations decreased almost significantly ($P<0.06$) with age in both groups (Figs. 9i, 10h). Plasma Na concentrations decreased significantly in calves ($P<0.05$) over the period of observation and did not do so in the heifers (Figs. 9j, 10j). The PK concentration increased significantly ($P<0.05$) in calves from 3 to 7 months of age and then declined up to the end of the observation period (Fig. 9k). In the heifers it showed no particular trend (Fig. 10k).

(b) Comparisons between the mean concentrations of blood components in calves (2-13 months) and heifers (13 - 24 months)

The average coefficient of variation (Table 28) show there was considerable among animal variation in both groups especially for plasma concentrations of glucose, Hb, albumin, globulin, Mg, Pi and Ca. The mean glucose concentrations were significantly ($P<0.01$) higher in calves than in the heifers, while Hb, PCV, TPP and globulin ($P<0.01$) and albumin ($P<0.05$) concentrations were significantly higher in heifers than in the calves (Table 28). The mean concentrations of blood consituents in these two groups are compared to those reported in the literature (Table 28).

(c) Correlation between blood chemistry and growth rate (calves and heifers) and production rank (heifers)

The mean concentrations of blood parameters which had significant ($P<0.05$) simple correlation coefficients with growth rate and production rank are shown in Tables 29 (calves) and 30 (heifers).

(d) Multiple linear regression analyses of the effects of blood parameters (concentrations and changes with age) on growth rate (calves and heifers) and production rank at first lactation (heifers)

These were carried out to assess whether blood parameters

Table 28. The mean (± SEM) concentrations of blood components of 10 calves (2-13 months) and 10 heifers (12-24 months) compared with normal values for calves and adult dairy cows reported in the literature.

Blood Components	Calves Mean ± SEM[+]	Average[†] "a" C of V		Heifers Mean ± SEM[++]	Average[†] "b" C of V	
Glucose (mmol/l)	4.1 ± 0.26	3.3	9.3	3.0 ± 0.12**	2.50	15.3
Hb (g/l)	108 ± 1.6	-	6.6	121 ± 0.8**	-	10.0
PCV (l/l)	0.33 ± 0.004	0.30	6.2	0.36 ± 0.004**	0.30	9.5
TPP (g/l)	65.9 ± 6.60	-	5.2	72.8 ± 3.00**	-	5.5
Albumin (g/l)	34.5 ± 5.50	30.8	6.4	36.3 ± 3.70*	32.0	8.9
Globulin (g/l)	31.5 ± 7.60	34.3	11	36.3 ± 2.50**	44.0	12.3
PMg (mmol/l)	0.90 ± 0.09	1.11	9.1	0.91 ± 0.08	1.03	13.1
Pi (mmol/l)	2.51 ± 0.21	2.74	10	2.30 ± 0.08	1.94	9.4
Ca (mmol/l)	2.39 ± 0.17	2.52	8.3	2.25 ± 0.10	2.38	10.4
PNa (mmol/l)	140 ± 1.11	140	1.7	139 ± 0.24	139	2.8
PK (mmol/l)	4.44 ± 0.40	5.38	7.1	4.43 ± 0.38	5.00	8.7

* $P<0.05$; ** $P<0.01$ (significance of difference from calves)

[+] Mean ± SEM of monthly means of 9 calves

[++] Mean ± SEM of the monthly means of 10 heifers

"a" Mean blood concentrations of calves 3-9 months old (Kitchenham et al, 1975)

"b" Mean blood concentrations of cows >1.5 years of age (Rowlands et al, 1974)

[†] Average of the coefficients of variation calculated monthly

Table 29 Mean concentrations (± SEM) and changes in blood parameters that gave a significant (P<0.05) simple correlation coefficient (r) with growth rate in calves (2-13 months)(n=10)

Blood parameters	Mean	(r)
Hb (g/l) overall mean	108 ± 1.6	0.768**
PCV (l/l) overall mean	0.33 ± 0.004	0.610*
Albumin (g/l) mean at 7 months	32.1 ± 1.20	0.812**
Albumin (g/l) overall mean	34.5 ± 0.70	0.782**
Globulin (g/l) mean at 13 months	33.9 ± 1.10	0.579*
Ca (mmol/l) mean at 7 months	2.52 ± 0.08	0.578*
Ca(mmol/l) change from 7-13months	-0.43± 0.18	-0.747**
PK(mmol/l) change from 7-13months	-1.26± 0.33	0.581*

* P<0.05, ** P<0.01.

Table 30 Mean (± SEM) concentrations and changes in blood parameters which gave significant (P<0.05) simple correlation coefficients with growth rate (r1) and milk production rank at first lactation (r2) in heifers (12-24 months) (n=10)

Blood components	Mean	r1	r2
TPP(g/l) mean at 12 months	71.8 ± 1.30	0.681**	0.700**
TPP(g/l) mean at 18 months	74.3 ± 2.10	0.878**	0.771**
TPP (g/l) overall mean	72.8 ± 3.00	-	0.691**
Globulin(g/l) mean at 12 months	34.7 ± 1.50	0.587*	0.817**
Globulin(g/l) mean at 18 months	37.8 ± 1.80	0.594*	-
Globulin(g/l) change 18-24months	2.30± 2.00	-	0.525*
Globulin (g/l) overall mean	36.3 ± 2.50	-	0.638*
Globulin(g/l) mean at 24 months	38.3 ± 2.20	-	0.575*
Hb (g/l) overall mean	109 ± 2.8	-	0.609*
PMg(mmol/l) mean at 24 months	0.97± 0.01	-	0.788**
PMg(mmol/l) overall mean	0.91± 0.08	-	0.683*
PNa(mmol/l) change 12-18 months	-0.80± 1.17	0.665**	-
PNa(mmol/l) overall mean	139.2 ± 0.42	0.683**	-

* P<0.05 ** P<0.01

measured at a particular age, over a 12-month period and the changes between particular ages can be used to predict growth rate in calves and heifers and production rank at first lactation. Separate multiple regression analyses for each "part" (see above) were carried out to find out if changes and (or) concentrations of a particular blood component could be used to predict growth rate and production rank at first lactation in young replacement dairy stock.

Attached to each multiple regression equation are the multiple coefficient of determination (R^2), multiple correlation coefficient (R), standard error of estimate (SEE) and standard error of individual partial regression coefficients (SEB) for each independent variable, Student's t values for each partial regression coefficient (T value), F value for multiple regression (F value) and the standardized regression coefficient (B) for each independent variable. The T value and B value show which of the independent variable(s) were most important in accounting for variation in the dependent variable.

Calves

(1) Growth rate (G.R.)

No blood component(s) concentration(s) at 2 months of age yielded a significant linear regression relationship with growth rate.

The best equation found for predicting growth rate in calves using concentrations of blood components at 7 months of age was:

Growth rate = -13.03 + (Albumin x8.64) (1)

(R^2 = 0.59, R=0.77, SEE=2.69, SEB=1.36, F value=10.26**).

Thus albumin concentration at 7 months of age accounted for 59.0% of the variation in growth rate. (growth rate expressed as kg/month over 12 months).

The best equation using concentrations of blood components at 13 months of age found for predicting growth rate was:

Growth Rate = 3.61 + (Globulin x 3.06) (2)

(R^2 = 0.43, R=0.65, SEE=1.62, SEB=1.35, F value=5.18*.

The concentration of globulin at 13 months of age accounted for 43.0% of the variation in growth rate.

The changes in blood components from 2 to 7 months of age did not yield a significant regression equation, however the changes from 7 months to 13 months - gave a significant linear regression relationship with growth rate as follows:

Growth Rate = 15.0+(Ca x -2.33)+(PK x 0.78) (3).

(R^2=0.68, R=0.83, SEE=1.30, SEB1=0.83, SEB2=0.51

T value b1=2.82*, T value b2=1.5, F value =6.39*,

B1 = 0.67, B2=0.36).

Where Ca is the change (in mmol/L) from 7 to 13 months, and PK is the change (in mmol/L) from 7 to 13 months.

The change in plasma Ca concentration from 7 to 13 months accounted for the greater part of the 68% variation in growth rate accounted for by this regression equation (on the basis of the B value).

The overall mean concentrations of the blood components did not yield any significant regressions on growth rate.

The concentrations of blood components at 13 months of age and the changes from 7 to 13 months that produced the best multiple linear regression equation for predicting growth rate were:

Growth Rate = 8.18+(Glob x 2.07)+(Ca + -1.72)+(PK x 0.87). (4

(R^2=0.89, R=0.94, SEE=0.86, SEB_1=0.76, SEB_2=0.58, SEB_3=0.34, T value b1=2.99*, T value b2=2.95*, T value b3=2.57*, F value=12.88**, B1=0.484, B2=0.490, B3=0.401).

Where Glob is the plasma globulin concentration (in g/l) at 13 months.

Ca is the change (in mmol/l) from 7 to 13 months (decrease) and

PK is the change (in mmol/l) from 7 to 13 months.

This multiple regression equation accounted for 89% of the variation in growth rate, and all the independent variables contributed significantly (T values above) to this variation.

The concentrations of blood components at 7 weeks of age and the changes from 7 to 13 months that produced the best multiple linear

regression equation for predicting G.R. were:

Growth Rate=-6.10+(Alb x 6.98)+(Ca x 3.09)+(PK x 1.11) (5).

(R^2=0.85, R=0.92, SEE=0.99, SEB1=2.30, SEB2=1.71, SEB3=0.41, T value b1=2.53*, Tvalue b2=1.81, T value b3=2.70*, F value=9.16**, B1=0.52, B2=0.38, B3=0.51).

Where Alb is the plasma albumin concentration (g/l) at 7 months.

Ca is the change in calcium concentration (in mmol/l) from 7 to 13 months.

PK is the change in plasma potassium concentration (in mmol/l) from 7 to 13 months.

This regression equation accounted for 85% of the variation in growth rate, and the plasma albumin concentration at 7 months and PK change from 7 to 13 months accounted for most of this variation.

Heifers

(1) Growth Rate

No combinations of plasma levels of blood components at 12 months of age gave any significant linear regression relationship with growth rate.

The best-regression equation found for predicting growth rate using blood component concentrations at 18 months of age as independent variables was:

Growth Rate =-7.52+(TPP x 2.08)+(glob x 0.70) (6).
(R^2=0.75, R=0.87, SEE=0.95, SEB1=0.79, SEB2=0.91,
T value b1=3.38**, T value b2=1.07, F value=10.7**,
B1=0.89, B2=-0.43).

(Both TPP and globulin levels were expressed as g/l).

This multiple regression equation accounted for 75.0% of the variation in growth rate and TPP concentration accounted for most of this on the basis of both T and B values.

The concentrations of blood components at 24 months of age and the overall mean concentrations did not yield significant linear regression relationships with growth rate. Similarly changes from 12 to 18 months and from 18 to 24 months in any blood component did not give a significant linear regression relationship with growth rate. When all the independent variables were combined the best equation that was found for predicting growth rate was;

Growth Rate =-10.55+(TPP1 x 1.67)+(TPP2 x 1.76) (7).
(R^2=0.84, R=0.92, SEE=0.77, SEB1=0.77, SEB2=0.44,
T value b1=2.16*, T value b2=4.01**, F value =18.4**,
B1=0.37, B2=0.68).

Where TPP1 is the level (in g/l) at 12 months and
TPP2 is the level (in g/l) at 18 months.

This multiple regression accounted for 84% of the variation in growth rate and although both TPP levels at 12 and 18 months contributed significantly (T values) to the variation in growth rate the variation accounted for by the TPP level at 18 months of age was nearly twice that accounted for by TPP at 12 months (see B values).

(2) Milk production rank

The best equation found for predicting production rank at first lactation using the levels of blood components at 12 months of age (as independent variables) was:

Production Rank=-74.1+(TPP x 6.33)+(glob x 41.4). (8).
(R^2=0.68, R=0.82, SEE=16.4, SEB1=6.53, SEB2=12.78,
T value b1=0.97, T value b2=3.24**, F value=7.31*,
B1=0.22, B2=0.73).
(Both TPP and globulin expressed as g/l).

This regression equation accounted for 68.0% of the between heifer variation in P.R. and the plasma levels of globulin accounted for more of this variation on the basis of the B values.

The concentrations of blood components at 18 months did not give any significant regression equation with P.R., however when they were combined with the changes from 12 to 18 months the following equation was the best for predicting Production Rank:

Production Rank = -200.7+(TPP x 42.5)+(glob x 13.4)+
(PNa x 3.61) (9).

(R^2=0.64, R=0.80, SEE=7.43, SEB1=4.63, SEB2=6.23, SEB3=1.10, T value b1=9.17**, T value b2=2.15*, T value b3=3.29*, F value=33.2***, B1=0.80, B2=0.32, B3=0.43).

Where TPP is the concentration (in g/l) at 18 months;
Glob is the change (in g/l) from 12 to 18 months and
PNa is the change (in mmol/l) from 12 to 18 months

This regression equation accounted for 64.0% of the variation in P.R. Although all the independent variables contributed significantly (T values) to the variation in Production Rank, more than half of this was accounted for by the TPP levels at 18 months of age (see B values).

The best equation found for predicting Production Rank using the concentrations of blood components at 24 months of age (as independent variables) was:

Production Rank= -149.1+(Globulin x 12.5)+(PMg x 222.5) (10).

(R^2=0.69, R=0.83, SEE=10.96, SEB1=6.04, SEB2=38.1, T value b1=2.07*, T value b2=5.83**, F value=20.81**, B1=0.30, B2=0.84).

(Where Globulin is expressed as g/l and PMg as mmol/l).

This multiple regression equation accounted for 69% of the variation in Production Rank. Both the plasma globulin and Mg concentrations at 24 months of age contributed significantly

(T values) to this variation. However the contribution of PMg to the variation was considerably greater than that of plasma globulin (B values). Thus most of the Production Rank variation between the heifers was due to differences in PMg concentrations.

The best equation found for predicting Production Rank when the overall mean blood component concentrations were used as independent variables was:

Production Rank= -268.7+(Hb x 8.75)+(TPP x 4.1)+
(glob x 10.3)+(PMg x 243) (11).
(R^2=0.719, R=0.848, SEE=10.87, SEB1=13.6, SEB2=2.28
SEB3=3.44, SEB4=54.1, T value b1=0.98, T value b2=1.21
T value b3=3.60**, T value b4=4.489**, F value=11.1**
B1=0.141, B2=0.181, B3=0.439, B4=0.701).

(Hb, TPP and globulin expressed as g/l and PMg as mmol/l).

This multiple regression equation accounted for 71.9% of the variation in Production Rank at first lactation with PMg and mean plasma globulin concentrations accounting for most of this variation (see B values).

When the concentrations of blood components at all (i.e. 12, 18 and 24 months) ages, overall mean concentrations and the changes for both periods were combined (independent variables) the following was the best equation for predicting Production Rank.

Production Rank = -171.2+(Hb x 6.92)+(TPP x 13.35) +
(PMg x 115.0) (12).

(R^2=0.719, R=0.848, SEE=9.92, SEB1=3.51, SEB2=6.63, SEB3=54.06, T value b1=1.97, T value b2=2.01*, T value b3 =2.13*, F value=17.78**, B1=0.335, B2=0.345, B3=0.436).

Where Hb is the level (in g/l) at 12 months.

TPP is the level (in/gl) at 18 months and
PMg is the level (in mmol/l) at 24 months.

DISCUSSION

(a) The relationship between blood chemistry and age

The effects of age on the concentration of blood components have previously been reported for calves (Dalton 1967, Reece and Wahlstron 1972, Kitchenham et al. 1975) and for adult cattle (Gartner et al. 1966, Tumbleson, Wingfield, Johnson, Campbell and Middleton 1973a, Tumbleson, Burks and Wingfield 1973b). There is a divergence in the results reported and this is probably a function of differing management, nutrition and the environment in the various studies (Tumbleson et al. 1973a).

The trends in plasma glucose and Hb concentration and PCV levels observed in calves in this study were similar to those reported by Kitchenham et al. (1975) and Little et al.

(1977) but differed from those observed by Gartner et al. (1966). A fall in plasma glucose concentration was observed with age in calves reared under both conventional and rapid rearing systems (Kitchenham et al. (1975). The mean Hb and PCV values were higher than those previously reported for dairy calves (Table 28). The difference could have been due to differences in the environment and nutrition.

The increases in TPP and plasma globulin concentrations with age in both groups were similar to those previously reported (Larson and Touchberry 1959, Gartner et al. 1966, Little 1974). The increases in TPP and plasma globulin concentrations are mainly due to increases in gammaglobulins (Larson and Touchberry 1959, Kaneko and Cornelius, 1970).

The lack of any significant relationship between PMg, PCa and PNa concentrations with age is in agreement with the previous work (Gartner et al. 1966, Pascoe 1967, Mylrea and Bayfield 1968), but differed from some other observations (Kitchenham et al. 1975, Payne et al. 1964). An increase in PMg and no change in plasma Ca concentrations (Gartner et al. 1966) and decrease in serum Ca concentration (Payne and Leach 1964) have been reported with increasing age in calves. The decrease of Pi concentration with age in the heifers is consistent with previous work (Payne and Leach 1964, Gartner et al 1966, Mylrea and Bayfield 1968, Kitchenham et al. 1975). The concentration of Pi was found to decrease with increasing age in calves in a conventional rearing system but not in a rapid rearing system (Kitchenham et al. 1975). Thus the decrease

in Pi concentrations in both the calves and heifers would suggest that in normal (conventional) rearing systems phosphorus intake may be inadequate for maintainance of plasma Pi levels in growing animals.

The increase in PK concentration in the calves with age differed from the findings of Little et al. (1977) who found that PK concentration decreased with age. Kitchenham et al (1975) found a greater decrease in PK concentration in calves in a conventional system compared to a rapid rearing system, thus the decrease in PK concentrations after 7 months of age was similar to his results especially in the latter system.

(b) Variation in blood component concentrations among calves and heifers.

The differences in concentrations of several blood components found among the calves and heifers could be partly attributed to genetic effects. Significant differences among offspring of different sires were found for PCV's and plasma concentrations of glucose, Ca and K (Rowlands et al. 1974). Most of the differences in plasma concentrations of these blood components were due to inheritable factors (Table 27) (Wiener and Field 1971, Rowlands et al. 1974, Kitchenham et al. 1975).

The average coefficient of variation in both groups (Table 28) was reasonably high for all parameters except TPP and PNa. It is difficult to explain this variation other than on the basis of genetic differences mentioned above. Part of this

variation could also be due to considerable between calf differences in their rates of maturation or development of ruminant type metabolism. This could certainly influence the variation in the plasma glucose concentration (Nicolai and Stewart 1964) and perhaps Pi. Calves and yearlings are also probably more susceptible to gastro-intestinal parasitism and variation in such susceptibility might explain the variation in plasma proteins and Hb even though the calves here were treated for internal parasites at 4-weekly intervals.

(c) The relationship between blood chemistry and growth rate

In two groups of calves, one growing at an average rate of 0.50 kg/day and the other at 1.1 kg/day, Payne et al. (1973) found that growth rate was related to blood glucose in the slower growing animals but not in the more rapidly growing group. A similar correlation was found in calves (1-12 weeks) growing at an average of 0.56 kg/day (Rowlands et al 1974). Correlations have not been found to occur in older animals (over 6 months) growing at faster rates (Arthaud et al 1959, Payne et al. 1973b). Thus it appears that correlations between growth rates and glucose concentrations are likely to occur in early life (from birth to 3 months of age), and may only occur when dietary intake of energy is limiting growth (Payne et al. 1973b). Thus the non significant correlation between growth rates and plasma glucose concentration in both groups of animals observed in this study may have been due to the age of the animals being studied or to the fact that the dietary intake of energy was not a limiting factor for growth.

The overall mean Hb concentrations and PCV levels were positively correlated with growth rate in the calves, that is, calves with high Hb concentration and high PCV levels had higher growth rates. The relationship between Hb concentrations and growth rate has been previously reported (Koubicek and Ray 1972, Kitchenham et al. 1977).

The plasma albumin concentration at 7 months of age and the overall mean concentrations (in each animal) were positively correlated ($P<0.01$) with growth rate. Thus calves with high albumin levels had higher growth rates. The relationship between albumin concentration and growth rate has been observed in a number of different environments and feeding systems (Rowlands et al 1974, Kitchenham et al. 1977). Faster growing calves have been found to have significantly higher plasma albumin concentrations than slower growing calves (Rowlands et al. 1974). Similar findings have been observed in bulls reared on a barley beef system (Kitchenham et al. 1977). This relationship between albumin concentrations and growth rate under different environments suggests that this relationship may be free of environmental and nutritional effects and hence may be used to prediot growth rate under different husbandry systems. Regression equations 1 and 5 enhance this view.

The plasma globulin concentrations at 13 months of age in calves and at 12 and 18 months of age in heifers were positively correlated with growth rate (Tables 29 and 30). Thus it seems that high plasma globulin concentrations are associated with high growth rate in animals after one year of age. The heritability of globulins in calves (3-9 weeks) has been shown to be very low

(0.01) (Rowlands et al. 1974) compared to that in animals over one year old (0.63) (Kitchenham and Rowlands 1976). Thus this could explain why plasma globulin concentration in the calves before 13 months of age was not significantly related to growth rate. Plasma globulins are composed mainly of immunoglobulins (Larson and Touchberry 1959, Kaneko and Cornelius 1970) and thus it could be that animals which have high globulin concentrations are those which have better humoral immune responses and hence are able to overcome infections which would otherwise limit their growth rate. High plasma globulin concentrations in animals between 1 and 2 years of age could well be a physiological characteristic of highly productive animals which could be used to predict growth rate (multiple regression equations 2 and 4).

The TPP concentrations at 12 and 18 months of age were positively correlated ($P<0.01$) with growth rate in the heifers (Table 30) that is, heifers with high TPP levels at these ages had higher growth rates. This relationship may have been due to both high albumin synthesis in the liver and high immunoglobulin production in response to various environmental infections (Larson and Touchberry 1959, Williams and Miller 1975). These would lead to increases in TPP concentrations. The relationship between TPP and growth rates could also have been an indirect one due to the positive correlation between TPP and globulin concentrations (Kitchenham and Rowlands 1976). However, this would be unlikely since TPP levels gave significant partial

regression coefficients (multiple regression equation 6 and 7) with growth rate and plasma globulin concentration did not.

In calves the growth rate was positively correlated with plasma Ca concentrations at 7 months of age and negatively correlated with the changes from 7 to 13 months of age (Table 29). The implication of these relationships is that calves which were able to maintain Ca concentrations had better growth rates. This relationship is not easy to explain, but it might be related to optimal digestion and absorption of nutrients in some calves thus allowing plasma Ca to be maintained at normal or slightly elevated levels.

The changes in PK concentration from 7 to 13 months of age were positively correlated with growth rates in calves (Table 29) i.e. calves which showed the greatest decreases in PK concentration in this period had higher growth rates. This would suggest that calves which did not maintain the high PK levels observed between 3 and 10 months (Fig. 9k) had higher growth rates. The relationship between low PK levels and high growth rates in cattle has been reported under different environmental and husbandry systems (Kitchenham et al. 1975, Rowlands and Manston 1976). For this reason low PK concentrations may be a physiological characteristic of fast-growing calves and decreases between 7 and 13 months might be used to predict growth rate (multiple regression equations 3 to 5).

The overall mean concentration (in individual animals) of PNa was positively correlated ($P<0.05$) to growth rate in the heifers, thus heifers with high PNa levels had higher growth rates. Similar observations were reported by Rowlands et al. (1974). A significant relationship between PNa concentrations and body weight at 6 and 9 months has been previously reported (Rowlands et al. 1974). Supplementation of sheep and beef cattle with sodium chloride has been found to increase their growth rate (Joyce and Brunswick 1975). Thus heifers which maintain high PNa levels should have better growth rates.

PMg concentrations at 24 months of age were positively correlated ($P<0.05$) with growth rates in the heifers i.e., heifers with high PMg concentrations had higher growth rates. This relationship may have been due to the influence of Mg on cellular energy utilization, for example, glucose metabolism via pathways of oxidative phosphorylation and transketolase reaction of the pentose monophosphatase shunt requires Mg (Pike and Brown 1975). Magnesium is also involved in protein synthesis systems in the body (Wacker 1969). Thus low PMg levels may affect an animal's performance by altering energy metabolism and protein synthesis.

Multiple regression analyses were carried out to relate concentrations and changes in blood components to growth rate in both calves and heifers. The combinations of blood component variables which gave significant multiple regressions with

growth rate differed between the calves and heifers. This suggests that the relationship of blood components and growth rate differs with age i.e. certain blood components may be related to growth rate in calves and not in heifers and vice versa. The blood parameters that gave significant regression coefficients with growth rate in calves were plasma concentrations of globulin at 13 months of age (equations 1 to 5). In heifers they were the concentrations of TPP at 12 and 18 months of age (equations 6 and 7).

The multiple regression equations (1 to 5) suggest that the blood parameters showing significant linear relationships can be used to predict growth rate in calves. Plasma albumin concentration would seem to be a better parameter than the others since its prediction of growth rate can be carried out earlier (7 months of age) than with the other parameters. The relationship between TPP concentrations (at 12 and 18 months - equation 7) and growth rate after one year of age suggests that these variables can be used to predict growth rates in heifers under the conditions applying in the present investigation.

The multiple regression equations described above show that growth rate of animals below 13 months may be predicted by sampling the animals at 7 and 13 months of age, and that of animals between 1 and 2 years may be predicted by sampling them at 12 and 18 months of age. The application of these multiple regression equations in predicting growth rate, however, may be limited, since relationships between blood components and

growth rate are to some extent influenced by nutrition and environment (Payne et al. 1973b). Thus it may be that some relationships may only occur when the dietary intake of a particular nutrient is a limiting factor to the growth rate. The rate of change in blood chemistry with age may also be affected by nutrition and the system of husbandry (Kitchenham et al. 1975). Thus relationships between the change in blood chemistry with age and growth rate observed in one husbandry system may not be observed in another system. Therefore to utilize blood chemistry to predict growth rate of calves or heifers would require reference values based upon a population mean obtained from animals which grew at a "target" (acceptable) growth rate applicable to the system of husbandry under investigation. However since correlations between some blood components (e.g. albumin, K) and growth rate appear to occur in a variety of environments (Rowlands and Manston 1976), the relationships may be physiological and hence could be used in many husbandry systems to predict growth rates.

(d) The relationship between blood components and production rank at first lactation

The positive correlation between Hb concentration at 12 months of age and milk production rank shows that heifers with high Hb levels at 12 months of age are likely to have higher milk production. Thus estimation of Hb concentrations in heifers at 12 months of age may be useful in predicting milk production rank at first lactation.

The mean TPP concentrations at 12 and 18 months and its overall mean concentration were positively correlated to production rank. This suggests that heifers with higher TPP concentration at these ages had higher production ranks. This relationship between TPP concentrations and production rank may be a reflection of protein synthesis and/or utilization for milk production. Proteins are required for milk synthesis (Rook and Line 1961) thus animals with high TPP levels probably had ample proteins for milk synthesis and hence would have produced more milk than those which had low TPP concentration. The animals with high TPP concentrations are probably those which preferentially utilize more body fat reserves and other non-protein nutrients for energy and hence are able to meet (or nearly meet) their energy requirements for milk production, since these produce more energy per gram than proteins (Webster 1978). Total plasma protein concentrations were found to have a high heritability (53%) (Kitchenham and Rowlands 1976). Hence it could be that high TPP concentration is a physiological characteristic of high milk producing heifers which might be manifested between 1 and 2 years. Therefore it could be used to predict milk production rank in heifers (multiple regression equations 8 and 9).

Plasma globulin concentrations at 12 and 24 months, the overall mean concentration and the change from 18-24 months were positively correlated with production rank in heifers. Thus heifers with high plasma globulin concentration or which showed greater increases in globulin concentration between 18

and 24 months had higher milk production. This relationship between plasma globulin and milk production may indicate that heifers which have high immunoglobulin (which composes most of the plasma globulin) production are better milk producers. That is, heifers which are able to produce greater amounts of immunoglobulins in response to environmental infections may have a better resistance to infections and hence be able to combat them before they advance to a stage where they can affect production. This contention may be supported by the positive correlation between the increase in plasma globulin concentrations from 18 to 24 months and production rank.

A high heritability (63%) and repeatability (84%) for plasma globulin levels in older cattle (over one and half years) was found by Kitchenham and Rowlands (1976). On the contrary, Rowlands et al. (1974) found no evidence of the heritability of plasma globulins in young (3-9 weeks) calves (Table 27). Thus this may suggest that the relationship of high plasma globulin concentrations and milk production in heifers could be a physiological characteristic manifested between 1 and 2 years of age which together with other blood parameters might be used to predict milk production rank (multiple regression equations 8, 9 and 10).

The change (fall) in PNa concentration between 18 and 24 months was negatively correlated ($P<0.01$) with production rank in heifers. That is, heifers with the greatest decrease in PNa

concentration between 18 and 24 months had the lowest production rank. This may suggest that heifers which were not able to maintain their PNa concentrations had low production ranks. Sodium is required in large amounts in lactating animals and young growing stock and its decrease may be associated with reduced milk production (Smith and O'connor 1983). Thus it could be that heifers which were not able to maintain their PNa levels between 18 and 24 months were not able to maintain their PNa levels during lactation and this may have been manifested in lower milk production. Thus the decrease in PNa concentration between 18 and 24 months may be useful in predicting production rank in heifers (multiple regression equation 9).

PMg concentration at 24 months and its overall mean concentration were positively correlated with milk production rank, i.e. heifers with high PMg levels had higher production rank than those which had low PMg levels. The individuality and repeatability estimates of PMg concentration have been shown to be higher in cows over one and a half years of age (heritability 32% and repeatability 65%)(Kitchenham and Rowlands 1976). This implies that animals tend to maintain their PMg levels over a long period of time, thus heifers with high PMg levels and therefore possibly better milk producers, could be identified before the first lactation (multiple regression equations 10 and 11).

The relationship between PMg and milk production rank could have been due to the association of Mg with energy metabolism and protein synthesis (Pike and Ward 1975). This contention may be supported by the observation that PMg levels accounted for most of the variation in production rank in multiple regression equation 10 and 11. Thus in heifers it seems that PMg levels are important in determining their milk production rank. A significant relationship between PMg concentrations and milk production has also been reported (Kitchenham et al. 1975b, Young 1978). A significant positive partial regression relationship was found between PMg and milk production (Kitchenham et al. 1975b). Magnesium supplementation in lactating cows resulted in increased production of milk and milk fat (Young 1978).

The multiple regression equations 8-11 show that blood components (concentrations and/or change) in heifers (12-24 months old) may be used to predict production rank at first lactation. Multiple regression equations 8, 9 and 10 would be more practical in the dairy farming industry since they would only need one or two samplings. These prediction equations would need to be tested in other herds under different environmental conditions to further assess their suitability for practical application.

CHAPTER 8

BLOOD CHEMISTRY CHANGES DURING EXPERIMENTALLY-INDUCED HYPOMAGNASAEMIA IN YOUNG CALVES

INTRODUCTION

There are conflicting reports on the relationship between PMg and EMg concentrations in the literature. Some workers have reported that Mg ions do not exchange between plasma and erythrocytes (Greenberg et al. 1933, Salt 1950, McAleese et al. 1961). Others have suggested that cell Mg is determined by the level of Mg supplied to bone marrow at the time of erythrocyte formation and does not change thereafter (Tufts and Greenberg 1937, Salt 1950). It has been shown that reticulocytes and young erythrocytes have higher Mg levels than older erythrocytes (Bernstein 1954, Ginsburg et al. 1962). Using radioactive Mg28 it has been found that Mg ions exchanged between plasma and erythrocytes both in vitro and in vivo in sheep blood (Care et al. 1959, McDonald et al. 1959). However, the addition of Mg into rabbit's blood did not result in any appreciable uptake of the Mg by the erythrocytes (Ginsburg et al. 1962).

Relatively few studies have been published on the effect of Mg on thyroid function in animals. Both the basal and

TSH-stimulated adenylate cylase activity of thyroid cell plasma membranes were found to require an optimal Mg concentration (Wolff and Jones (1971). Hypomagnesaemia results in reduced thyroxine synthesis in rats while hypermagnesaemia has the reverse effect (Humphray and Heaton 1972, Heaton and Humphray 1974).

The present study was undertaken to determine the effects of induced hypomagnesaemia and moderate hypermagnesaemia on the EMg levels and thyroxine levels in young calves to determine;

(a) Whether EMg was a better indicator of the Mg status of calves than PMg.

(b) Whether the responses of EMg and PMg levels to a Mg load could be a useful indicator of Mg status and

(c) Whether metabolic rate (assessed in terms of blood thyroxine levels) was influenced by Mg status in young calves.

MATERIALS AND METHODS

Experimental animals

Seven male dairy calves (4 Friesian and 3 Jerseys) 1-2 weeks old were used. They were randomly allocated into experimental (4 calves) and control (3 calves) groups and each group was put into a concrete stall with rubber floor matting. The calves were weighed once a week during the period of observation.

Feeding regime

The calves were fed twice a day, 2 litres of whole milk per feed in the first week, 1 litre whole milk and 1 litre skimmed milk per feed in the second week and 2 litres of skimmed milk per feed thereafter. They had access to water and a barley straw mix containing 5% urea, 10% soyabean oil and 5% potassium chloride (KCl) ad libitum throughout the experimental period. The control calves were each given 1 g of magnesium oxide (MgO) and experimental calves were each given 1g of KCl and 1g of urea in their morning milk feed.

Blood collection

Blood samples were collected once a week from the jugular vein until the PMg concentrations in experimental calves fell to a level approximately 0.5 mmol/l less than the control calves at which time all calves were subjected to a magnesium load by injecting them with a solution of magnesium sulphate ($MgSO_4$).

Magnesium load

Urine was collected for 24 hours (pre-load) and a blood sample taken before the calves were injected intramuscularly with $MgSO_4$ solution at a dose rate of 40 mg/kg body weight. The total urine was collected in the following 24 hours (post-load) and blood samples were taken every 4 hours for 24 hours.

Urine collection

Urine was collected into a bottle connected to a plastic bucket by a rubber tube. The bucket was suspended on the calf beneath the preputial area by rubber bands tied around the calf's back. The position of the bucket was monitored constantly to maintain accurate urine collection. 2.0 ml of urine were also obtained at each blood collection time.

Preparation of samples and analytical methods

These have been described earlier in the section of general materials and methods. The blood samples were analysed for PCV, Hb, RCC, plasma Mg, Na, K, Ca, Pi, glucose, alkaline phosphatase (AP), thyroxine and erythrocyte levels of Mg, Na and K. The urine was analysed for Mg concentration.

Statistical analysis

The mean weekly concentrations of each blood component for each group were obtained and significance of differences estimated by Student's T-test. The significance of mean differences in blood component levels between initial and final sampling in the two groups was assessed by Student's T-test.

RESULTS

(a) Changes in blood chemistry during experimental induction of hypomagnesaemia in calves

The mean changes in levels of blood components during the induction of hypomagnesaemia are shown in Fig. 11. Table 31 shows the comparisons between the initial and final values of blood components in both groups.

Plasma magnesium (PMg)

The PMg levels decreased significantly ($P<0.01$) in the experimental calves. The decrease was slow in the early stages of the experiment. The Mg levels in the control group did not show any significant change, and they were significantly higher ($P<0.01$) than those of the experimental calves by the end of the experiment (Fig. 11a, Table 31).

Erythrocyte magnesium

The mean levels of EMg decreased significantly in both the experimental calves ($P<0.01$) and control ($P<0.05$) groups. The decrease was very similar in both groups upto 4 weeks and then slowed down in the control group such that by the end of the experiment the mean level of EMg in the control group was significantly higher ($P<0.01$) than that of the experimental group (Fig. 11b, Table 31). The decrease in the EMg concentration

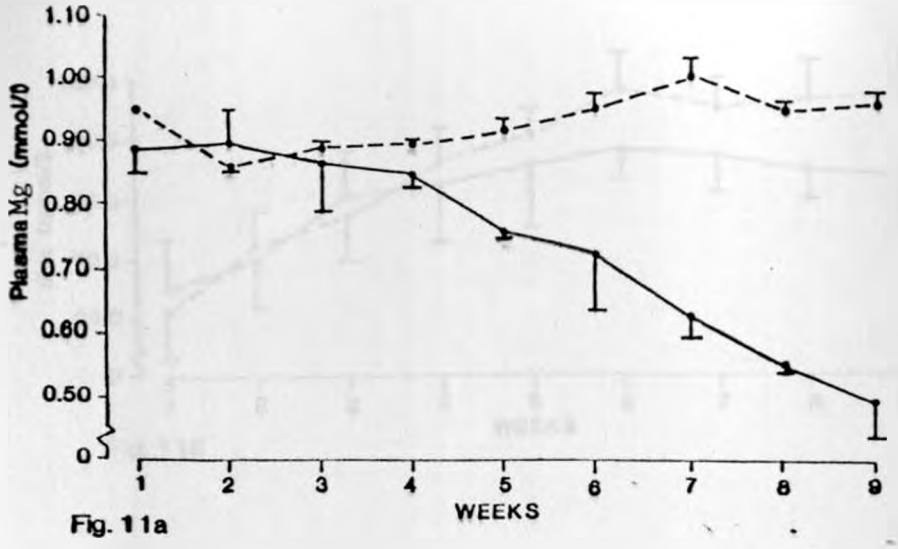


Fig. 11a

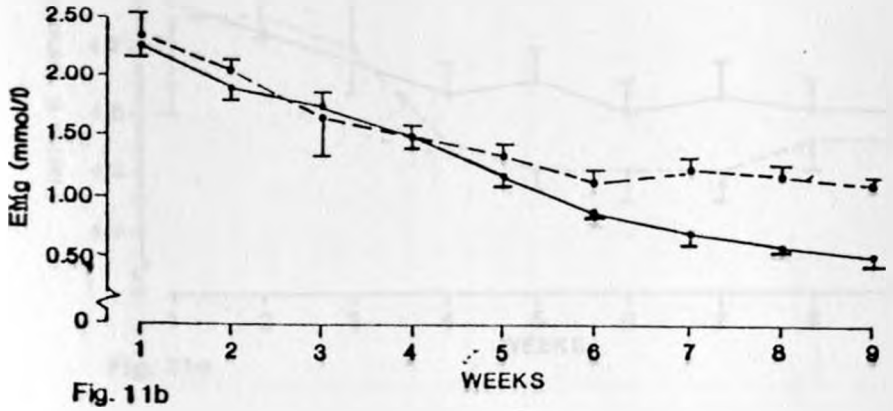


Fig. 11b

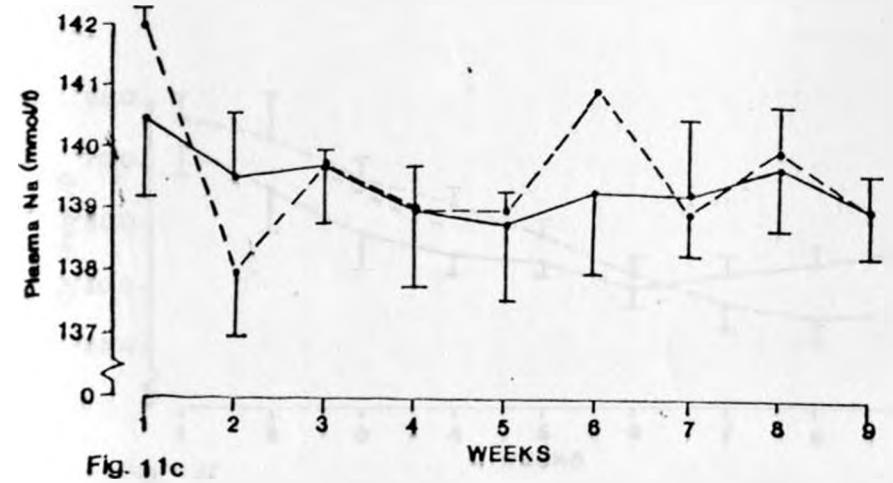
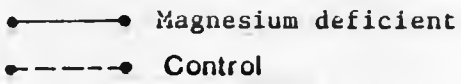


Fig. 11c



Figures 11a-11c. Changes in plasma Mg, EMg and plasma Na (weekly mean ± SEM) values during experimental hypomagnesaemia in young calves and in control calves

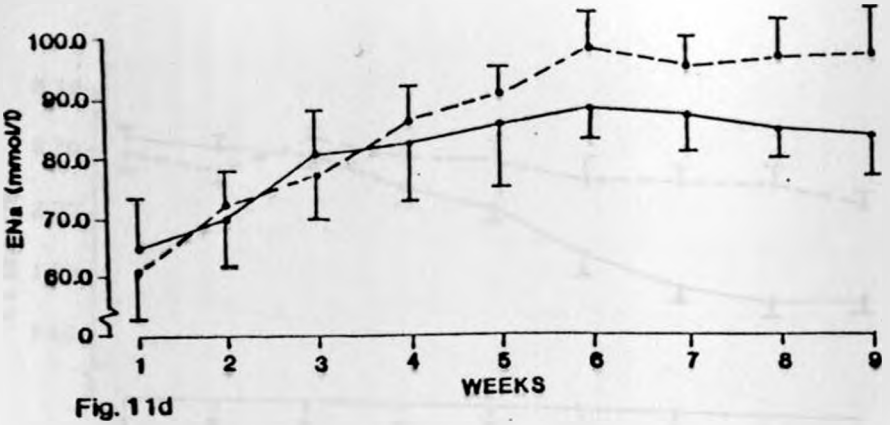


Fig. 11d

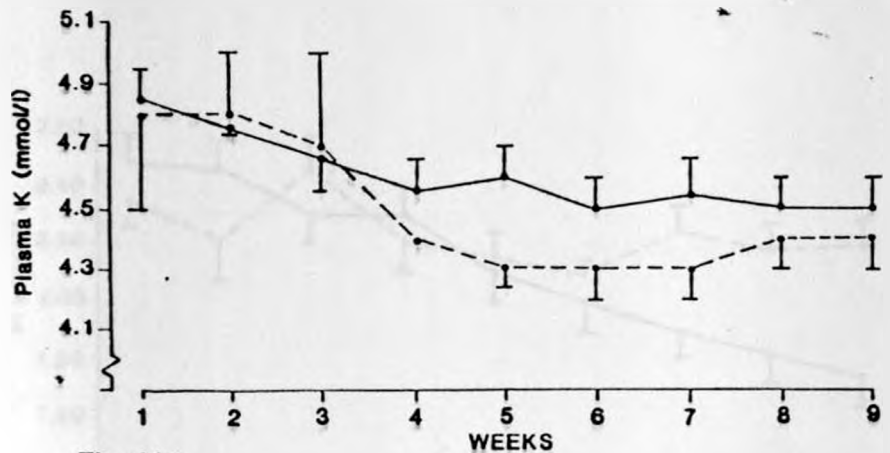


Fig. 11e

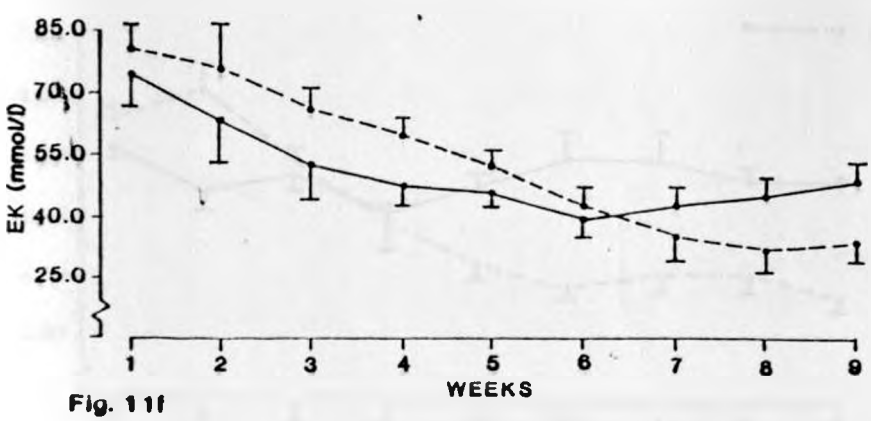


Fig. 11f

Magnesium deficie

Control

Figures 11d-11f. Changes in ENa, plasma K and EK (weekly mean ± SEM) values during experimental hypomagnesaemia in young calves and in control calves

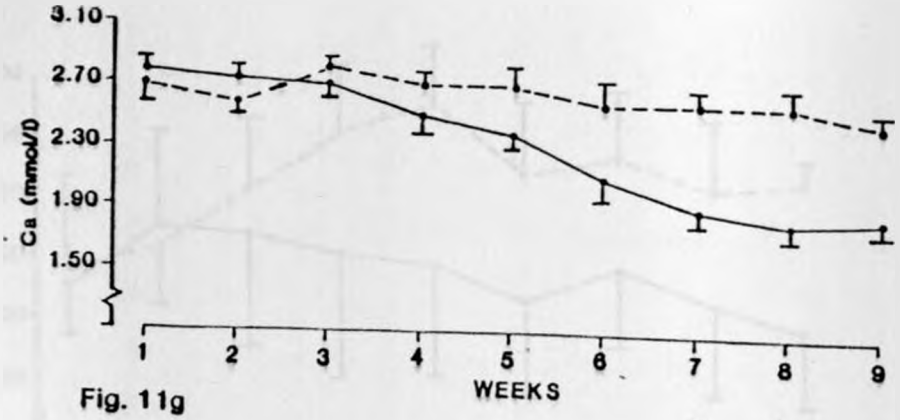


Fig. 11g

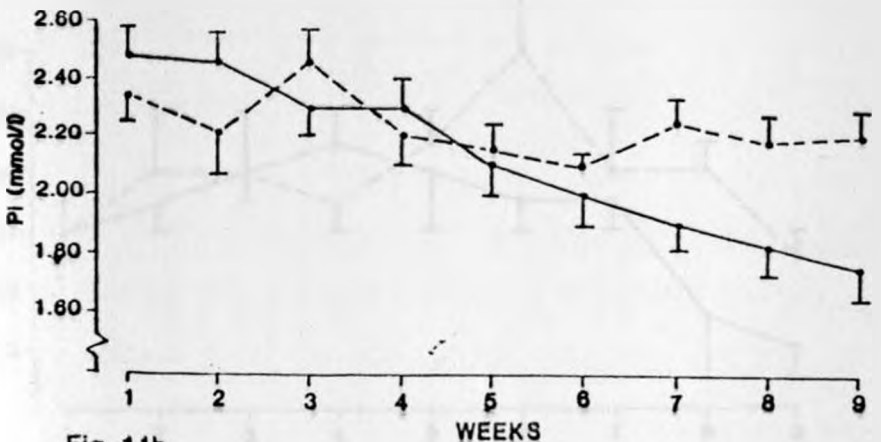
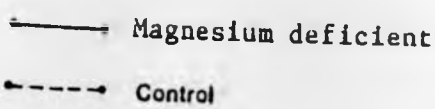


Fig. 11h



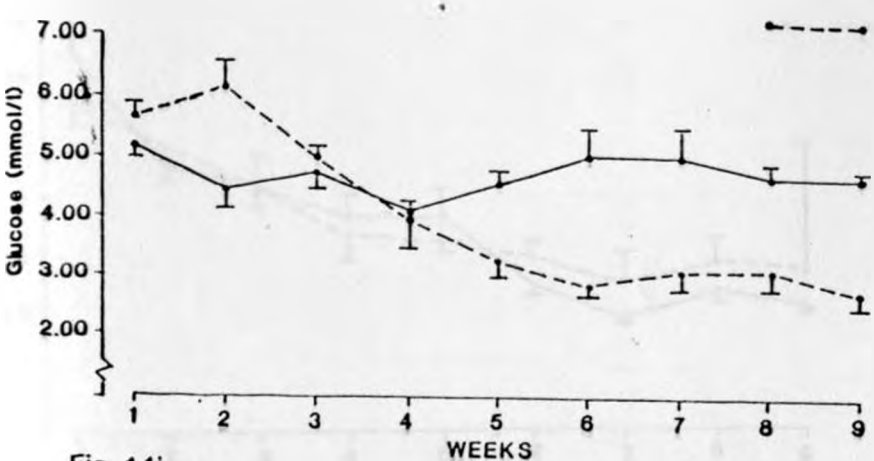


Fig. 11i

Figures 11g-11i. Changes in Ca, Pi and plasma glucose (weekly mean ± SEM) values during experimental hypomagnesaemia in young calves and in control calves

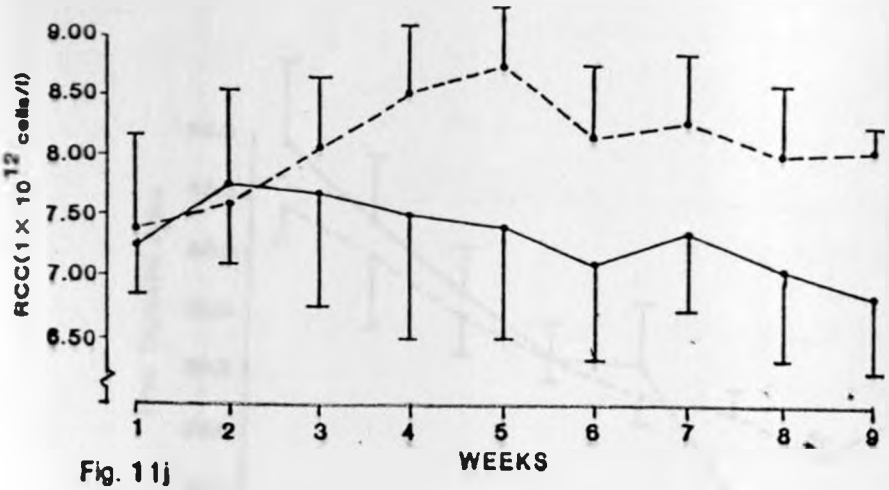


Fig. 11j

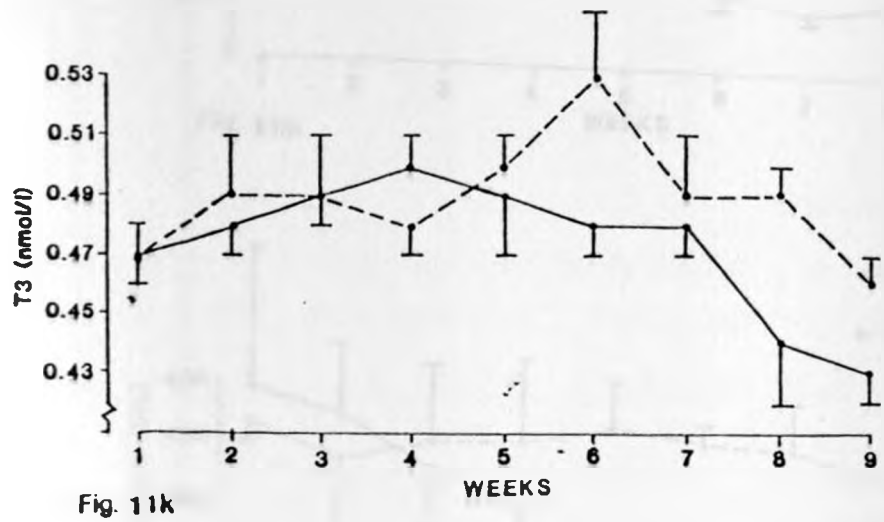


Fig. 11k

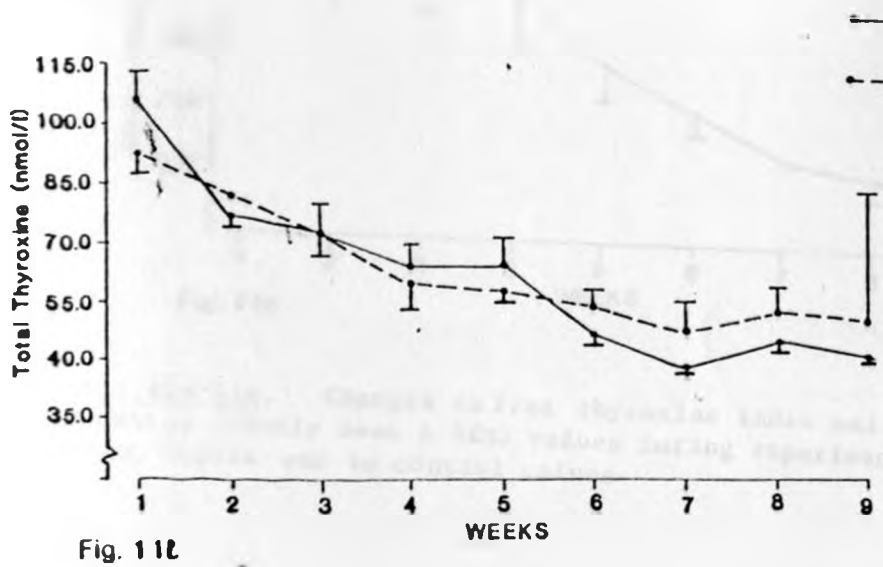
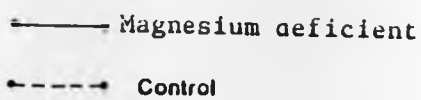


Fig. 11l



Figures 11j-111. Changes in RCC, T_3 and total thyroxine (weekly mean ± SEM) values during experimental hypomagnesaemia in young calves and in control calves

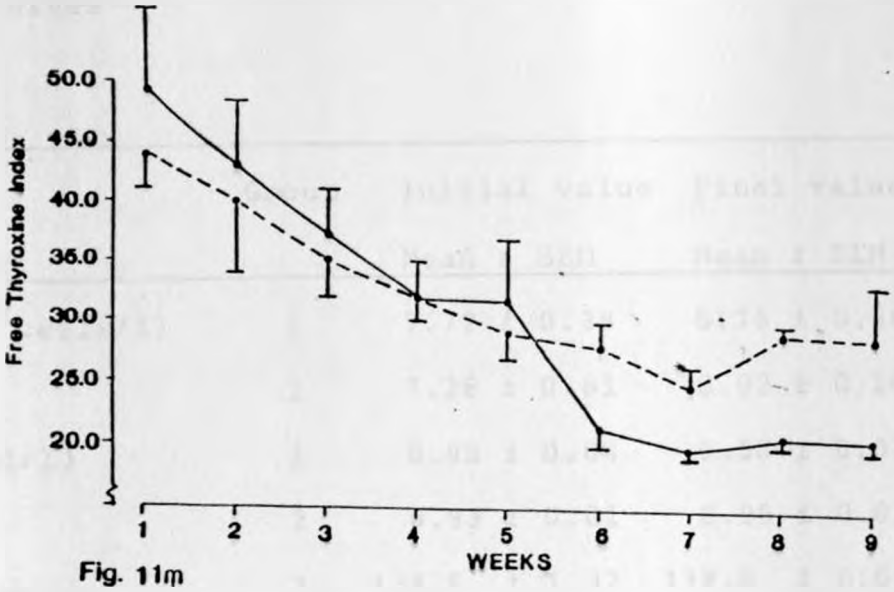


Fig. 11m

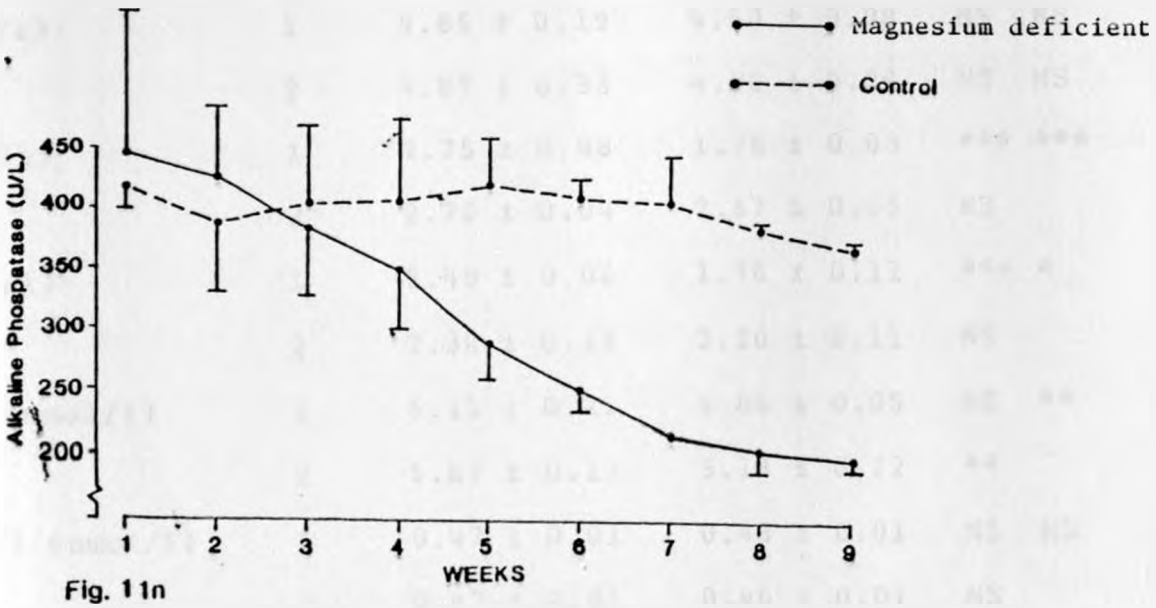


Fig. 11n

Figures 11m-11n. Changes in free thyroxine index and alkaline phosphatase (weekly mean ± SEM) values during experimental hypomagnesaemia in young calves and in control calves

Table 31 Comparisons between initial and final values of blood components in both experimental (hypomagnasaemic) and control calves

Blood Component	Group	Initial value Mean ± SEM	Final value Mean ± SEM	T-Test a	T-Test b
RCC($\times 10^{12}$ cells/l)	1	7.72 ± 0.38	6.70 ± 0.56	NS	NS
	2	7.26 ± 0.81	8.02 ± 0.50	NS	
PMg (mmol/l)	1	0.89 ± 0.04	0.50 ± 0.03	**	***
	2	0.93 ± 0.01	0.96 ± 0.02	NS	
PNa (mmol/l)	1	138.5 ± 1.32	138.5 ± 0.65	NS	NS
	2	141.7 ± 0.33	139.0 ± 0.58	NS	
PK (mmol/l)	1	4.85 ± 0.19	4.60 ± 0.08	NS	NS
	2	4.87 ± 0.33	4.46 ± 0.06	NS	NS
Ca (mmol/l)	1	2.75 ± 0.08	1.78 ± 0.03	***	***
	2	2.70 ± 0.04	2.57 ± 0.05	NS	
Pi (mmol/l)	1	2.49 ± 0.06	1.76 ± 0.11	***	*
	2	2.34 ± 0.11	2.20 ± 0.11	NS	
Glucose (mmol/l)	1	5.15 ± 0.22	4.65 ± 0.05	NS	**
	2	5.67 ± 0.17	3.18 ± 0.22	**	
Plasma T3 (nmol/l)	1	0.47 ± 0.01	0.43 ± 0.01	NS	NS
	2	0.47 ± 0.01	0.46 ± 0.01	NS	
Plasma T4 (nmol/l)	1	106.5 ± 8.9	46.3 ± 2.30	*	NS
	2	94.0 ± 4.0	52.3 ± 5.50	*	
PTI	1	49.3 ± 4.32	20.8 ± 1.85	**	*
	2	44.3 ± 3.33	28.7 ± 1.88	*	

Table 31 Contd.

Plasma alkaline phosphatase (μ/l)	1	450.0 ± 27.5	198.5 ± 5.80	**	***
	2	431.3 ± 19.3	366.0 ± 5.51	*	
EMg (mmol/l)	1	2.23± 0.24	0.51± 0.08	**	**
	2	2.38± 0.20	1.34± 0.06	*	
ENa (mmol/l)	1	65.3 ± 7.12	80.1 ± 6.03	*	NS
	2	61.4 ± 10.7	96.6 ± 8.9	*	
EK (mmol/l)	1	76.8 ± 7.0	45.7 ± 3.95	*	NS
	2	82.8 ± 8.06	34.5 ± 5.21	*	

* P<0.05, ** P<0.01

1 = Experimental calves

2 = Control calves

T-Test a = between initial and final values within each groups

b = between the final values of each group

in the experimental group (adjusted for the change apparently due to increasing age as observed in the controls) was nearly twice that of PMg (Table 32).

Plasma sodium (PNa) and potassium (PK)

The mean levels of PNa and PK did not show any significant changes in either group (Figs. 11c, 11e, Table 31).

Erythrocyte sodium (ENa)

The mean levels of ENa increased significantly ($P<0.05$) in both the experimental and control calves. The increase was more rapid upto the 6th week after which it slowed down in the control calves and started decreasing in the experimental calves and by the end of the experiment the experimental calves had lower ($P<0.07$) ENa levels than the control calves (Fig. 11d, Table 31).

Erythrocyte potassium (EK)

The mean levels of EK decreased significantly ($P<0.05$) in both the experimental and control calves. The decrease was more rapid upto the 6th week and then slowed down in the control calves and started to increase in the experimental calves. The final mean level in experimental calves was non-significantly higher than that in the control group (Fig. 11f and Table 31).

Table 32: Decreases in PMg and EMg concentrations in four calves in which hypomagnesaemia was induced over 9 weeks.

	Initial value	Final value	Decrease	+ Adjusted Decrease
EMg(mmol/l)	2.25 ± 0.24	0.51 ± 0.08	1.74	0.70
PMg(mmol/l)	0.89 ± 0.04	0.50 ± 0.03	0.39	-
*(EMg mmol/l)	(2.28 ± 0.20)	(1.24 ± 0.06)	(1.04)	(-)

* Decrease in 3 control calves (mainly due to increase in age)

\+ Adjusted for change due to increase in age (i.e. 1.04)

Plasma calcium

The plasma mean Ca levels decreased significantly ($P<0.01$) in the experimental calves only. The final mean value of the experimental calves was significantly ($P<0.01$) lower than that of the control calves (Fig. 11g, Table 31).

Plasma inorganic phosphate (pi)

The mean levels of plasma Pi decreased significantly ($P<0.01$) in the experimental calves and non-significantly in the control calves. The mean level in the control calves was significantly ($P<0.05$) higher than that in the experimental calves by the end of the experiment (Fig. 11h, Table 31).

Plasma glucose

The plasma glucose levels decreased in both groups, however the decrease was significant ($P<0.01$) in the control calves only. The mean level in the experiment calves was significantly ($P<0.01$) higher than that in the control calves by the end of the experiment (Fig. 11i, Table 31).

Red cell counts

The mean RCC's did not show any significant change in either group. However, they showed a tendency to decrease in the experimental calves and to increase in the control calves,

and the difference between the final mean levels of both groups was nearly significant ($P<0.06$) (Fig. 11j, Table 31).

Plasma tri-iodothyroxine (T3)

The mean levels of T3 showed a non-significant decrease in both the experimental calves and the control calves (Fig. 11k). The decrease in the experimental calves was greater than that in the control calves and by the end of the experiment the differences between the final mean levels of both groups approached significance ($P<0.06$) (Table 31).

Plasma total thyroxine (T4)

The mean levels of T4 decreased significantly ($P<0.05$) in both the experimental calves and control calves. The decrease was similar in both groups and although by the end of the experiment the mean levels did not differ significantly between the two groups, the mean level of the experimental calves was lower than that of the control calves (Fig. 11l, Table 31).

Free thyroxine index (FTI)

The mean FTIs (i.e. index of T3xT4) decreased significantly in both the experimental ($P<0.01$) and control ($P<0.05$) groups. The decrease was similar in both groups up to the fifth week and then slowed down in the control calves such that by the end of the experiment the mean value in the controls was significantly ($P<0.05$) higher than that of the experimental calves (Fig. 11m, Table 31).

Plasma alkaline phosphatase (AP)

The mean AP level decreased significantly in both the experimental calves ($P<0.01$) and the control calves ($P<0.05$). The decrease was however greater (251.5u/l) in the experimental calves than in the control calves (66.3u/l), such that by the end of the experiment the mean level in the experimental calves was significantly ($P<0.01$) lower than that in the control calves (Fig. 11n, Table 31).

(b) Changes in blood chemistry and urine magnesium concentrations after magnesium sulphate ($MgSO_4$) loading in hypomagnesaemic and normomagnesaemic calves

The results are shown in Figures 12a-h and 13 and Tables 33, 34 and 35.

Plasma magnesium

The PMg levels increased significantly in both the experimental (hypomagnesaemic)($P<0.01$) and control ($P<0.05$) groups within 4 hours of $MgSO_4$ administration, and the increase was greater ($P<0.05$) in the experimental calves than in the control calves (Table 34). After the peak (4 hours) the PMg levels decreased in both the experimental calves ($P<0.01$) and the control calves ($P<0.05$) 24 hours after $MgSO_4$ administration (Table 33). The PMg levels of the control calves had reached the pre-load Mg level by 24 hours post-Mg injection while the

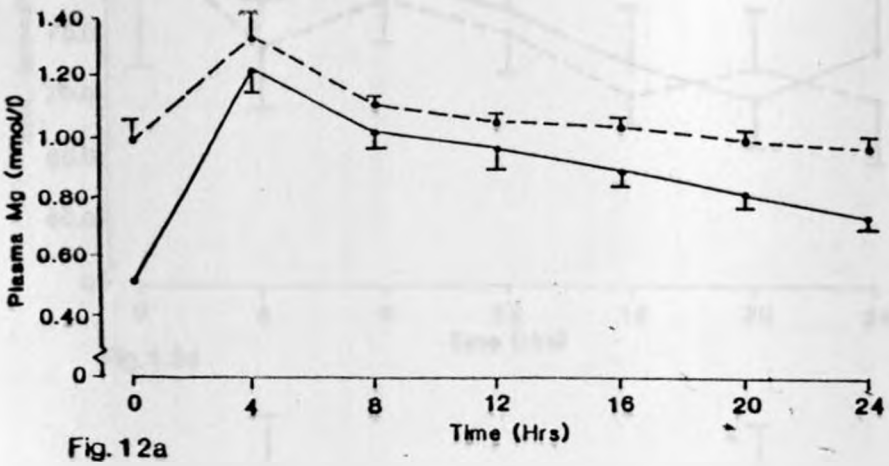


Fig. 12a

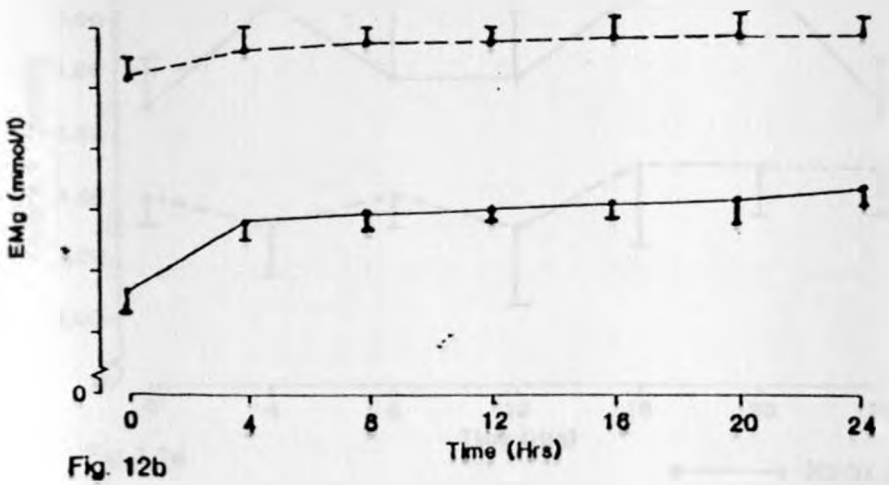


Fig. 12b

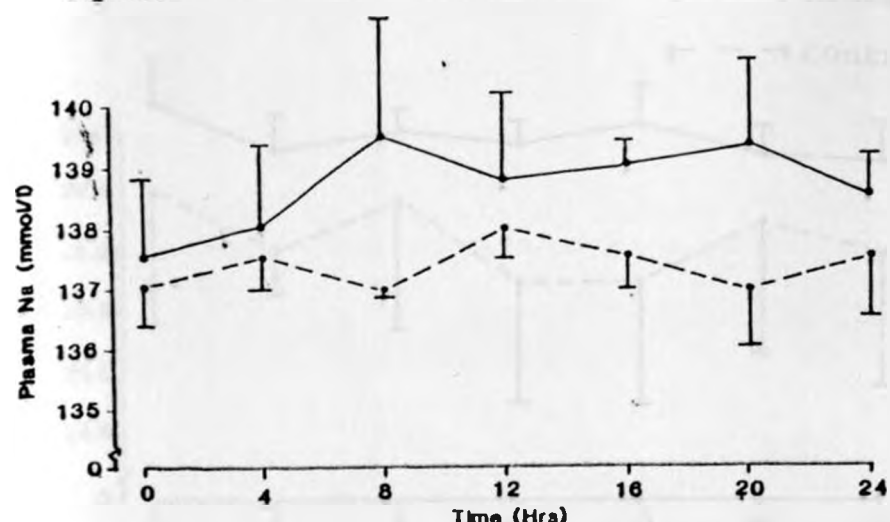


Fig. 12c

Magnesium deficient

Control

Figures 12a-12c. Changes in plasma Mg, EMg and plasma Na (mean ± SEM) values after magnesium sulphate administration in hypomagnesaemic and control calves

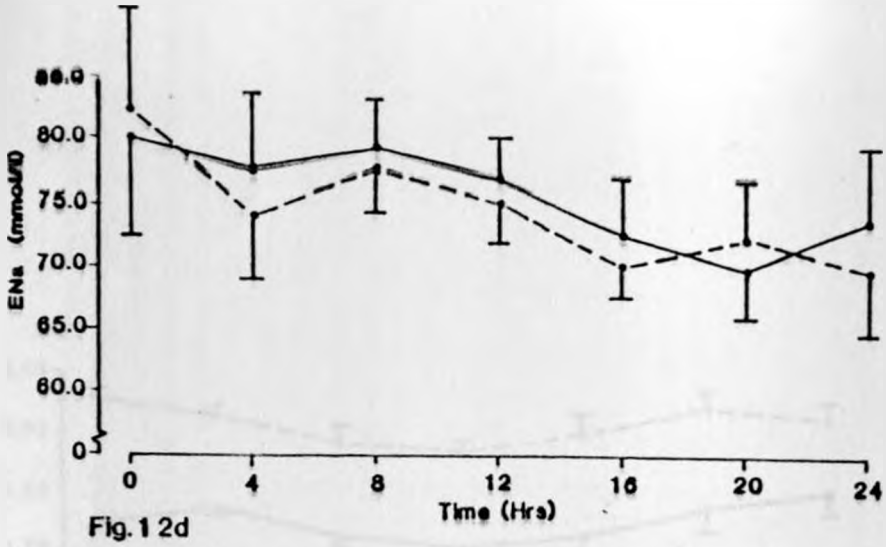


Fig. 12d

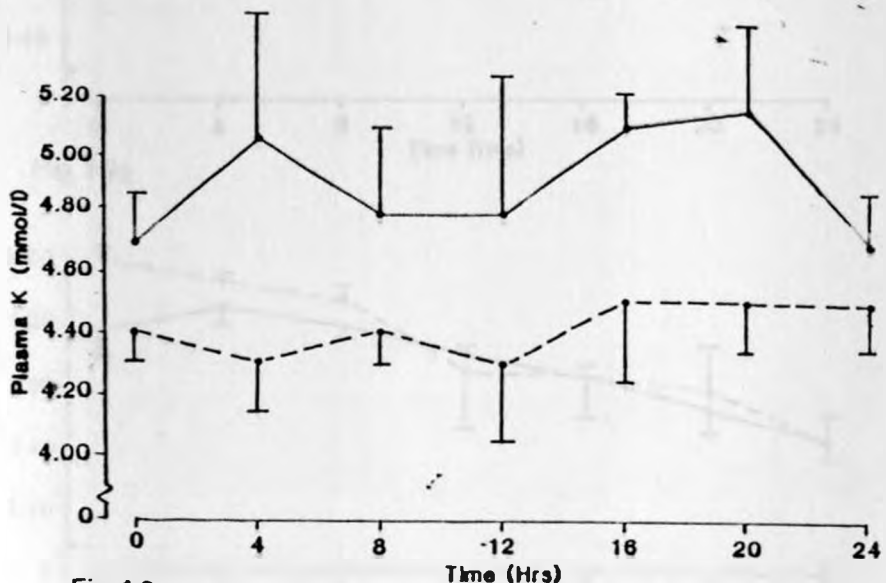


Fig. 12e

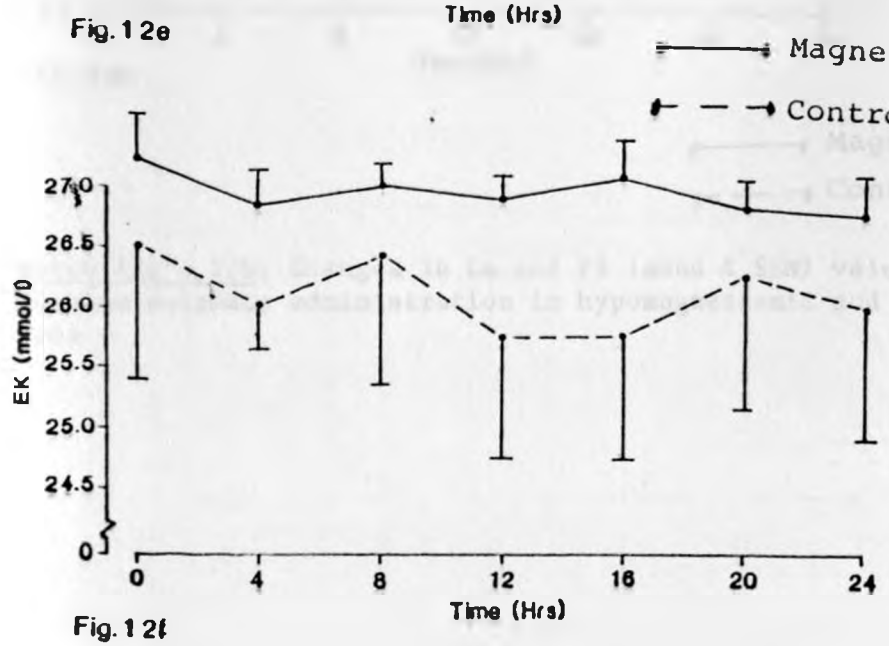


Fig. 12f

Magnesium deficient

Control

Figures 12d-12f. Changes in ENa, plasma K and EK (mean ± SEM) values after magnesium sulphate administration in hypomagnesaemic and control calves

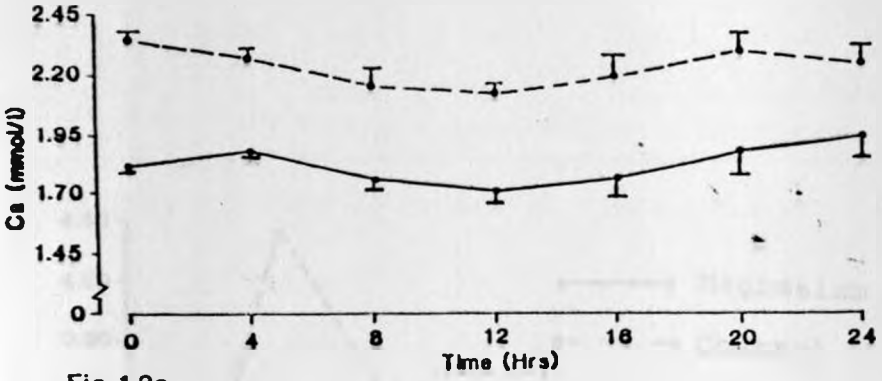


Fig. 12g

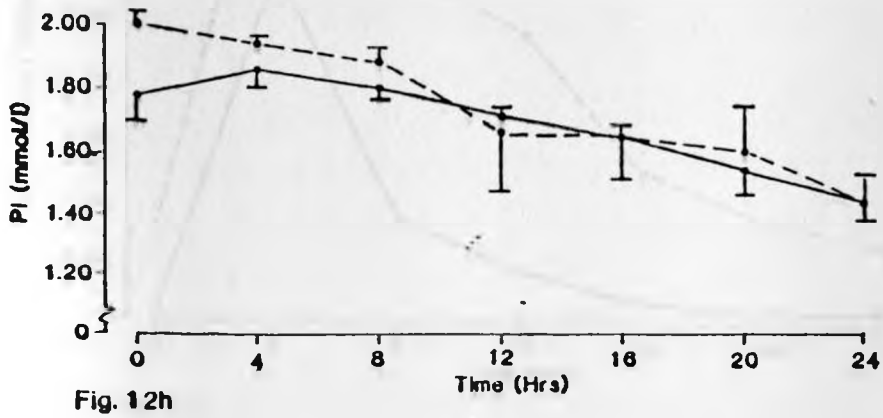


Fig. 12h

Magnesium deficient

Control.

Figures 12g - 12h. Changes in Ca and Pi (mean ± SEM) values after magnesium sulphate administration in hypomagnesaemic and control calves

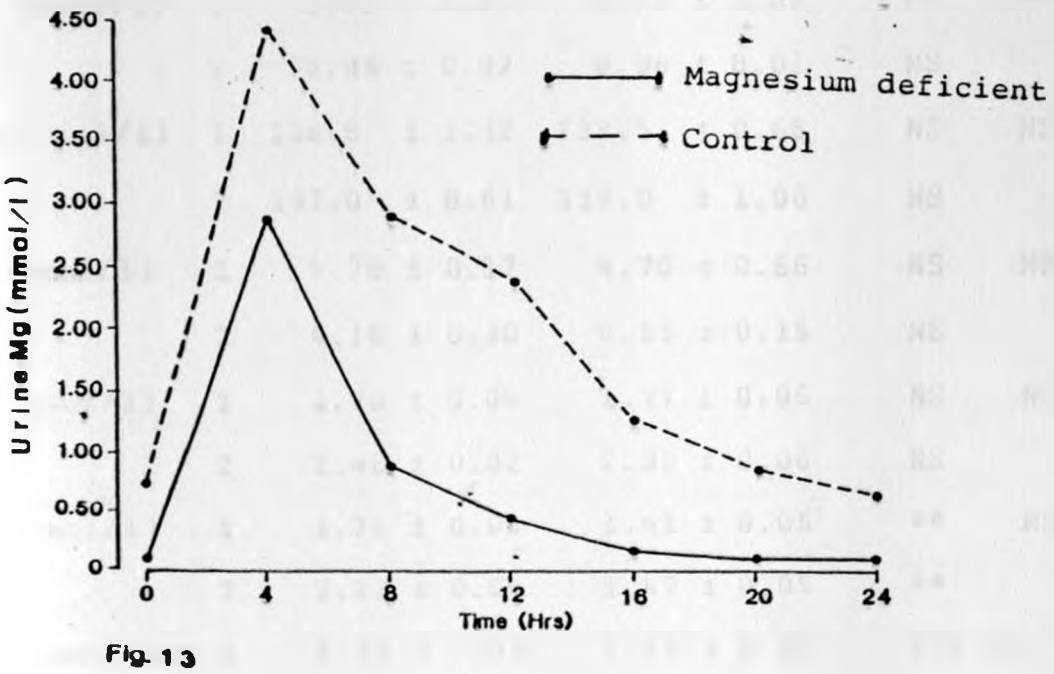


Figure 13. Urinary magnesium concentration after magnesium sulphate administration (40 mg/kg body weight intramuscularly) in hypomagnesaemic and control calves

Table 33 Comparisons between initial (pre-load) and (24 hr post-load) blood component values after $MgSO_4$ administration in hypomagnesaemic (experimental) and control calves

Blood Component	Group	Initial value Mean ± SEM	Final value Mean ± SEM	T-Test a	T-Test b
PMg (mmol/l)	1	0.51 ± 0.01	0.75 ± 0.02	**	**
	2	0.99 ± 0.02	0.96 ± 0.01	NS	
PNa (mmol/l)	1	136.8 ± 1.32	138.5 ± 0.66	NS	NS
	2	137.0 ± 0.61	137.0 ± 1.00	NS	
PK (mmol/l)	1	4.70 ± 0.17	4.70 ± 0.66	NS	NS
	2	4.10 ± 0.30	4.55 ± 0.15	NS	
Ca (mmol/l)	1	1.78 ± 0.04	1.77 ± 0.05	NS	NS
	2	2.46 ± 0.02	2.39 ± 0.06	NS	
Pi (mmol/l)	1	1.76 ± 0.06	1.41 ± 0.05	**	NS
	2	2.23 ± 0.04	1.47 ± 0.05	**	
EMg (mmol/l)	1	0.55 ± 0.09	0.83 ± 0.07	P<0.06	**
	2	1.24 ± 0.02	1.37 ± 0.08	NS	
ENa (mmol/l)	1	129.7 ± 5.88	118.9 ± 5.45	NS	NS
	2	122.3 ± 2.30	116.1 ± 4.46	NS	
EK (mmol/l)	1	27.9 ± 2.57	26.1 ± 3.11	NS	NS
	2	23.6 ± 5.45	21.5 ± 4.77	NS	

* P<0.05, ** P<0.01

1 = Experimental calves

2 = Control calves

T-Test a = between initial and final values within group

b = between the final values of each group

Table 34: Changes between 0 and 4 hrs in PMg, EMg and urine Mg concentrations in hypomagnesaemic (experimental) and control calves after Mg loading

Parameter	Group	Mean change from 0 - 4 hrs Mean ± SEM	T-Test a	T-Test b
PMg (mmol/l)	1	0.70 ± 0.04	*	**
	2	0.29 ± 0.04	*	
EMg (mmol/l)	1	0.22 ± 0.06	NS	NS
	2	0.11 ± 0.06	NS	
Urine Mg (mmol/l)	1	2.34 ± 0.4	**	*
	2	3.85 ± 0.3	**	

* $P<0.05$

** $P<0.01$

1 = Experimental calves

2 = Control calves

T-Test a = Significance of the difference between the means (i.e. between 0 and 4 hrs) in each group

b = Significance of the group differences in changes from 0 to 4 hrs

Table 35: Urine volume and Mg concentrations during 24 hrs before and 24 hrs after $MgSO_4$ loading in hypomagnesaemic (experimental) and control calves

24 hr urine volume (1)

Group	Before loading Mean ± SEM	After loading Mean ± SEM	Difference before and after Mean ± SEM	T-Test 1	T-Test 2
1	12.46 ± 1.92	13.27 ± 1.01	0.81 ± 0.78	NS	NS
2	13.08 ± 1.28	13.86 ± 1.32	0.78 ± 0.71	NS	

24 hr Urine Mg concentration (mmol/l)

	Mean ± SEM	Mean ± SEM	Mean ± SEM		
1	0.08 ± 0.02	0.12 ± 0.03	0.04 ± 0.02	NS	*
2	0.50 ± 0.02	0.98 ± 0.03	0.48 ± 0.03	**	

* $P<0.05$, ** $P<0.01$

1 = Experimental calves

2 = Control calves

T-Test a = Significance of the difference of the means (i.e. before and after load) in each group

b = Significance of the difference of the changes (before and after) between the two groups

experimental calves were still higher ($P<0.05$) than the pre-load Mg levels (Fig. 12a). In the experimental calves the PMg levels were still lower ($P<0.05$) than those of the control calves 24 hours after $MgSO_4$ injection (Table 33).

Erythrocyte magnesium

The EMg levels increased in both the experimental calves and control calves, and this increase approached significance ($P<0.06$) in the experimental calves only by 24 hours post $MgSO_4$ injection (Table 33). The increase was greatest within 4 hours of Mg injection (Fig. 12b) and this did not differ significantly between the two groups (Table 34). The EMg levels of the experimental calves were still lower ($P<0.05$) than those of the control calves 24 hours after Mg injection (Table 33).

Plasma and erythrocyte sodium and potassium

The concentrations of PNa, ENa, PK and EK did not show any significant change after $MgSO_4$ injection in calves in either group (Figs. 12c, to 12f, Table 33).

Urine magnesium

The 24 hour urine volumes did not differ significantly between pre-load and post-load volumes in the two groups (Table 35). The pre-load urinary Mg concentration in the control group was significantly ($P<0.01$) higher than that in the experimental calves (Table 35). After $MgSO_4$ injection the urinary Mg

concentration increased significantly ($P<0.01$) in both groups within 4 hours of injection and this increase was higher ($P<0.05$) in the control calves than in the experimental calves (Table 34). After the peak response (at 4 hrs) the urinary Mg concentration decreased ($P<0.01$) to values slightly above the pre-load values in the experimental calves, while in the control calves the values remained higher than the pre-load values upto 24 hours post $MgSO_4$ injection (Fig. 13).

The 24-hour post-load urine Mg concentration was significantly ($P<0.01$) higher than the pre-load 24 hour concentration in the control calves only. The change (increase) in the urine Mg concentration 24 hours after Mg injection was greater ($P<0.05$) in the control calves than in the experimental calves (Table 35).

DISCUSSION

(a) Blood chemistry changes during experimental induction of hypomagnesaemia in calves

The results show that hypomagnesaemia can be induced in young calves housed indoors by feeding a skimmed milk ration plus cereal chaff containing high levels of K, urea and soyabean oil. Blaxter, Cowlishaw and Rook (1960) failed to induce hypomagnesaemia in calves by feeding them a high K ration. Thus it is probable that the addition of urea and soyabean oil to the diet in this experiment may have aided the induction of hypomagnesaemia. Urea and soyabean oil depress Mg uptake from the alimentary tract (Erschoff 1948, Head and Rook 1955).

The initial slow decrease in PMg concentrations in the experimental calves could have been due to the high rate of Mg absorption from the large intestine in young calves (Smith 1959). As K is absorbed higher in the alimentary tract (rumen, abomasum and small intestine) than Mg (Steward and Moodie 1956, Suttle and Field 1969) an adverse effect of K on Mg absorption might have been avoided. In calves Mg was found to be absorbed from the large intestine up to five weeks of age after which time no further absorption occurred at that site (Smith 1959). The slow decrease of PMg levels in the early stages of induction of hypomagnesaemia could also have been due to the large labile reserve of Mg in the skeleton of young calves which can be mobilized (Blaxter, Rook and macDonald 1954). Upto 30% of skeletal Mg in young calves can be mobilized (Blaxter, Rook and McDonald 1954) and so this

could counteract the decrease in PMg concentration in a short period of dietary Mg deficit. The high Mg levels in the ration of the control calves should have increased Mg availability (Rook and Balch 1958) in spite of the high levels of K, urea and oil, thus enabling them to maintain their PMg levels.

The lack of any significant change in PNa and PK levels in either group was similar to previously reported results (Suttle and Field 1969).

The decrease in plasma Ca levels in the experimental calves could have been due to a reduced Ca release from bone and/or impaired action of parathyroid hormone (PTH) on bone. An impaired action of PTH on bone could explain the continued decline in plasma Ca levels with hypomagnesaemia, when both hypocalcaemia and hypomagnesaemia would have led to an increased secretion of PTH (Care, Sherwood, Potts and Aurbach 1966 , Buckle, Care, Cooper and Gitelman 1968).

Studies with bone cultured in vitro have shown that Mg increases Ca release from bone by displacing it from the hydration shell and by stimulating processes which involve the simultaenous catabolism of the matrix and mineral phase (Pak and Diller 1969, MacManus and Heaton 1970). Thus Mg deficiency would lead to a decrease in Ca release from the bone and hence reduce its concentration in the plasma. The decrease in plasma Ca concentration during hypomagnesaemia has been found to be due to

a decrease in the release of Ca from the bone (Larvor and Brocharrt 1964). These workers suggested that Mg could be involved in PTH activity on the bone. Later reports in rats showed that Mg deficiency reduced Ca release from the bone and inhibited the action of PTH on bone (MacManus and Heaton 1970, MacManus, Heaton and Lucas 1971). Thus the continued decrease in plasma Ca levels in Mg-deficient calves could have been due to reduced action of PTH on the bone.

The decrease in Pi level in the experimental calves could have been due to increased secretion of PTH as a result of both hypomagnesaemia and hypocalcaemia (Care, Bell and Bates 1971). The increase in PTH levels would have reduced the Pi levels either by increasing renal phosphate excretion or increasing salivary excretion of phosphate (Mayer et al. 1966).

The higher plasma glucose levels observed in the experimental calves could have been partly due to impaired cellular glucose utilization. Mg is required for hexokinase reactions and is also involved in other aspects of energy utilization (Wacker 1969). Glucose metabolism via pathways of oxidative phosphorylation and the transketolase reaction of the pentose monophosphate shunt require Mg (Pike and Brown 1975). Thus the observed high plasma glucose levels in the experimental calves may be partially reflective of the dependence of energy-related enzyme systems on Mg.

The high plasma glucose levels in the Mg deficient calves could also have been partly due to delayed development of the rumen micro-organisms, which utilize dietary glucose for their energy and break-down of other carbohydrates to rumen volatile fatty acids (Roe et al. 1966) and a ruminant-type metabolism. Mg is required in the development of rumen microflora (Martin et al. 1964, Ammerman et al. 1971). Thus Mg deficiency in the experimental calves could have led to delayed development of the rumen microflora and hence higher glucose absorption from the digestive system into the blood circulation.

The plasma AP levels (mean 440u/l) in both the experimental and control calves at start of the experiment were more than five times the normal upper levels (10-80 u/l) of adult cattle. Plasma AP activities are normally very high during periods of high bone metabolism (Russel et al. 1974). Thus the high plasma AP levels observed in both groups at start of the experiment could have been due to a higher activity of bone formation which would be expected in young growing animals. The plasma AP levels decreased significantly in both the experimental ($P<0.01$) and control calves ($P<0.05$) with age. Similar observations have been reported in Jersey cattle (Agergaard and Larsen 1974). The much greater decrease of plasma AP level in the experimental calves suggests that the decrease was due not only to an increase in age but also to Mg deficiency. Mg is required in bone formation (Blaxter and Rook 1954, Blaxter, Rook and MacDonald 1954), and its decrease in the experimental calves could have been associated with decreased bone

formation and a decrease in bone AP synthesis and subsequently a decrease in plasma AP levels. Inhibition of bone formation has been found to result in a decrease in bone AP isoenzyme synthesis (Larvor and Brochart 1964, Elin et al. 1971).

Mg is required in the synthesis of AP (Elin et al. 1971) and its deficiency has been shown to lead to a decrease in AP levels (Larvor and Brochart 1964). Thus the much greater decrease in the experimental calves could have also been due to decreased AP synthesis as a result of the Mg deficiency.

However, although AP levels decreased in the Mg-deficient calves, lower than normal levels of AP can not be used as an indicator of body Mg status in calves since AP levels can be affected by many other factors. For example, zinc deficiency, decreased total feed intake and stress have been reported to decrease AP levels in calves (Miller et al. 1969).

The RCC's showed a tendency to decrease in the experimental calves, however it was not significant (P=0.06). RCC levels have been reported to decrease in Mg deficient calves (Larvor et al. 1965). The lack of a significant decrease in RCC levels in the Mg-deficient calves could be because the plasma Mg levels did not reach levels as low as those (0.12 mmol/l) reported by Larvor et al. (1965).

The mean EMg levels were very high (mean 2.40 mmol/l) in both groups at the start of the experiment. These high levels could

have been due to several factors. It could be that in early life young calves have a higher number of reticulocytes and young erythrocytes which contain higher EMg levels than old erythrocytes (Bernstein 1959, Ginsburg et al. 1962). It could also be that foetal haemoglobin (HbF) has a higher affinity for Mg leading to higher levels of non-exchangeable bound EMg (Care 1960) and thus higher total EMg levels. Most of the EMg is found in the cell sap in association with Hb where it is bound to organic phosphates (mostly ATP) and enzymes (Rose 1968, Bunn et al. 1971). It is also possible that the foetal erythrocytes which are replaced within 2-3 months after birth (Grimes et al. 1956) have a higher metabolic rate and hence are able to maintain higher EMg levels than adult erythrocytes. Young erythrocytes with high EMg concentration have been shown to have a higher metabolic rate than old erythrocytes (Bernstein 1959).

The decrease in the EMg levels in both groups with time was probably due to the factors discusssed above which also might explain why EMg concentrations started falling earlier than PMg concentrations.

The significantly lower ($P<0.05$) final EMg levels in the experimental calves could have been due to the formation of erythrocytes with reduced EMg values especially from 6 weeks (when the decline in EMg levels in the control calves stopped) upto the end of the experiment as a result of low Mg availability during erythropoiesis. Erythrocytes formed during periods of low Mg status have been shown to have lower EMg levels (Tufts and

Greenberg 1937, Salt 1950, Hellerstein et al. 1970). The greater decrease in EMg levels in the experimental calves could also have been due to an increase in the exchangeable Mg in the erythrocyte and increased erythrocyte permeability. During Mg deficiency erythrocyte ATP concentration is significantly reduced (Welt 1964, Elin 1973) and this could lead to an increase in the free (exchangeable) EMg. Depletion of erythrocyte ATP also decreases erythrocyte membrane stability (Elin 1973), thus this could lead to a decrease in EMg levels during Mg deficiency. The greater decrease in EMg concentrations compared to PMg concentration in the experimental calves (Table 31) was similar to the observation of Tufts and Greenberg (1937). The smaller decrease in PMg levels could have been due to one or simultaneous effects of the following; decreased Mg excretion (urinary Mg fell to almost zero - Table 35), drop in the absolute amount of Mg deposited in the bone during this period and/or some diffusion of EMg into the plasma.

The results of this study show that EMg levels decrease with age in young calves and the decrease is almost complete by 8 weeks after birth. This implies that in order to eliminate the effect of age on EMg levels, assessments of EMg should be carried out after 2 month of age. The greater decrease of EMg levels in the experimental calves suggests that EMg concentrations could be used to assess the Mg status of an animal and detect chronic Mg deficiency. Also the greater decrease in EMg concentrations than in PMg concentrations suggests that estimation of EMg levels may be a better indicator of body magnesium status

than PMg estimation during periods of prolonged marginal Mg deficiency.

The concentration of ENa increased ($P<0.05$) while that of EK decreased ($P<0.05$) in both groups. This confirmed the earlier finding (chapter 4) that ENa concentrations are lower and those of EK higher in young calves than in adult cows. The larger changes observed from week 1 to week 6 for both ENa and EK concentrations indicate that changes in these cations are considerable in the first two months of life (Table 11). The results also show that in Mg-deficient calves, EK concentrations tended to increase (and PK did not) while those of ENa tended to decrease after a six-week period. The changes could have been brought about by an increased number of young erythrocytes with high EK and low ENa concentrations (Evans 1963a, Timms and Murphy 1980) coming into circulation. The decline in RCC's in the experimental calves although not significant could have been enough stimulus to increase erythrocyte production and release into circulation of young erythrocytes (Evans 1963).

The initial high T4 concentrations (mean 100.2 nmol/l) and the decrease with age in both groups were similar to previous work in young growing calves (Kahl et al. 1977, Khurana and Madan 1984). Mg deficiency in rats has been found to inhibit iodine uptake by the thyroid gland and to decrease protein bound iodine concentration in the serum (Heaton and Humphray 1974). Thus the slightly greater decrease in T3 and T4 levels

in the the experimental calves could have been due to reduced iodine uptake by the thyroid gland leading to a lower production of T3 and T4. The decrease in FTI values in both groups was reflective of the decrease in T3 and T4 concentrations with age. However, the significantly ($P<0.05$) lower final FTI in the experimental calves than in the control calves suggests that during hypomagnesaemia in calves FTI values could be a better indicator of thyroid activity than either T3 or T4 and that hypomagnesaemia does reduce thyroid activity in calves.

(b) Changes in blood chemistry and urinary magnesium after magnesium sulphate administration (magnesium loading) in hypomagnesaemic and control calves

The initial increase and subsequent decrease in PMg concentration in both groups after $MgSO_4$ administration was predictable. The rapid fall in PMg concentration in the control calves was probably due to urinary Mg excretion (Table 34). In normal animals (with no Mg deficiency) most of the parenterally administered Mg is rapidly excreted in urine within 24 hours (McAleese, et al. 1961, Simesen et al. 1962). The rapid decrease in PMg concentration in the experimental calves could have been due to tissue uptake, since very little of the injected Mg was excreted in the urine (Table 34). During periods of Mg deficiency Mg is lost from body tissues into the extracellular fluid (Blaxter and Rook

1954, McAleese, et al. 1961). Subcutaneous Mg injection into Mg-deficient calves did not increase fecal Mg excretion (Smith 1959).

Although the increase in the EMg concentration approached significance ($P<0.06$) in the hypomagnesaemic group 24 hrs after Mg load the change was not significantly different from that in the control group (Fig. 12b). The increase in EMg concentration was not significantly different between the two groups 4 hours after Mg load when most of the Mg uptake occurred (Table 34). The increase was higher in the hypomagnesaemic group than in the control group (Table 33). This indicates that Mg uptake by erythrocytes even in Mg deficiency is not great. There are variable reports on the in vivo and in vitro studies on Mg uptake by the erythrocytes. Wallach et al. (1962) found no change in EMg levels following 2-4 fold increases in PMg levels. McAleese, et al. (1961) found only slight uptake of Mg28 by sheep erythrocytes in contrast to McDonald et al (1959) who found a very marked uptake of Mg28 following intravenous Mg28 injection. A marked increase in EMg concentration after addition of Mg to blood has been observed by some workers (Tufts and Greenberg 1937, Care et al. 1959) while other workers observed only a slight increase (Ginsburg et al. 1962). The reason(s) for these varying reports is not clear, however it may be that erythrocyte uptake of Mg varies under differing physiological states in the animal.

In the present study erythrocytes did take up some Mg and although the rate of uptake was not significant it was twice as high in the erythrocytes with low EMg concentrations. The small number of observations may have mitigated against obtaining significant differences. In Mg deficiency associated with low intracellular and extracellular Mg levels the erythrocyte's membrane stability decreases and is restored when Mg is supplied (Elin et al. 1971, Elin 1973). Thus the slightly higher uptake of Mg by the erythrocytes of the hypomagnesaemic group 4 hrs after Mg injection may have been possibly due to an increased erythrocyte membrane permeability as a result of decreased erythrocyte membrane stability.

The implication of these results could be that EMg concentrations may be a better indicator of the long-term Mg status of an animal than PMg concentrations. This is because sudden increases in dietary Mg which would elevate the PMg concentration would have comparatively little effect on EMg concentrations (Table 34).

The $MgSO_4$ injection did not have any significant effect on PNa, PK, ENa and EK concentrations in either group which suggests that a sudden increase in PMg concentration does not affect these components. The lack of any significant change in plasma Ca concentration in both groups indicates that it was not influenced by the increase in PMg concentration. High PMg concentration stimulates release of both calcitonin (Care et al.

1971) and parathyroid hormone (PTH) (Rude et al. 1976). Thus the effects of calcitonin on plasma Ca (which decreases plasma Ca concentration) could have been counteracted by PTH (which increases plasma Ca concentration) hence resulting in no significant change in plasma Ca concentration. The significant ($P<0.01$) decrease in Pi levels in both groups following MgSO4 injection could have been due to increased phosphorus excretion in the urine and saliva (Mayer et al. 1966) as a result of increased PTH secretion (Rude et al. 1976) and/or increased utilization for energy metabolism reactions.

The pre-load urinary Mg concentration was higher ($P<0.01$) in the control group than in the experimental calves. This was as expected since in periods of Mg deficiency very little Mg is excreted in urine (Field 1961, McAleese et al. 1961). Urinary Mg levels have been suggested to be better indicators of body Mg status and a more reliable means of monitoring the adequacy of dietary Mg intake in dairy cows than PMg levels (Collins and Brophy 1980). Thus latent Mg deficiency (i.e. without decrease in PMg levels) can be detected by the level (or absence) of urinary Mg excretion.

The significantly ($P<0.05$) higher increase in urinary Mg concentration 24 hrs after Mg load in the control group than in the hypomagnesaemic group (Table 34) indicates that changes in urinary Mg concentrations following a Mg load could be used to determine Mg status of calves and perhaps other animals. Animals with low body Mg status will have less urine Mg concentration

than animals with normal body Mg status. Caddell (1975) found that the amount of Mg excreted in urine within 24 hours could be used to detect latent Mg deficiency in infants. She found that children with Mg deficiency excreted less Mg in urine than those with a normal Mg status following parenteral Mg administration.

The significantly ($P<0.05$) lower 4 hour urine Mg concentration in the hypomagnesaemic calves than in the control calves (Table 34) also indicates that 4 hour urine Mg concentration after Mg load can be used to detect Mg deficiency in calves provided fluid intake and urine output are comparable. To overcome the latter problem a urine creatinine ratio technique or a urine magnesium/solute ratio technique as suggested by Caple and Halpin (1985) could be utilized. In the former method the concentration of magnesium is expressed as the ratio of creatinine clearance or as the percent creatinine clearance ratio while in the latter, the urine magnesium concentration is expressed as a ratio of the total urine solute concentration determined either by measurement of urine osmolality or indirectly by urine specific gravity. Urine magnesium output would then be expressed as µmol/mosmol.

CHAPTER 9

GENERAL DISCUSSION AND CONCLUSIONS

This thesis incorporates several studies on changes in blood chemistry in dairy cattle under various physiological conditions and the relationships of such changes to production indices (milk production, fertility and growth rate).

The studies included;

(1) Changes in blood chemistry in late pregnancy and early lactation in dairy cows and the relationship of such changes with fertility and milk production

(2) Changes in blood chemistry during a short period of starvation (40 hours) in heifers (11 months old) and relationship of the observed changes to growth rate

(3) Changes in blood chemistry after ACTH administration in heifers (14 months of age) and the relationship of the changes to growth rate

(4) Changes in blood chemistry over a 12-month period in calves from 2-13 months old and heifers from 12-24 months old and the relationship of mean values, values at selected ages and the changes in the values over selected periods with growth rate and production rank (heifers only) in first lactation and

(5) Changes in blood chemistry associated with the long-term induction of hypomagnesaemia in young calves. The development of a Mg load test based on the interpretation of changes in erythrocyte, plasma and urine Mg levels after Mg loading to determine the Mg status in young calves.

Each study has been described separately, together with a detailed discussion giving particular reference to related studies carried out by other workers. The present discussion identifies and where possible, collates those findings of practical significance to the dairy industry.

The results of this study (Chapters 3, 4 and 5) have produced new relationships between blood chemistry and fertility and milk production rank which could be used to predict the latter in dairy cows. These are mainly attributable to the use of both slopes (i.e. changes with time) and mean concentrations of blood components for both the 8 weeks pre-partum and 8 weeks post-partum periods separately and combined in correlation and multiple linear regression analyses. The determination of erythrocyte Mg, K and Na also increased the number of variables which could be used in the relationships between blood chemistry and production indices. Since EMg and EK gave significant simple and partial regression coefficients with fertility and milk production rank (Tables 14, 15 and multiple regression equations 11-16 in chapter 5) they should be included in future MPT studies in dairy cows. The results of the studies show that to exploit

the full potential of blood chemistry relationships with fertility and milk production the approach undertaken in this study might be better than the conventional MPT (Payne et al. 1970). The observation that age did not show up in any of the multiple regression equations for predicting fertility and milk production rank means that these equations may be used in herds containing cows of mixed ages. Production ranks are in themselves adjusted for age (see general material and methods).

The pre-partum slopes of globulin, albumin and WCC and the mean pre-partum albumin concentrations showed that they could be used to predict fertility in dairy cows (multiple linear regression equations 1, 2 and 7 in chapter 5). The slopes of globulin and albumin and the mean albumin concentration were particularly important in that they accounted for most of the variation in the fertility especially in herd II which had the greater number of cows. The cows with greater pre-partum slopes in globulin, smaller slopes in albumin and high albumin concentrations had better fertility (fewer services/conception and shorter days open).

Plasma globulin concentrations decreased from the 8th week to the last week of pregnancy (Fig. 1f). This suggests that the change between 8 weeks and 1 week before calving may be related to fertility. The plasma albumin concentration increased from 8 to 3 weeks before calving and then fell in the last two weeks of pregnancy to values higher than those at 8

weeks before calving (Fig. 1e). Since high pre-partum albumin concentrations were associated with better fertility, it is suggested that the concentration of plasma albumin in the last week of pregnancy and the change between 8 weeks and 1 week before calving may be related to fertility. That is, cows with high plasma albumin concentration 1 week before calving and a smaller difference in concentration between values at 8 weeks and 1 week before calving may have better fertility. The pre-partum WCC's 1 week before calving were higher than the WCC's at 8 weeks before calving (fig. 1p). Since cows with high slope (change with time) pre-partum had better fertility, it could be suggested that the change in WCC's between 8 weeks and 1 week before calving may be related to fertility in dairy cows.

From the multiple linear regression equations 1, 2 and 7 (chapter 5) it may be concluded that slopes of plasma albumin and globulin and WCC's and the mean concentration of albumin 8 weeks pre-partum may account for approximately 62 to 82% of the variation in services/conception and 44% of that in days open and thus could be useful in predicting these parameters in dairy cows. Possibly plasma albumin concentration at one week pre-partum could replace the mean concentrations over 8 weeks and the differences in concentrations of albumin, globulin and WCC's between 8 weeks and 1 week pre-partum could replace the above slopes. This would permit a more practical method of fertility predictions in a dairy herd since the need for the weekly samplings would be overcome.

The mean post-partum concentrations of glucose, Ca and globulins, and the post-partum slopes of RCC and albumin showed that they could be used to predict fertility in dairy cows (multiple linear regression equations 3, 4, 8 and 9 chapter 5). The mean concentrations of glucose and globulin accounted for most of the variation in fertility. The cows with higher glucose concentrations had better fertility a reverse situation to that of globulin. The relationship of plasma glucose concentration and fertility may have been due to its association with energy balance (Hewett 1974) which influences ovarian activity (Butler et al. 1981). Thus it could be that cows with high glucose concentrations have a positive energy balance necessary for ovarian activity and manifestation of oestrus as reported by McClure (1966, 1968). The relationship between post-partum plasma globulin concentration and fertility may have been due to its association with inflammatory processes (especially those of the reproductive tract) as suggested by Amiel (1970).

The relationship of high post-partum mean plasma glucose and globulin concentrations with fertility and their (glucose and globulin) increase over 8 weeks to values higher than at 1 week (globulin) and 2 weeks (globulin) and 2 weeks (glucose) after calving could suggest that their levels at 8 weeks post-partum may be related to fertility. That is, cows with high plasma glucose and low plasma globulin concentrations 8 weeks after calving may be likely to have better fertility.

Therefore from the multiple linear regression equations 3, 4, 8 and 9 (chapter 5) it may be concluded that post-partum mean concentrations of plasma glucose and globulin were the most important blood parameters influencing fertility and together with the pre-partum slopes of RCC's and plasma albumin and the mean concentration of Ca, they can account for approximately 71 to 87% of the variation in services/conception and 57 to 66% of that for days open. It also suggests that plasma glucose and globulin concentrations 8 weeks post-partum may be related to fertility in dairy cows, however further investigations are warranted to assess the usefulness of this simpler approach in predicting fertility.

The pre-partum mean concentrations of EMg and albumin and RCC's showed that they could be used to predict milk production rank in dairy cows (equations 11 and 12 chapter 5). The pre-partum EMg concentration was the most important blood parameter as it contributed significantly to the variation in production rank in both herds. The cows with low pre-partum plasma albumin concentrations and RCC's were probably those in which large amounts of protein (which is required for albumin and RCC formation) were drained by the foetus for its development and possibly led to a greater development of the foetal placenta. Greater development of the foetal placenta would lead to higher production of placental lactogen which could then lead to greater mammary gland growth in the dam and possibly higher milk production (Skjervold 1979, Gasu and Tomar 1981).

Since the concentration of EMg did not show any significant changes before calving (Fig. 2a) and the lowest RCC's were observed 1 week before calving (Figs. 1q) it is possible that their levels (and that of plasma albumin - see above) 1 week before calving may be related to production rank in dairy cows.

Therefore it may be concluded that the pre-partum mean concentration of EMg is an important factor in determining production rank, and together with the pre-partum mean concentrations of albumin and RCC's (equations 11 and 12, chapter 5) they could account for 54 to 77% of the variation in milk production rank in dairy cows. It could also be suggested that their mean concentrations 1 week before calving may be related to subsequent milk production rank and this suggestion is worthy of further investigation.

The post-partum mean concentrations of EK, EMg and Hb showed also they could be used to predict milk production rank in dairy cows (equations 12-14 chapter 5). The post-partum mean concentration of EK was the most important blood parameter i.e. it accounted for most of the variation in milk P.R. in both herds. The post-partum concentration of EMg and EK increased while that of Hb decreased after calving. This suggests that concentrations of EMg, EK and Hb at 8 weeks after calving may be related to milk production rank, since high EMg and low EK and Hb concentrations post-partum were associated with high milk production rank.

It is therefore concluded that the mean post-partum concentration of EK is an important factor in influencing milk production, and when combined with mean post-partum concentrations of EMg and Hb it can account for approximately 53 to 58% of the variation in production rank in dairy cows and hence could be useful in its prediction. The relationship between low EK concentration and production rank may be through the association of EK concentrations with the endocrine system system suggested by Evans (1963). He found that British breeds of cattle had lower EK concentrations than Zebu type of cattle and suggested that this could be due to differences in the functioning of the endocrine system especially the thyroid and adrenal glands. Thus it could be that cows with low EK concentrations have higher thyroid gland activity which is associated with higher milk production (Wilson 1975).

The relationship between EMg concentrations and milk production rank and also with fertility (Tables 14 and 15) may have been due to the association of Mg and thyroid gland activity found in chapter 8 and also reported by Heaton and Humphray (1972). In chapter 8 it was found that thyroid gland activity (FTI) decreased in hypomagnesaemic calves. Decreased thyroid gland activity has been associated with infertility (subnormal oestrus activity), low milk production, low resistance to infection and reduced adrenal cortical response to ACTH (Robertson et al. 1957, Wilson 1975). Thus cows with high EMg (pre-partum and post-partum) concentrations and heifers

with high PMg concentrations may have had greater thyroid gland activity which is necessary for better fertility and milk production.

The results presented in chapter 6 show that there were significant between animal differences in blood chemistry responses to starvation and ACTH injection and the responses could be used to predict growth rate. The changes in PMg, EMg, and albumin concentrations and PCV's between 0 and 40 hours of starvation could account for approximately 53 to 63% of the variation in growth rates. The changes in glucose, BUN and PNa concentrations and WCC's and absolute eosinophil counts between 0 and 4 hours (glucose 6 hours) after ACTH injection account for 57 to 63% of the variation in growth rates. Therefore these relationships suggest that blood chemistry changes in response to starvation (40 hours) or after ACTH injection (4 or 6 hrs) may be used as "growth potential markers" in replacement dairy stock. However to be of practical use further studies would be necessary to assess how well later performance (i.e. growth rate) compares with the predicted one based on blood chemistry changes (due to starvation and/or ACTH injection) since growth rates were only recorded over specific periods i.e. 2-13 months in calves and 13-24 in the heifers studied.

The results in chapter 7 show that plasma protein components (TPP, albumin, globulin and Hb) were the most important parameters in the multiple regression equations found for predicting growth rate (in both calves and heifers) and production rank (heifers). Animals with high concentrations

of these components had both high growth rates and production ranks. This may be due to the reported long-term high individuality of these components in cattle (Payne et al. 1973, Kitchenham et al. 1977). In the adult cows (chapter 5) however, the pre-partum plasma protein components (i.e. RCC's, Hb and albumin concentrations) had a negative relationship with production rank i.e. cows with low RCC's and Hb and albumin concentrations before calving had higher milk production ranks. This inverse relationship between these protein components and milk production in older cows might suggest that protein utilization (for foetal growth and milk production) increases more with age in potentially high milk producing cows. High concentrations of PMg (at 24 months of age and the overall 12 month mean) were also associated with high production rank at first lactation (the possible association between Mg and production rank has been discussed).

The results in chapter 8 show that EMg concentrations decrease significantly ($P<0.01$) with progressive decrease in PMg concentration and show a much smaller increase than PMg following a Mg load. Also the EMg concentrations did not show significant changes during starvation or after ACTH injection (induced stress). Thus these observations indicate that EMg concentrations do not show significant changes as a result of physiological stresses (e.g. short-term decrease in feed intake and ACTH injection). Nor do they respond immediately or markedly to a Mg loading (chapter 8) and therefore they could be better indicators of the long-term body Mg status in an animal than PMg concentrations.

The decrease in thyroid gland activity (PTI) in Mg deficient calves suggests that EMg concentration which could indicate the long-term Mg status in an animal may also give an indication of long-term thyroid gland activity. Such a relationship (i.e. between EMg levels and long-term thyroid gland activity) could be used to identify animals with better thyroid gland activity and hence better production (milk production, fertility and growth rate).

The results of chapter 8 also show that the amount of Mg excreted in urine within 4 and 24 hours after a Mg load (intramuscular $MgSO_4$ solution injection) may be used to show body Mg status in calves i.e. those with lower Mg status appear to excrete much less Mg in the urine after the Mg load than those with normal Mg status. This method of determining Mg status of calves would be useful in identifying calves (and possibly adult animals) with a marginal protracted Mg deficiency (which is less likely to be shown by determination of PMg concentrations). The lower urine Mg concentration in the Mg deficient calves than in the control calves before the Mg load also shows that urine Mg concentration may be used to indicate the level of Mg intake in calves. Thus a combination of determinations of PMg, EMg and urine Mg concentrations before and after a Mg load may give a broad picture of long- and short-term Mg status in an animal.

Magnesium concentrations have an important relationship with energy metabolism. Therefore the results in this thesis in respect to significant relationships between EMg, production rank, fertility and the relationship between Mg status and FTI support and enhance the importance of Mg in energy metabolism.

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A P P E N D I X

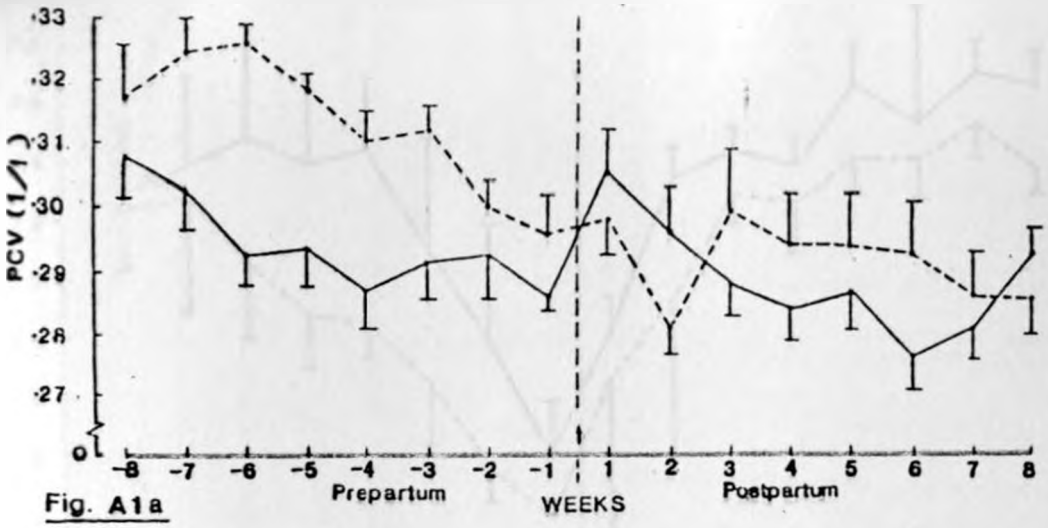
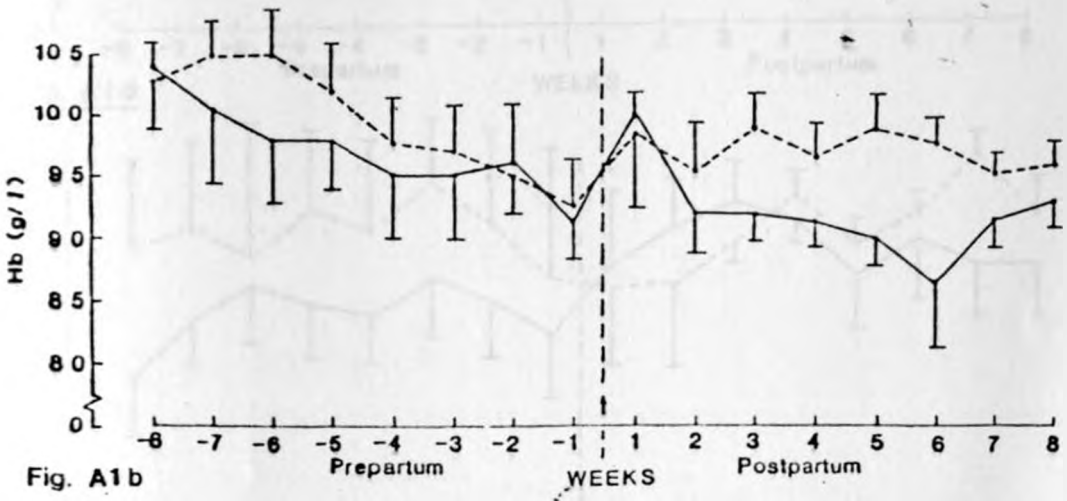


Fig. A1a



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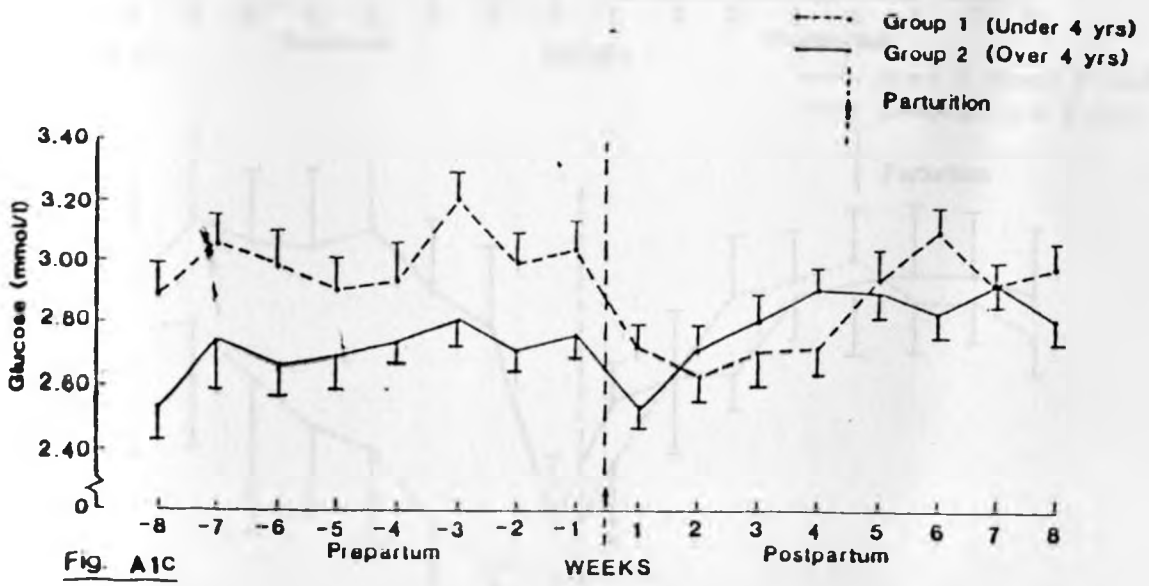


Fig. A1c

Figures A1a-A1c. Changes in PCV, Hb and glucose (mean ± SEM) values in dairy cows 8 weeks before and 8 weeks after calving according to age groups (Herd II) (Group 1, n = 32 cows; Group 2, n = 30 cows)

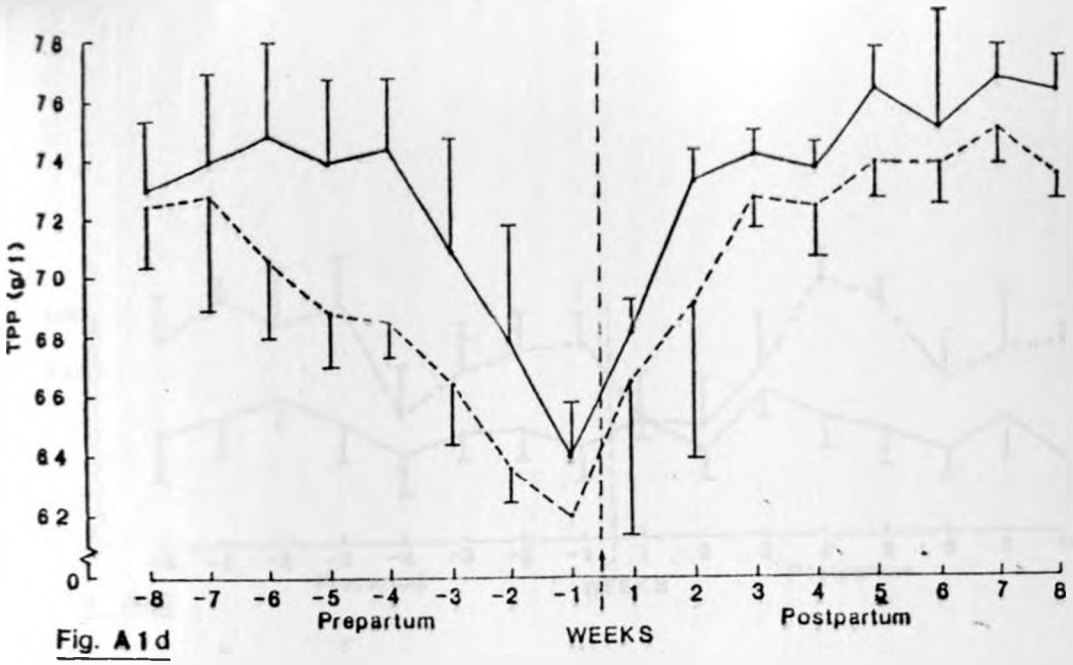


Fig. A1d

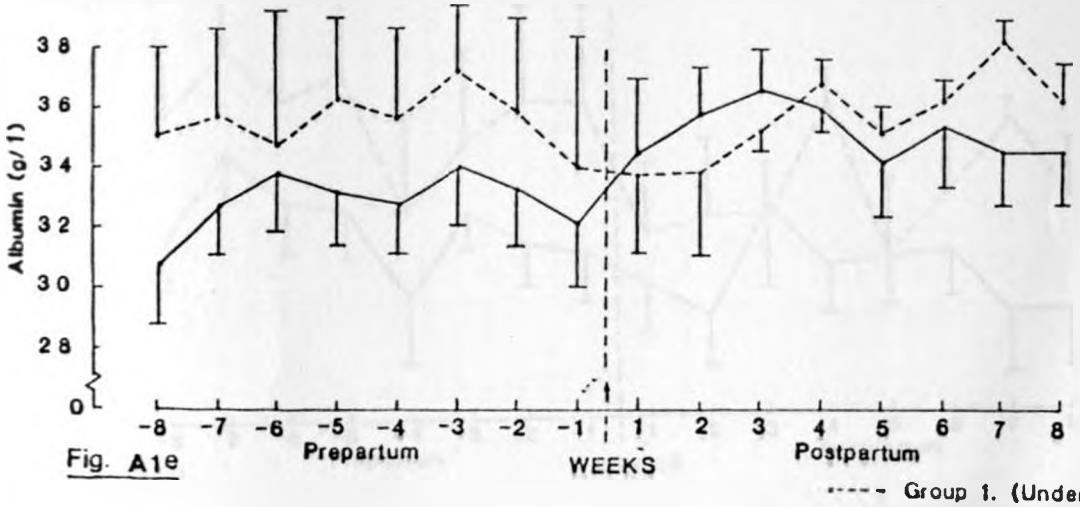


Fig. A1e

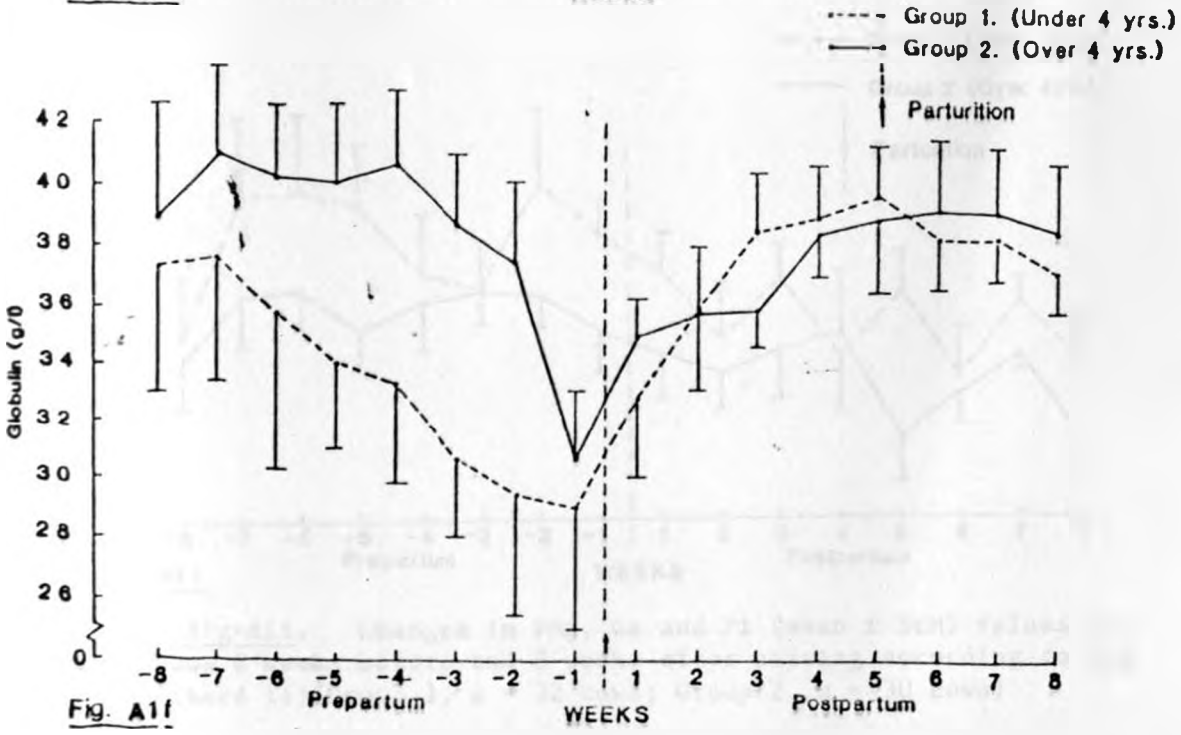


Fig. A1f

Figures A1d-A1f, Changes in TPP, albumin and globulin (mean ± SEM) values in dairy cows 8 weeks before and 8 weeks after calving according to age groups (herd 11) (Group 1, n = 32 cows; Group 2, n = 30 cows).

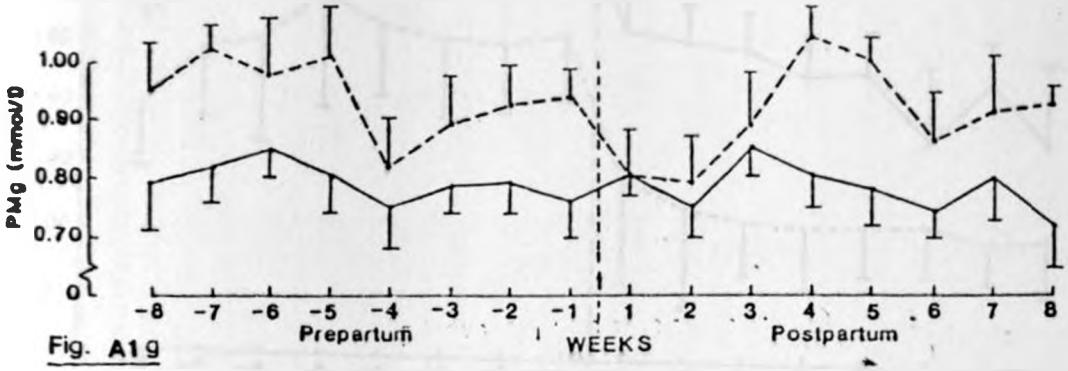


Fig. A1g

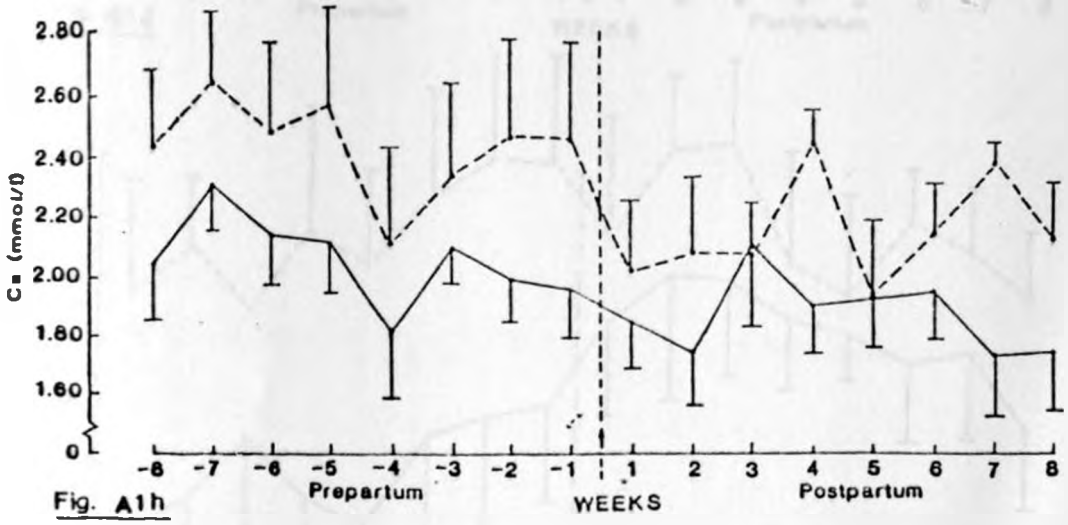


Fig. A1h

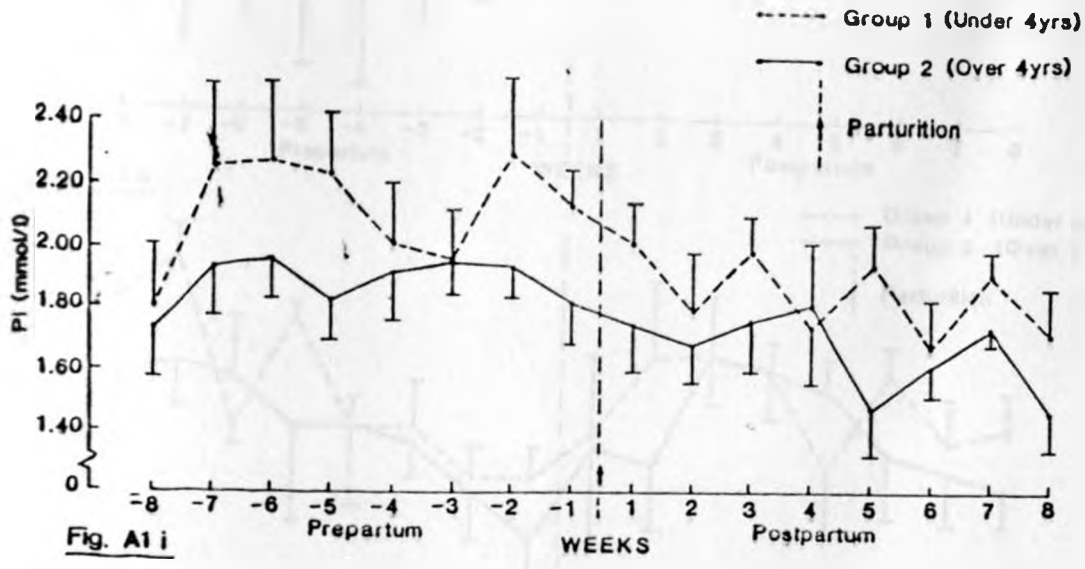


Fig. A1i

Figures A1g-A1i. Changes in PMg, Ca and Pi (mean ± SEM) values in dairy cows 8 weeks before and 8 weeks after calving according to age groups (herd 11)(Group 1, n = 32 cows; Group 2, n = 30 cows)

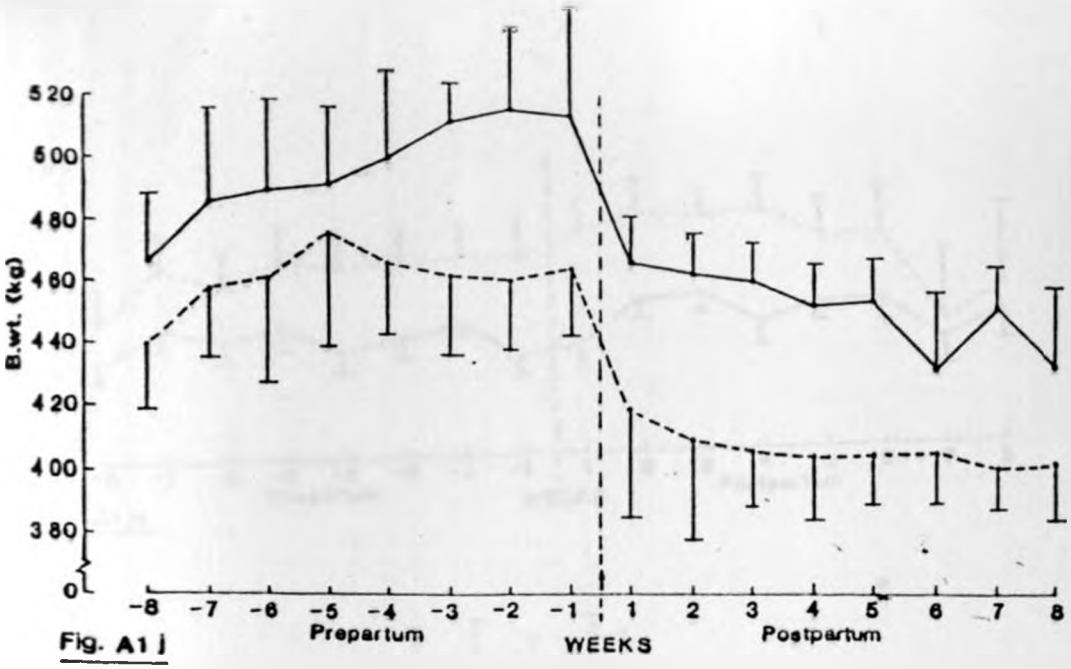


Fig. A1 j

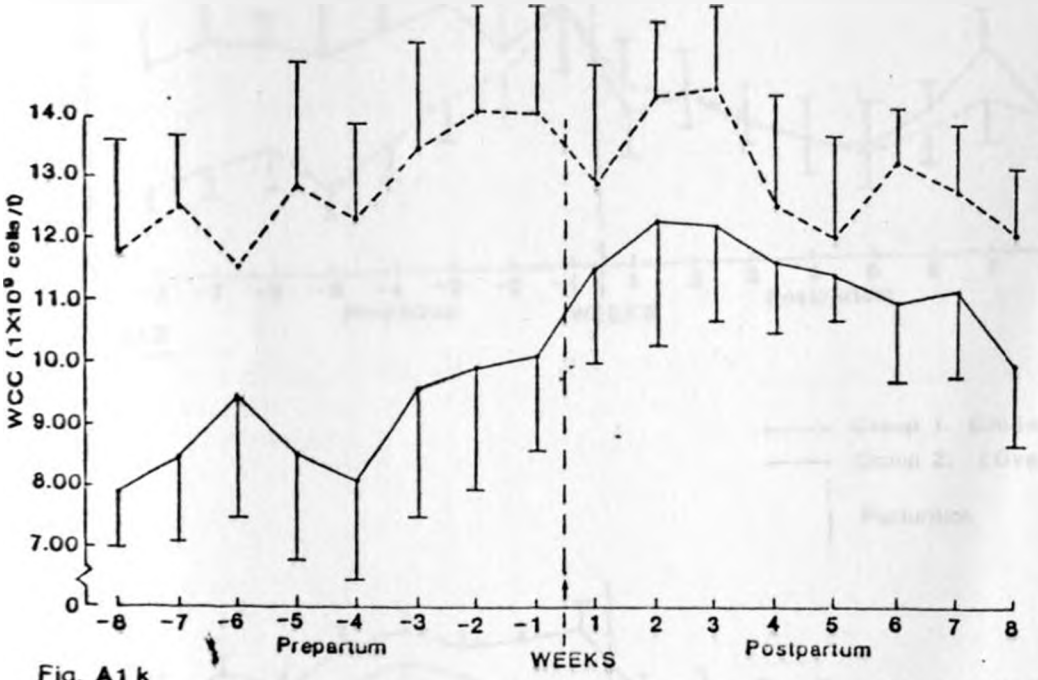


Fig. A1 k

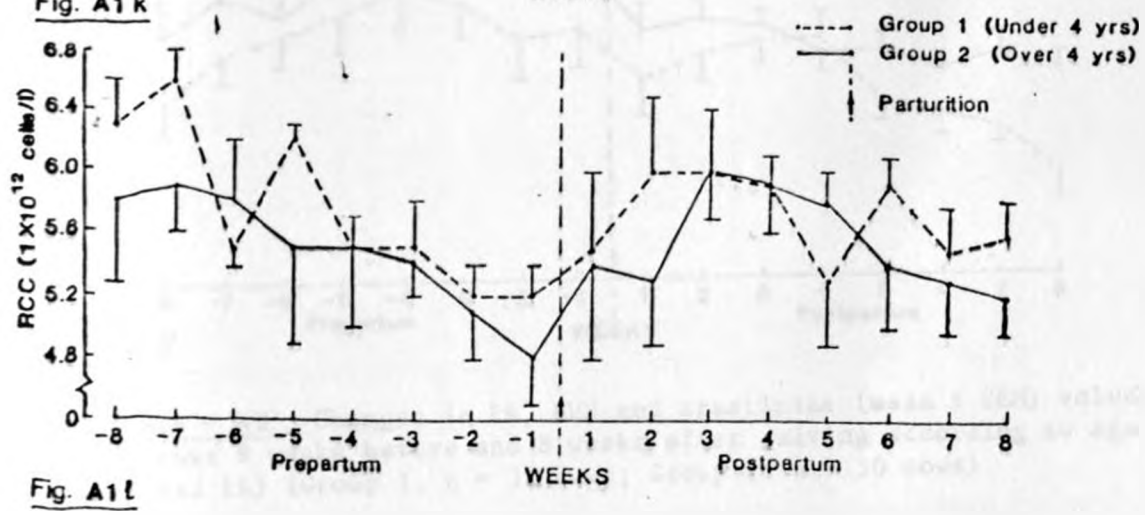


Fig. A1 l

Figures A1j - A1l. Changes in B. wt, WCC and RCC (mean ± SEM) values in dairy cows 8 weeks before and 8 weeks after calving according to age groups (herd II) (Group 1, n = 32 cows; Group 2, n = 30 cows)

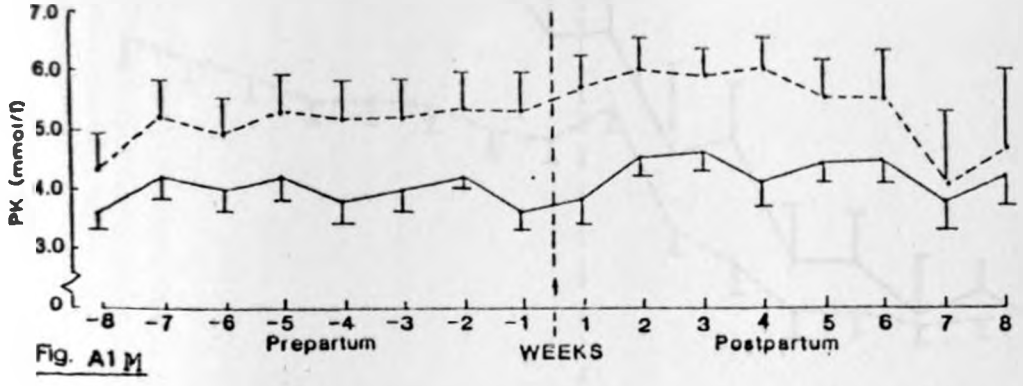


Fig. A1 M

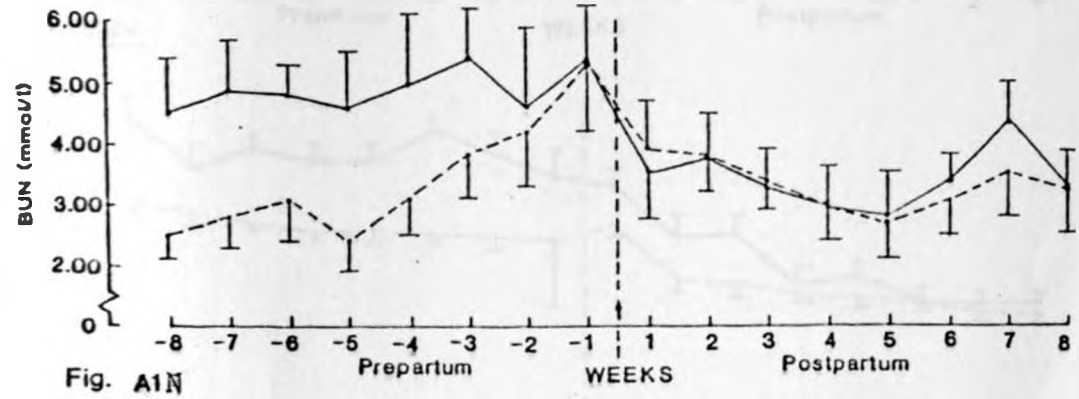


Fig. A1 N

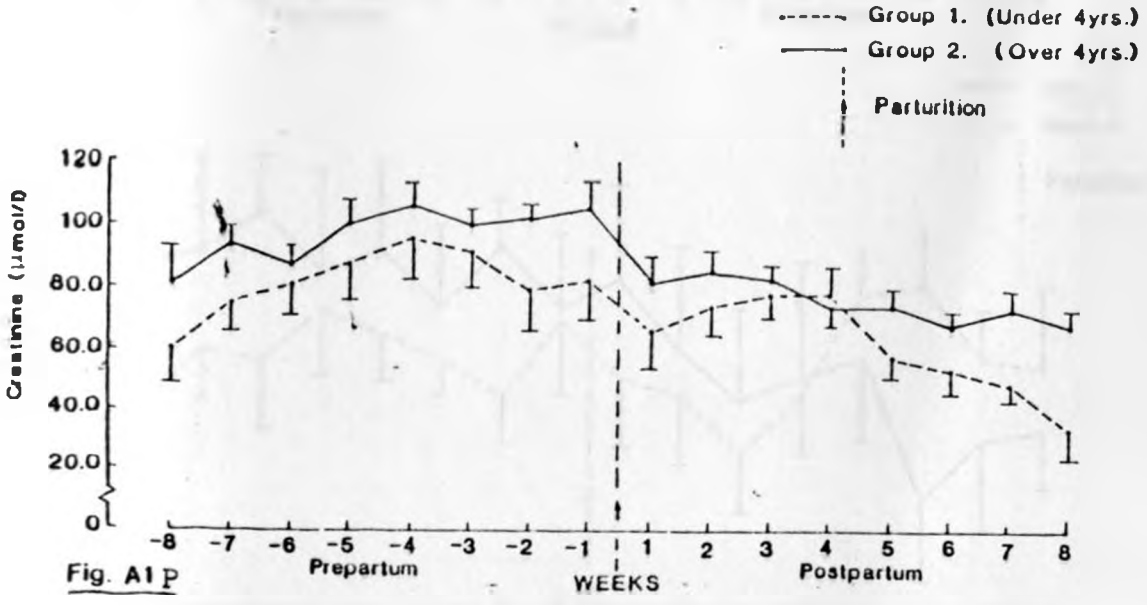


Fig. A1 P

Figures A1M - A1P. Changes in PK, BUN and creatinine (mean ± SEM) values in dairy cows 8 weeks before and 8 weeks after calving according to age groups (herd 11) (Group 1, n = 32 cows; Group 2, n = 30 cows)

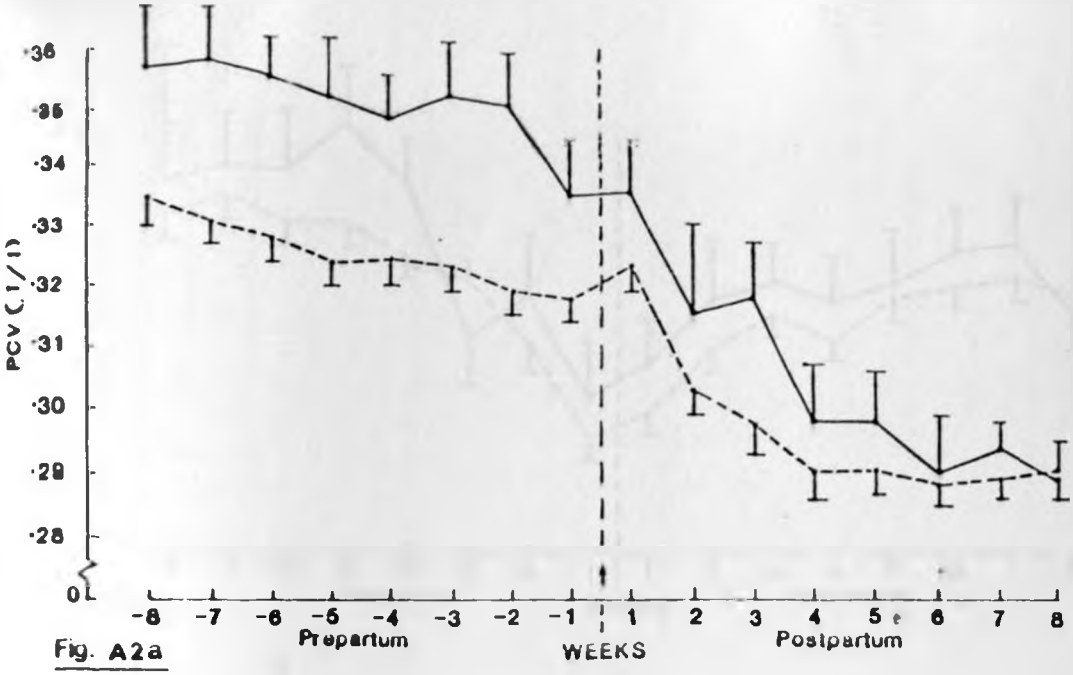


Fig. A2a

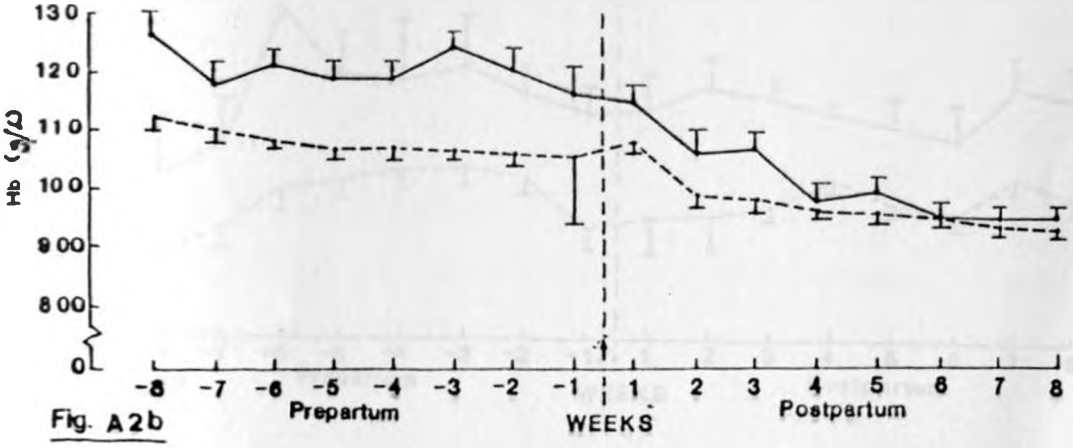


Fig. A2b

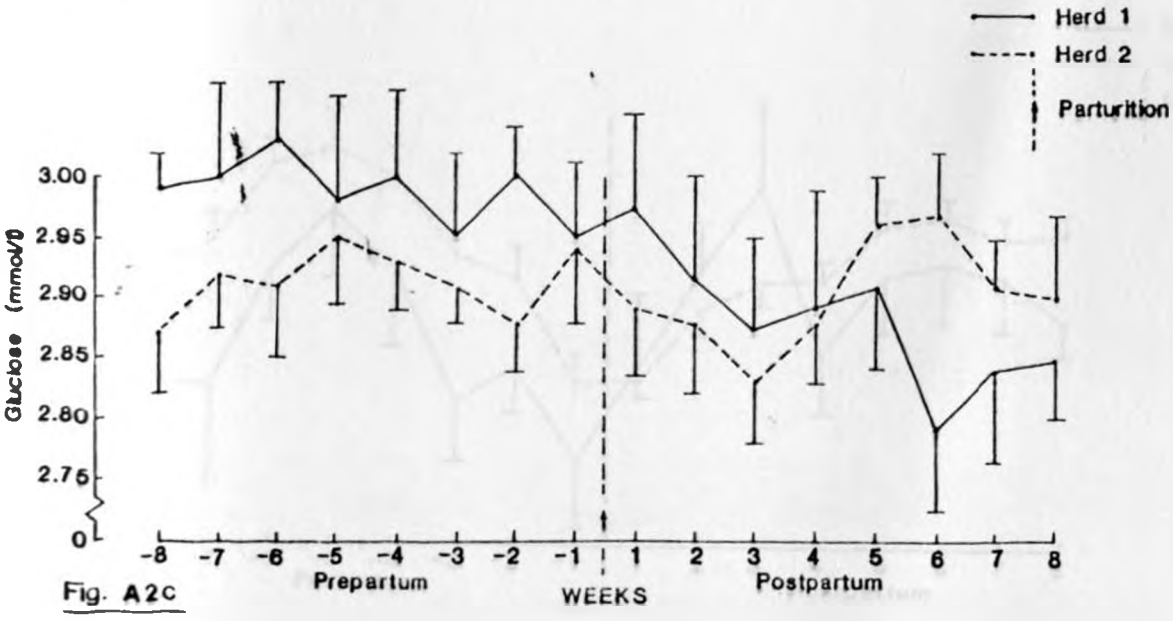


Fig. A2c

Figures A2a - A2c. Changes in PCV, Hb and plasma glucose (mean ± SEM) values in dairy cows 8 weeks before and 8 weeks after calving according to herds (herd I, n = 23; herd II, n = 62)

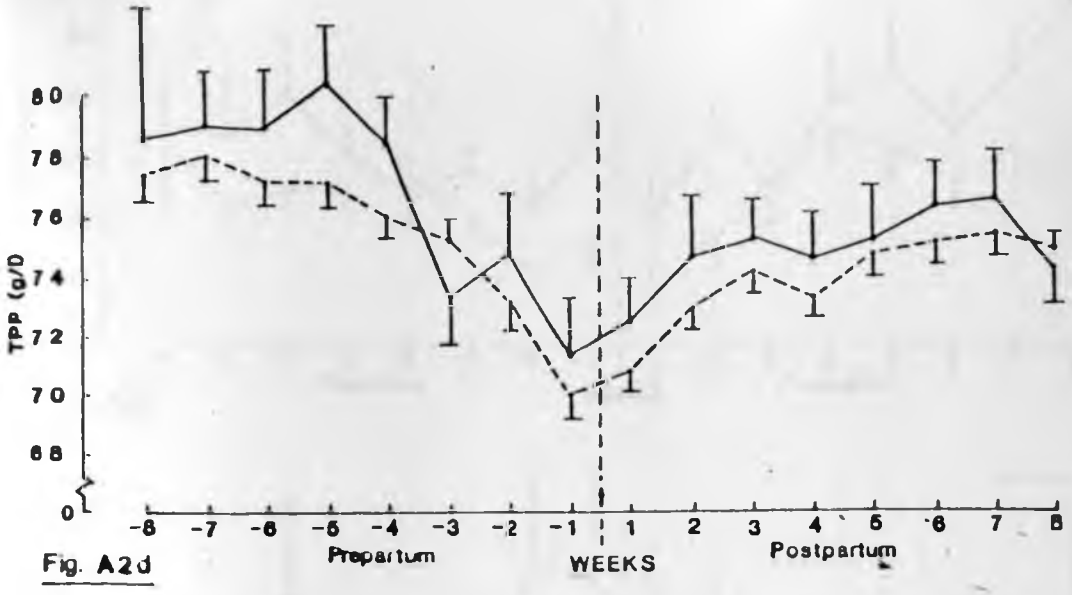
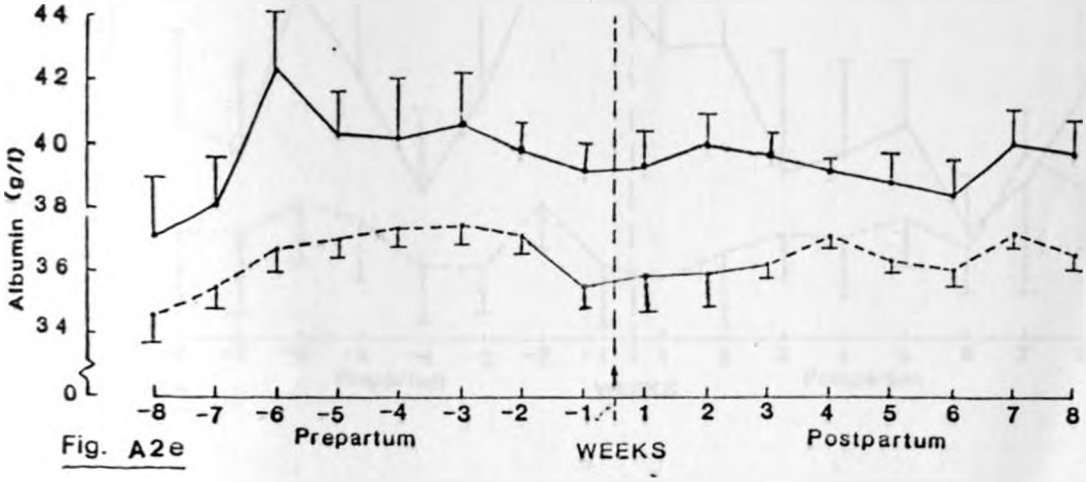


Fig. A2d



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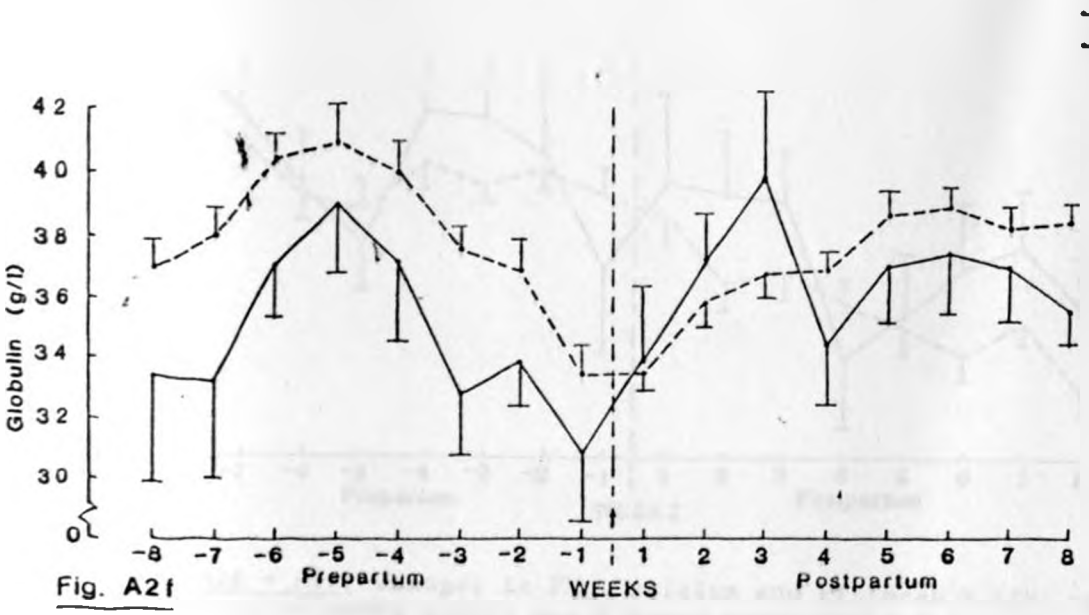

Fig. A2f

Figures A2d - A2f. Changes in TPP, albumin and globulin (mean ± SEM) values in dairy cows 8 weeks before and 8 weeks after calving according to herds (herd I, n = 23, herd II, n = 62)

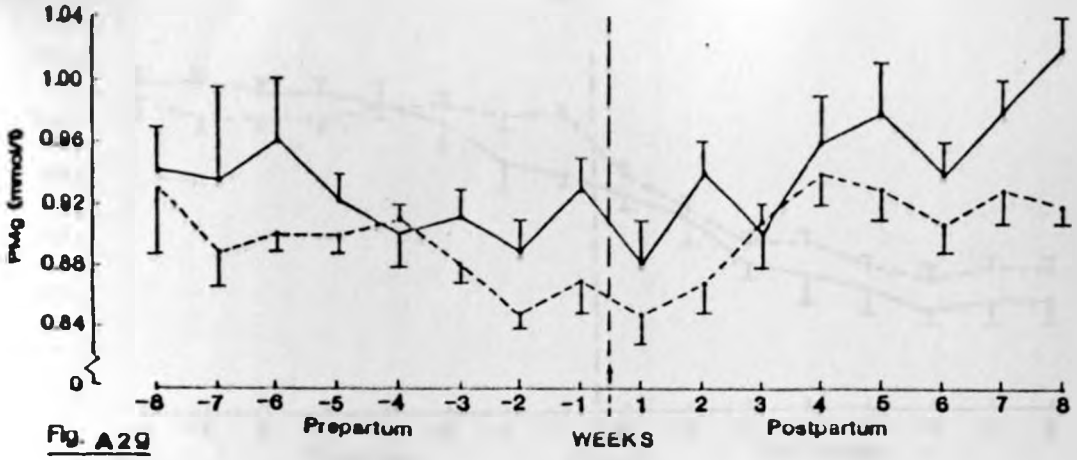

Fig. A2g

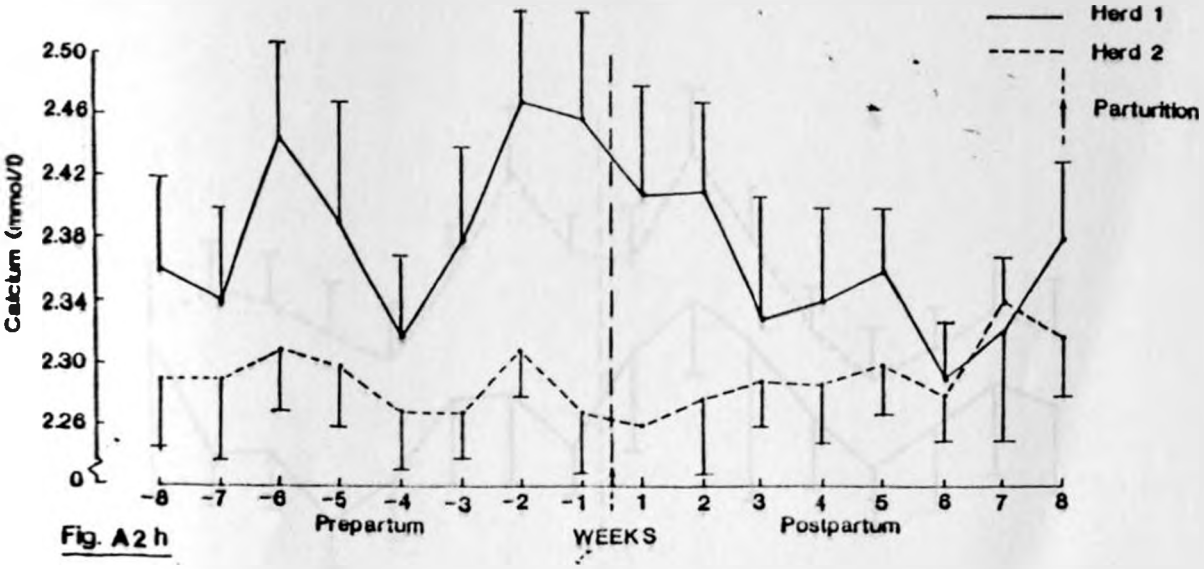

Fig. A2h

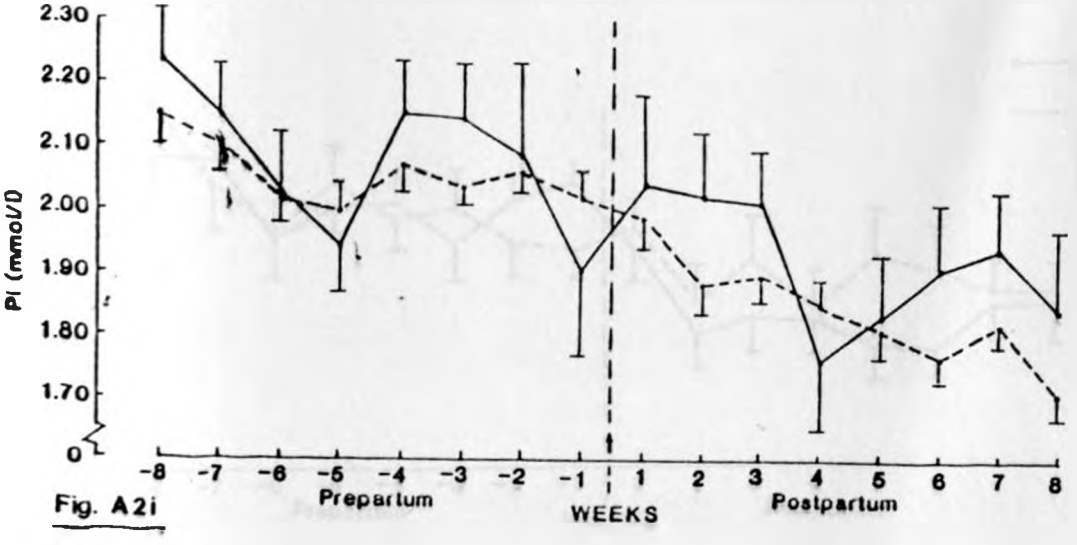

Fig. A2i

Figures A2g - A2i. Changes in PMg, calcium and PI (mean ± SEM) values in dairy cows 8 weeks before and 8 weeks after calving according to herds (herd I n = 23, herd II, n = 62)

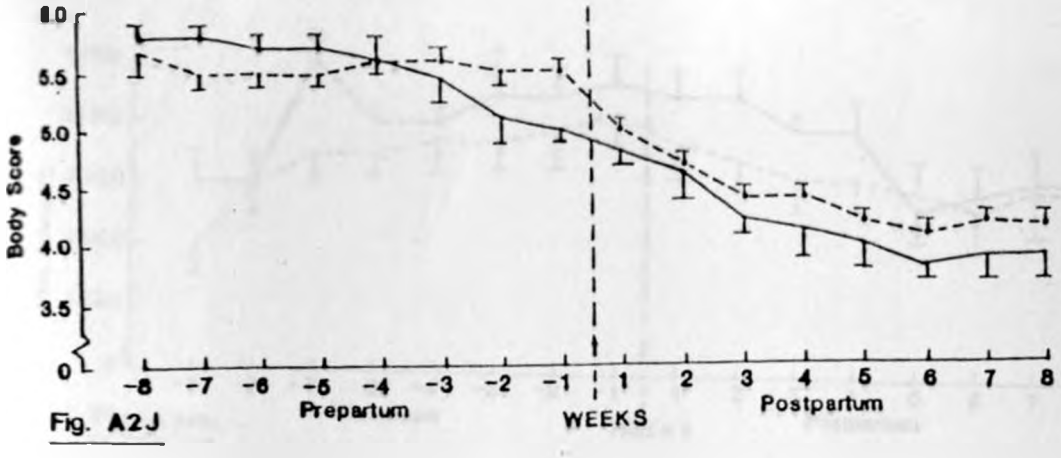

Fig. A2J

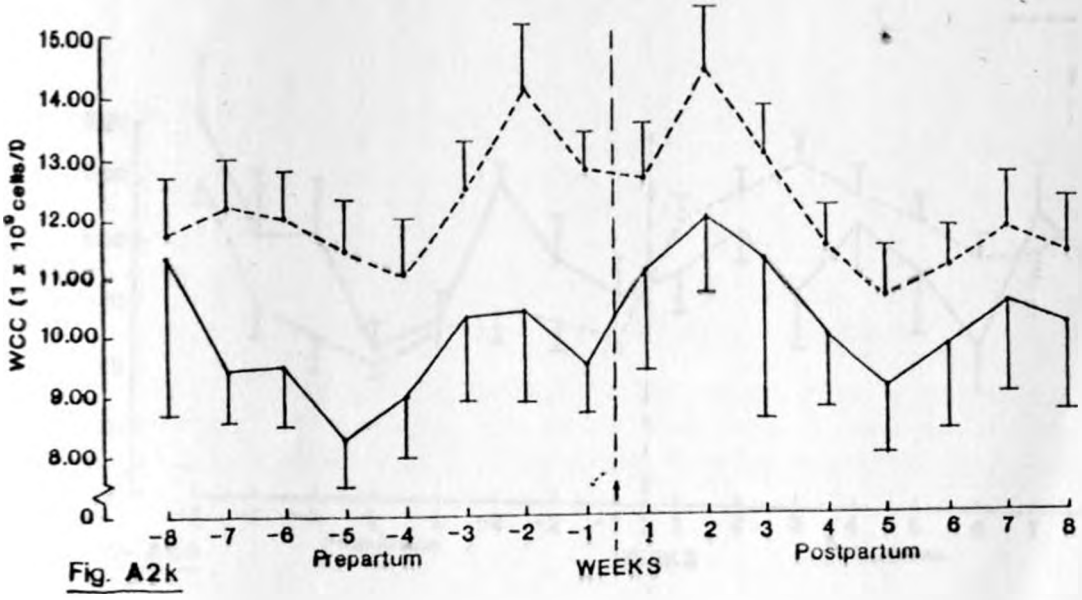

Fig. A2k

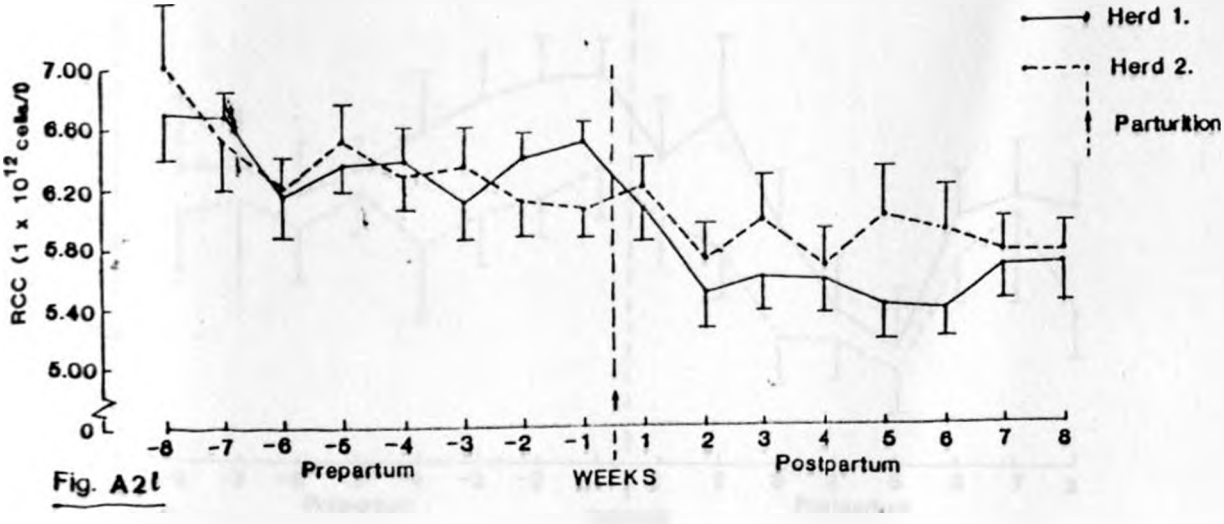

Fig. A2l

Figures A2j - A2l. Changes in body score, WCC and RCC (mean ± SEM) values in dairy cows 8 weeks before and 8 weeks after calving according to herds (herd I, n = 23, herd II, n = 62)

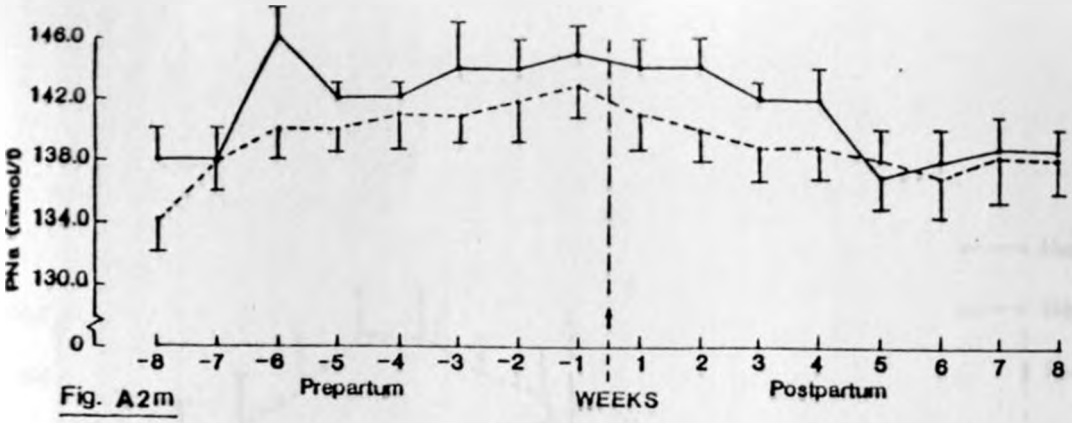

Fig. A2m

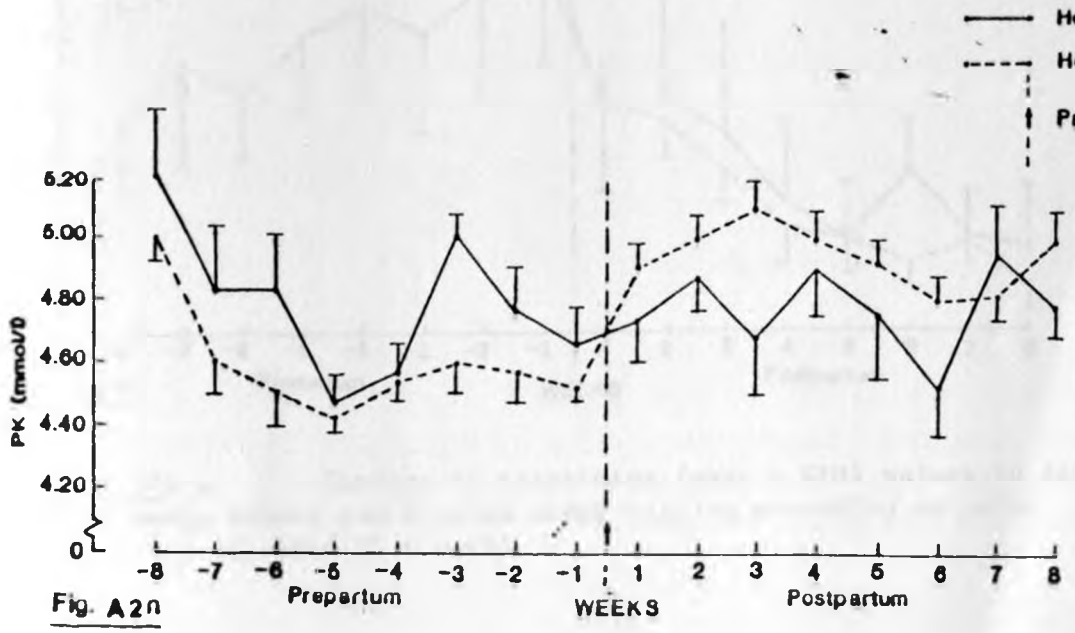

Fig. A2n

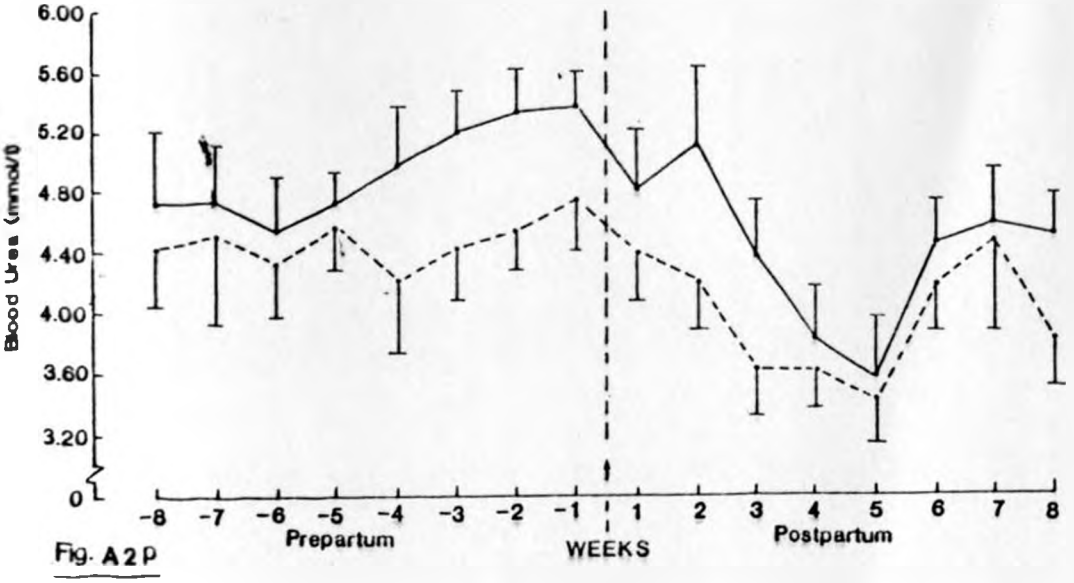

Fig. A2p

Figuers A2m - A2p. Changes in PNa, PK and blood urea (mean ± SEM) values in dairy cows 8 weeks before and 8 weeks after calving according to herds (herd I n = 23, herd II n = 62)

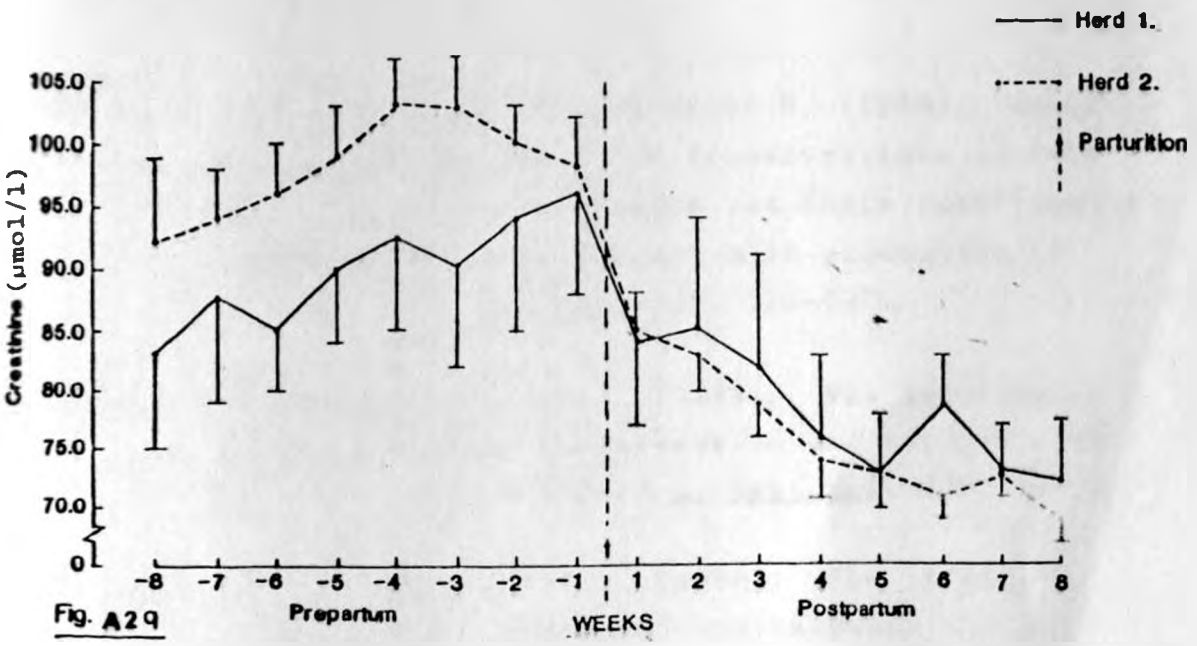

Figure A2q . Changes in creatinine (mean ± SEM) values in dairy cows 8 weeks before and 8 weeks after calving according to herds (herd I n = 23, herd II n = 62)

PAPERS FROM RESEARCH PRESENTED IN THIS THESIS ALREADY PUBLISHED.

MULEI, C.M., Daniel, R.C.W. and Green D. (1988). Changes in erythrocyte Mg, Na and K concentrations in late pregnancy and early lactation and their relationship with subsequent fertility and milk production in dairy cows. J. Vet. Med. A35, 522-528.

MULEI, C.M. and Daniel, R.C.W. (1988). The association of plasma magnesium concentration and thyroid gland activity in young calves. J. Vet. Med. A35, 516-521.

MULEI, C.M. and Daniel, R.C.W. (1988). The effect of induced hypomagnesaemia and hypermagnesaemia on the erythrocyte magnesium concentration in cattle. Vet. Res. Commun. 12(4-5), 289-293.