THE USE OF SOME BIOCHEMICAL PARAMETERS IN THE STUDY OF **PHYSIOLOGICAL CHANGES ASSOCIATED WITH PREGNANCY IN SHEEP**

BY.

KOGO, EMMANUEL JIMOH

A thesis submitted to the Postgraduate School, Ahmadu Bello University, Zaria, in partial fulfillment of the requirements for the award of the degree Master of Science in Biochemistry.

Department of Biochemistry
Faculty of Science
Ahmadu Bello University.
Zaria

JULY, 1987

DECLARATION

I hereby declare that this thesis is original and has been written by the author. It is a record of my own research work and has not been presented before in any previous application for a higher degree.

Emmanuel Jimoh Kogo

25/9/87
Date

CERTIFICATION

This thesis entitled the *The use of some biochemical parameters in the study of physiological changes associated with pregancy in sheep* by Mr Emmanuel Jimoh Kogo meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

Dr. J.A. Abalaka
Dean, Faculty of Science and Head of
Biochemistry Department, A.B.U, Zaria

25/9/87.
Date

Dr. A.I. Ukoha,
Supervisor/Internal Examiner
Biochemistry Dept., A.B.U., Zaria

25th Sept'87
Date

Professor E.C.I. Molokwu
Supervisor/Internal Examiner,
Department of Vet. Physiology and
Pharmacology, A.B.U., Zaria

July 28, 1987
Date

Professor G.E. Osuide
Dean, Post Graduate School,
A.B.U., Zaria.

16-2-88
Date

Dr. G.E. Ekeke
External Examiner,
University of Portharcourt
Portharcourt.

25TH JULY, 1987
Date

DEDICATION

This thesis is dedicated to my lovely wife Josephine for her unflinching and moral support. To my sweet children Emmanuella, MaryAnne, Edwin, Gabriel and Anthony for their tolerance and patience. To my mother-in-law Madam E.A. Olubiyo who kept my moral high throughout the period of this work. To my very good friend Alhaji Usman Abdurrahim who was always by my side at all times and very supportive.

Notation

Age is no barrier to knowledge. Advancement with age should go along with absolute patience, tolerance and maturity to withstand distractive pressures. I thank God for giving me all these, and also the will, my good health and the opportunity to do the course.

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ABSTRACT

In Nigeria, many animal foetuses particularly -from small ruminants are lost during slaughter in abattoirs- This is due lack of simple method for detecting early pregnancies.

In view of the need to develop simpler techniques which can detect early pregnancies, a study was conducted using eleven (11) Nigerian Yankasa sheep and five (5) rams to study levels of some serum biochemical parameters and haematology the gestation period. These parameters included electrolytes, trace elements, cholesterol, urea, proteins and alkaline phosphatases. Colbalt, copper and bicarbonate levels were lower in pregnant than none pregnant ewes while zinc and manganese levels were found to be raised. The total protein maintained a uniform low level all through pregnancy. The alkaline phosphatase level declined progressively from early pregnancy till lambing while the fall in urea was particularly marked from mid—pregnancy to lambing. Albumin level was low throughout the pregnancy period returning to almost normal levels at term while phosphorus levels increased during the first two months followed by lower levels the next two months, but returning to normal level at term.

Ihe albumin/phosphorus ratio was found, in this study, to be the most reliable parameter for prediction of various stages of pregnancy. The estimations of albumin and phosphorus are less expensive and could therefore be used as an alternative to the expensive methods currently available for the determinations of pregnancies in the sheep.

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CHAPTER ONE

INTRODUCTION

Sheep, a ruminant, forms one of the most commonly kept domestic animals reared by ordinary man in Nigeria. This animal, is found in every part of Nigeria and most particularly in the savanna grassland of the country. The vast grassland for feeding, with low rainfall favours this large population.

During some religious festivals large number of rams and ewes is used and more are imported from neighbouring countries to meet Nigeria's demand. Therefore a lot of Nigeria's foreign reserve is utilized for these purposes. In 1984, for example during the moslem festival of *ID EL KABIR* there was a newspaper report that over one million sheep mainly rams were imported from Niger Republic and other neighbouring countries to meet Nigeria's demand.

In the savanna region of Nigeria, the most common breed of sheep is the Yankasa. Ademosun *et al.*, (1984) and Jahnke (1982) reported in their papers that the population of domestic animals in Nigeria are estimated as follows:

(a) Sheep 10.00 million
(b) Goats 30.00 million
(c) Cattle 12.00 million
(d) Pigs 0.80 - 1.00 million
(e) Camels 0.60 - 0.70 million
(f) Exotic Poultry 11.00 million
(g) Other birds and local poultry 200 - 300 million.

From the report, it is clear that the population of goats is higher than any other set of animals. However, on religious ground , meat from goats does not enjoy wide acceptance for food when compared with cattle and sheep. For instance, Sokoto state keeps the highest population of goats and yet it is a common beleive that the meat is not accepted by the indigenes of that state on religious ground. Goats are therefore mainly reared for commercial purposes in this state. The same is also true in those states where sheep have not gained wide acceptance for food and are only reared for economic and religious purposes.

A visit to most abbatoirs in the urban and rural areas would reveal that during slaugthering and dressing of animal carcases, a large number of foetuses litter the floor. Although a very minute fraction of these foetuses are used for food and researches, a large portion are discarded. These would in no doubt constitute a big loss to the animal industries in Nigeria. The losses from cattle and camels can be reduced by rectal palpation and with suitable personnel, this method can simply be used to separate the pregnant animals before they are slaugthered. For instance in Kano state, the government buys off any detected pregnant cattle and camels to prevent their being slaughter. Despite this measure, some losses are still reported. In case of small ruminants the situation of these losses are worsened as there are no easy methods available for testing pregnancies. There are many methods for testing early pregnancies in animals Cunnigham *et al*., 1983; Pierson and Ginther, 1984; and Merilan 1983). Unfortunately these are not widely practiced in Nigeria, because they are expensive and there might be

inadequate personnel to operate them.

The demand for animal protein in Nigeria is fast increasing because of the rapid growth in population which stood at sixty five million in 1965 and is projected to be ninety-nine million in 1986 (National Bureau, 1984). In an attempt to meet this increasing demand it has become necessary to reduce if not completely eliminate these foetal losses that occur at the abbatoirs. It is therefore aimed in this project to study some biochemical changes that occur during the pregnancy of the sheep with a view to finding simplier methods of early detection of pregnancy in sheep to reduce these losses in the slaugther houses.

Yankasa sheep have been chosen for this experiment because the breed is widely spread across the savanna zone of Nigeria. The sheep can feed on a wide variety of grasses, they have averagely big stature and as such high meat yield. They have fast reproductive rate and if well fed and care for can lamb twice a year producing four lambs.

CHAPTER TWO

LITERATURE REVIEW

2.1 The mineral elements in animal nutrition

Plants and some organisms derive their nutrients from the soil and the concentrations of these nutrients in them are dependent on those in the soil of their localities (Underwood, 1966; Kapu, 1975). The herbivores derive most of their nutrients from plants and water, while the omnivores derived theirs from plants and animals (Suttle, 1975).

These nutrients function physiologically and when they are found in excess these elements can be toxic, while if too little can lead to various nutritional disorders (Mcdowell, *et al*., 1986). It has also been reported that whenever animals are defficient in some nutrients (minerals) they can travel long distances in search of these deficient nutrients. This is particularly true of wild animals but in domestic ones, such deficient nutrients can be incorporated in their feeds.

In most animals, apart from using these nutrients physiologically they are present in the bones or shells which acts as body supports or protectors in the cases of some snails and tortoise. The bones or shells are reservoir of elements from where requirements can be drawn when there is any shortage.

Most of the elements are found in the tissues and some are in such a micro-concentration that they could not be measured with precise accuracy using the analytical methods

available to the earlier workers. These elements are therefore referred to as trace elements and methods are now available for their precise measurements.

Trace elements, had been so commonly and popularly used that it has come to stay despite the fact that methods are available these days for their precise measurements. Many attempts were made to use other terminologies such as minor or oligo elements, but these failed to gain popularities. These trace elements occur in tissues of living organisms in low concentrations and the magnitude of their concentrations vary from organisms to organisms, and from place to place.

Elements found in plant and animals are grouped into:

(a) Macro-quantity elements such as potassium, sodium, *chloride, calcium, magnesium and phosphorous.*

(b) Micro-quantity elements such as cobalt, zinc, copper, *manganese and flouride and so on.*

The concept for this division was applied by Underwood (1977).

2.2 Potassium, sodium, chloride and bicarbonate

These ions are very important in maintaining the normal water distribution between cells, plasma, interstitial fluids and as such exert osmotic pressure of the body (Varley, 1963). In addition to the above, potassium and sodium are very important for normal muscular contraction.

Potassium, chloride and sodium are found to be of low concentration in grasses and herbages. Concentrations of sodium in grasses in Kenya were found to vary between 0.002 and 0.007 per cent (Howard *et al.*, 1962), while Denton *et al.*, 1961) recorded 0.008 per cent in Australian

inland grasses. Low values are also present in cereals and grains. Despite the low concentration of these elements in animal feeds, their deficiencies are not common. Animals can derive more of these elements from water containing more dissolved salts from the soil, which contain large deposits of various elements.

2.2.1 Sodium

Of the electrolytes, sodium is the major cations in the extracellular fluid. It plays a central role in the maintenance of normal distribution of fluid in the body and exerts the osmotic pressure of the body. Under normal condition all the sodium ingested as food are absorbed in gastrointestinal tract. The excess are excreted with urine and sweat. The body has a good threshold for sodium and this is followed by chloride ion. The tubules of kidney can reabsorb as much as ninety-nine per cent of the total daily intake. This re-absorption is controlled by aldosterone - and adrenocortical hormone. The mechanism of renal re-absorption of sodium forms an important mechanism in acid formation and in urine.

Hyponatremia (i.e low serum sodium) can be obtained in an unhealthy conditions such as kidney damage and disease of adrenal gland. These can lead to excessive fatigue, sense of exhaustion, muscle weakness and cramps, nausea, headache, dizziness, mental confusion and hyponatremia i.e low serum sodium, can be obtained (English, 1976a). Hypernatremia, a condition obtained when there is excessive concentration of sodium in the extracellular fluid, is not commonly seen and it can cause severe thirst or coma. The normal values in most animals as reported by Pugh (1966), is between

139.00 mM/L and 152.00 mM/L.

2.2.2 Potassium

Potassium body requirement is absorbed from the gastro-intestinal tract and since there is low renal threshold for this elements, a large quantity is excreted by the distal tubules of the kidney. Only very small quantities are reabsorbed into the cells from the plasma while the rest circulate with the plasma before they are excreted by the tubules of the kidney. With this mechanism the body can protect itself against high plasma potassium level referred to as hyperkalemia which could cause serious changes in the physiology of the body; one of such changes is manifested in the muscular irritabilities. Low values, hypokalemia, can cause excitation of the muscles (Howard *et al*., 1962

2.2.3 Chloride

Chloride ion is the most abundant in the extracellular fluid while a very small concentration exist in the intra-cellular fluid. The ion is passively distributed in the body and it always accompanies the sodium ion which is actively distributed in the body.

Chloride ion influences the ratio of bicarbonate ion in the cells and plasma. When there is less carbonic ions within the cells, there is a chloride ion shift, this would influence the pH of the blood. The body fluid has an efficient buffering power for the maintainance of a constant pH range in the blood.

2.2.4 Bicarbonate

The cells of the body of an animal can only function in healthy condition within a very narrow range of pH (pH 7.44 - 7.36), for this reason it has therefore got many means whereby this narrow range of pH can be maintained for effective working conditions of the cells. It has many buffer pairs of ions which control the pH; the most significant pairs are the bicarbonate - carbonic acid (HCO_3^-/H_2CO_3), monohydrogen phosphate - dihydrogen phosphate ($HPO_4^{2-}/H_2PO_4^-$); proteinate - protein, others are organic phosphate and haemoglobin. All these, in the blood, have a collective burden of preventing marked changes in the pH of the blood. Of the buffer pairs mentioned above, bicarbonate - carbonic acid is most important to the clinician; this is because it is easiest to measure and the body has the most control over the pair. The kidney is the principal organ for regulating bicarbonate, dihydrogen phosphate, and ammonium ions, to bring the pH of the body fluid within normal when there is an upsurge of hydrogen or alkali ions in the extracellular fluid of the body.

2.3 Urea

This is the principal end products formed by the liver from the catabolism of protein. Ornithine combine with carbamoyl phosphate from the blood to form citrulline. The citrulline in presence of ATP and amino acid (Aspartic acid) gives arginino succinate and in presence of an enzyme arginino-succinase would give off fumarate and arginine. The arginine is catalyse with arginase to give off urea and ornithine which is used once more in the cycle (Appendix A9).

Urea is the principal form in which amino - nitrogen of amino acids are eliminated from the body (Maxine, 1961); other forms that are less important than the urea are the creatinine, creatine, urates, ammonium salts, free amino acids and a number of other nitrogen containing substances that are present in the urine.

It has been observed that sheep maintained on a constant diet increase their turnover of urea during pregnancy and lactation, and reduce the renal excretion when compared with the non-pregnant and non-lactating sheep (Benlamlihs and Oukessou, 1985 and Benlamlihs, 1986). This observation has been attributed to the extra demand for protein during pregnacy and lactation.

Lower blood urea than normal is found in severe liver disease. While high values are obtained during renal diseases such as the presence of stones in the urinary tract, stricture of the urethra and tumor of the bladder affecting the ureters.

2.4 Cholesterol

It has been recognised that it is essential to have a constant supply of progesterone, oestrogen and some steriod hormones throughout the period of pregnancy in human and animals. These compounds maintain the uterus in healthy physiological condition for foetus to remain and keep. The basic requirements for the synthesis of these hormones by the adrenal cortical tissue, the placenta and corpus luteum are the acetate and cholesterol (Younglai and Solomon, 1969).

The liver is the main source of plasma cholesterol but most tissues are able to synthesize it. It is excreted with

the bile by the liver and the liver destroys it by converting it to cholic acid. The normal concentration in blood is very wide between 150 and 240 mg/100ml in human but 50 and 250 mg/100ml in animals (Maxine, 1961). It is present as free and ester form in the blood. It has been shown by many workers that the values of cholesterol increase with age, during pregnancies giving an increase of about 20-25 per cent above normal, during pathological conditions such as nephrosis, diabetis mellitus, obstructive jaundice, myxodema, xanthomathosis, hypothyroidism and cholestasis (Siede and Sieffort, 1983). Low values have as well been recorded in hyperthyroidism. Without taking all these conditions into considerations it would therefore be most misleading to make use of any information on cholestrol values in blood for any pregnancies diagnosis. However, Amin and Suseela (1983) use the ratio of total cholestrol and albumin in plasma as an alternative for the measurement of high density lipoprotein cholesterol in plasma because it is cheaper and easier to carry out.

2.5 Calcium, inorganic phosphorous and magnesium

Over 70 per cent of the ash of the body consists of calcium and phosphorous and these are mainly found in the skeleton and teeth. Magnesium is said to be poorly stored in the bone.

2.5.1 Calcium

The major portions of calcium ions in the body fluid, though negligible, play an important role in the maintainance of normal body physiological functions. Calcium ions in the

extracellular fluid have the following functions:

(a) Normal neuromuscular excitability

(b) Capillary and cell membrane permeabilities

(c) Normal muscle contrations

(d) Normal transmission of nerve impulses

(e) Normal blood coagulation.

Less than half of the calcium in the extracellular fluid is attached to protein and this averages 4.5 mg/100ml. This is physiologically inactive while the remaining averaging 5.5 mg/100ml is physiologically active and very important pathologically. In most animals, the calcium and phosphorus level tend to rise or fall with dietary calcium level.

It has been shown by Economides (1986) that the efficiency of absorption of trace elements in animals is greatly less than 0.1 and this does not apply to calcium in sheep because sheep absorbed calcium according to their need. The efficiency of absorption of phosphorus ranges between 0.6 and 0.7, of magnesium about 0.2 and of sulphur about 0.6. The efficiency of absorption of molybdnium is high (0.8) when there is no sulphur intakes from diets, or can be low (0.3) when there is high sulphur intake in the diet. The demand for all nutrients particularly calcium and phosphorus increases in late pregnancy and early lactation. Twardock *et al.*, (1973) showed that the maximum transfer rate of calcium and phosphorus from dam to foetuses was 2.8 and 1.4 grammes per day respectively.

Braithwaite and Blascock (1976) were able to show that low calcium diet during pregnancy resulted in increased absorption of calcium and higher resorption from bone. In dairy sheep, calcium requirement in early lactation are

higher than those in late pregnancy. Ruminants in general can tolerate a wide range of calcium or phosphorus in their diet if vitamin D, calcium, and phosphorus intake are adequate. Low phosphorus in the diet may enhance calcium excretion in the faeces and lower absorption efficiency (Economides, 1986). Parathyroid hormone plays an important role in the mobilization of calcium from bone when there is low dietary content. Also affecting the absorption of calcium from diet is the presence of oxalates, though these can be degraded by some ruminant microbes and this can lead to alkalosis which can upset mineral equilibrium and affect calcium metabolism (Watts, 1959). The presence of phylates which are high in cereals can reduce calcium and phosphorus absorption especially in monogastric animals and unless the presence of phytates is decompose by the enzyme phytase, phosphorus utilization can particularly be affected (Mellanby, 1949).

The serum concentration of calcium in normal adult animals averages 10 mg/100ml, the range being 9-12 mg/100ml. There is slight difference between the young and adult animals. Hackett *et al*., (1957) reported serum mean level as 12.16 ± 0.28 mg/100ml, while Kumaresan *et al*., (1982) found the level of calcium in Yankasa sheep to be average 10.10 ± 0.37 mg/100ml.

2.5.2 Phosphorus

Outside the skeleton and teeth, phosphorus is involved in vital cellular structure and served in degradation and synthesis of numerous carbon compounds. High energy phosphate bonds plays a fundamental role in storage and

transfer of energy. The ability of phosphorus to be excreted either as H_2PO_4 or HPO_4 in urine plays an important role in the acid-base maintainance in the body.

Phosphorus is found in the cells, present in the soft tissue, in some protein, lipids and in many enzymes. Most phosphorus of the blood is present as esters in the red cells. Serum contains 14-15 mg/100ml but only 5.8 mg/100ml of these are found in lipid. A trace of phosphorus is found as ester while the most significant portion occur as inorganic phosphorus, about 4-7 mg/100ml in horses (Mylrea and Bayfield, 1968), 5.2 ± 0.11 mg/100ml in sheep (Kumaresan, 1982). In young animals higher content and more variable concentration ranging 5-9 mg/100ml are found. The serum concentration would depend principally on the dietary content, its absorption and uptake from skeleton, vitamin D, urinary excretion and the hormonal level controlling this level in the body.

2.5.3 Magnesium

Sheep and cattle are both domestic animals known to be affected by deficiency of magnesium. Magnesium is an activator of numerous enzymes such as the phosphatase and those involving adenosine triphosphate (ATP). It is also required by those enzymes that need thiamine pyrophosphate for their activities (Alkawa, 1963). Magnesium plays a role in the production and destruction of acetylcholine needed for the transmission of impulses. Bone is the source of storage for magnesium from which the plasma level is raised when there is deficiency. There should be a continuous supply of magnesium which in sheep increases from daily intake of 0.70

grammes for body maintainance to 2.00 grammes during pregnancy (Economides, 1986). The normal serum level in sheep was found to be 2.50 ± 0.3 mg/100ml with a range of 2.00-5.00 mg/100ml (Christian and Williams, 1957). Blaxter and Rook (1954) found that low magnesium concentration in the body may result in tetany.

2.6 The trace elements

For over a century, science had realised the importance of trace or essential elements in physiology of animals and plants. Schwartz and Smith, (1967) worked in this field and added a great deal to the physiological importance of trace elements. In recent years, extensive studies in the field of absorption, retention, excretions, enzymology and improved methodology of various assays added much to the values of trace elements. The efficiency of absorption of trace elements are quoted by Economides (1986) as generally less than 0.01.

The following trace elements: copper, cobalt, manganese and zinc were estimated in this project.

2.6.1 Copper

Copper and iron were found to be essential for the formation of haemoglobin. O'dell *et al.*, (1961) found that copper played a vital role in the formation of aotic elastin.

In many parts of the world, animal diseases outbreak have been reported and found to be linked with copper deficiencies from grazing (Prasad, 1976; Underwood, 1977).

Normal blood serum levels in sheep and most domestic animals were found to lie between 0.50 mg/ml and 1.50 mg/ml. Wiener *et al*., (1969) demonstrated breed and sex deficiencies while Anastasiadis *et al*., (1981) reported higher values in pregnant females than non-pregnant females. Copper deficiencies in female rats resulted in reproductive failure due to foetal death and resorption (Hall, 1969).

The minimum requirement of copper and molybdnium in animals is difficult to establish because of the interaction between copper, molybdnium and sulphur to form copper-molybdnium-sulphur (Cu-Mo-S) complex. If molybdnium and sulphur levels in sheep diet are normal, then copper concentration should be 5 mg per kilogramme dry matter. If molybdnium intake is less than 1 mg per kg dry matter, copper retention would be high even if diet contains a normal level of copper. Sheep are said to be very sensitive to copper toxicity (Underwood, 1977 and Economides, 1986).

2.6.2 Cobalt

In various areas of the world animal diseases have been found to be linked with cobalt deficiencies from grazing; locally referred to as coast disease in Australia, bush sicknes in New Zealand and pining disease in Britain. The animals loose appetites, weights, become anaemic, weak, and finally die.

Cobalt is required for the promotion of the synthesis of vitamins B_{12} (Evans *et al*., 1965) and 0.10 mg cobalt per kg dry matter is recommended (Economides, 1986). Cobalt deficiency due to inadequate dietary cobalt in ruminant would result in vitamin B12 deficiency brought about by the

inabilities of the rumen micro-organisms to synthesise sufficient amounts of the vitamin to meet the needs of the host animals tissues. This is a unique nutritional situation in which animals utilise a trace element solely as integral part of a vitamin and completely dependent upon the symbiotic activities of their own micro-organisms for the supply of the vitamin.

If cobalt is added to animal feeds, there would be increase production of haemoglobin, erythrocytes and protein in blood. The reproductive capacity in cattle and sheep and the stimulation of lactation especially in ewes would improve. It would also increase the enzyme activities such as lipase, amylase, diastase, trypsin in sheep and arginase in cattle.

Some organisms such as *Archidoris tuberculata* and *Pleurobranchus Hollusik* are found to contain high cobalt content. Some grasses and swamp black gum, *Nyasa Sylvation* are known to contain high cobalt. It is also found to play an essential role in the fixation of nitrogen by nitrogenase through the root nodules of legumes (Ahmed *et al.*, 1959).

In blood constituent, variable cobalt level is found, higher in plasma than the red cells (Underwood, 1977). The major route of excretion from the body is through urine, faeces, sweat and hairs.

2.6.3 Manganese

As early as 1931, Hart, found manganese to be necessary for growth and normal ovarian activities in mice. It was shown to prevent testicular degeneration and ovarian

functions in rats (Underwood, 1977). It is an enzyme activator, a property shared by other bivalent element such as magnesium. It is found to be involved in glucose utilization and if deficient in guinea pig a diabetic like curve is obtained in reponse to glucose load (Everson *et al.*, 1968). It is not very clear yet whether manganese is involved in insulin synthesis or insulin need. Curran (1954) found that manganese stimulates the hepatic synthesis of cholesterol and fatty acids in rats.

Manganese is excreted from the body through hairs of animals and feathers of birds. In human serum, manganese is mostly bound to β_1 globulin and the serum and plasma level were found to be 1.42 ± 0.20 µg/L and 0.59 ± 0.18 µmg/l respectively (Cotzias *et al.*, 1966).

2.6.4 Zinc

Zinc had been known to be an essential element for the growth of *Aspergillus niger* over a century ago, but it was not until 1926 that it was established to be present in plants and haemosycotypin - (the respiratory pigment of snail *Sycotypus*, (Flynn *et al.*, 1974). O'Dell *et al.*, (1958) were able to demostrate the requirement of zinc for growth while Prasad *et al.*, (1961) were able to relate the lack or low quantity of zinc in the body to dwarfism and hypogonadism.

Vallee (1959) was able to show that the constituents of many metabolic enzymes such as carbonic anhydrase, alkaline phosphates, pancreatic enzymes and carboxydase contain zinc. Orten (1966) showed that zinc acts as co-factors in variety of enzyme system. Somers and Underwood (1969), Fujioka and

Liebernlan (1964) showed that zinc is vitally concerned with the processes of RNA and protein synthesis.

Zinc is excreted from the body through the nails, hairs, wool and sweats and it is poorly stored in the body. In this case, the body reserve cannot meet the demand of either the dams or the foetuses during pregnancy (Halsted *et al.*, 1968). Zinc in plasma is loosely bound to albumin but is more firmly bound to globulin in the erythrocyte, leucocytes and platelets. In zinc deficient animals, there is usually a decline in plasma protein and reduction in the alkaline phosphates activities. Pregnant ewes and cattle have lower plasma zinc during pregnancy than the non-pregnant animals (Mills *et al.*, 1967 and Halsted *et al.*, 1968).
Normal levels of zinc in lambs and calves lie between 0.80 and 1.2 µg/ml while in severely zinc deficient lambs, calves and goats values below 0.4 µg/ml were recorded (Mills *et al.*, 1967).

2.7 Protein

Proteins are essential component of all living cells, some fulfil structural functions, some act as biological catalysts and some engaged in transport activities. It plays an important roles as buffers in the maintainance of acid - base balance in the blood (Peters, 1972; Laurell, 1972). Most plasma proteins are synthesised in lymphoreticular system and they have varied functions such as:

(a) Distribution of extracellular fluids. Though plasma osmolarity is mostly due to sodium and its associate anions, the effective osmotic pressure (colloid osmotic pressure) is due to plasma proteins. The albumin is more

active than the globulin because of its lower molecular weight.

(b) The antibodies forming a strong defensive mechanism.

(c) Transport roles for the metabolites, hormones, metal ions, drugs and excretory products.

(d) Clotting factors

(e) A source of nutrients for the tissues

(f) Having a good buffering capacity

During foetus development protein metabolism is put into a severe test and as such several changes in the plasma proteins are observed (Larson and Hay, 1958). In ewes, Dunlap *et al.*, (1959) found a decrease in the concentration of albumin during the first half of pregnancy which returns to near normalcy at term. During the later half of pregnancy the concentration of globulin decreased markedly; this; may be due to production of globulin rich colostrum. Larson and Hay (1958) in their studies found decreased concentration of protein throughout the gestation period of the ewes. These losses they thought, were due to β_2 and γ globulin and to a lesser degree alpha-globulin. Gomez *et al.*, (1984) showed that albumin decrease while globulin fractions and total protein increased with age. The exceptions to this are the very old subjects.

In birds, production of eggs require large amount of protein and this consequently places the domestic fowls under great stress. Laying hens contain more total protein and alpha-globulin than non-layers. A similar report was given by Perk and Lobl (1961a) in ducks.

Protein levels in animals and birds are very sensitive to nutritional influences, and growth factors. Low protein-

diet in animals and birds could result in hypoproteinamia that would give symptom of oedema.

2.8 Alkaline phosphatases (ALP)

(ALP - EC. 3.1.3 orthophosphoric monoester phospho-hydrolase)

Alkaline phosphatase is present in all tissue cells, and/or in the cell membrane. It is found particularly at high level in the epithelium of the kidney tubules, bone, placenta and intestinal villi. When ALP and other enzymes leak through the cell walls into the plasma, they are cleared from the plasma either through the kidney, bile ducts or body sweats at the same rate. If there are adverse physiological changes in the body, there might be over production or under production of ALP, these are transported into the plasma to give it the raised or lowered levels. Different adverse physiological conditions give increased or decreased levels of the enzymes in the blood and this can be used to indicate of the physiological changes.

The first measurement of alkaline phosphatases activities in sera was carried out by Kay, early 1930. Since that time numerous substrates, buffer, incubation conditions were introduced as shown by McComb and Bower Jr. (1972). Several different unrelated methods had been use by various laboratories to measure the activities of this enzymes. As the activities of ALP depend on the medium in which the activities take place and the buffers conditions used, many buffers have been found to activate or inhibit the activities of these enzymes (Hausamen, *et al.*, 1967 and McComb and Bowers, 1972). Bower Jr. and McComb (1966) also

noticed that heat denaturation when incubated at 37°C under certain condition results in the failure of linearity of reactions with time and that some methods can become non-linear as a result of substrate exhaustion. From these studies, many workers preferred the use of p-nitrophenyl phosphate as substrate and 2-amino-2-methyl-1 Propanol (2A2MIP) as buffers. Nevertheless, carbonate buffer with their various modifications are still widely used. Various different working temperatures for best activities were tried, (Lowry *et al.*, 1954 and Bower *et al.*, 1966, 1975). They found that the best temperature and pH for serum ALP activities was at 30°C and 10.30 respectively. In addition some metal ions such as magnesium and zinc were found to enhance the activities.

In 1983, Tietz *et al.*, put up a reference method for the measurement of ALP in human serum using 2A2MIP as buffer, p-nitrophenyl phosphate (4NPP) as substrate, while magnesium and zinc ions as enzyme activators and incubating at 30°C were able to show that human serum ALP consists at least five tissue-specific enzymes that can be separated between pH 2.6 and 9. They also suggested that serum and heparinised plasma free of haemolysis could be used but not plasma from anti-coagulants such as the citrate, oxalate, and EDTA. They read the activities at wavelength of 405 nm using a spectro-photometer.

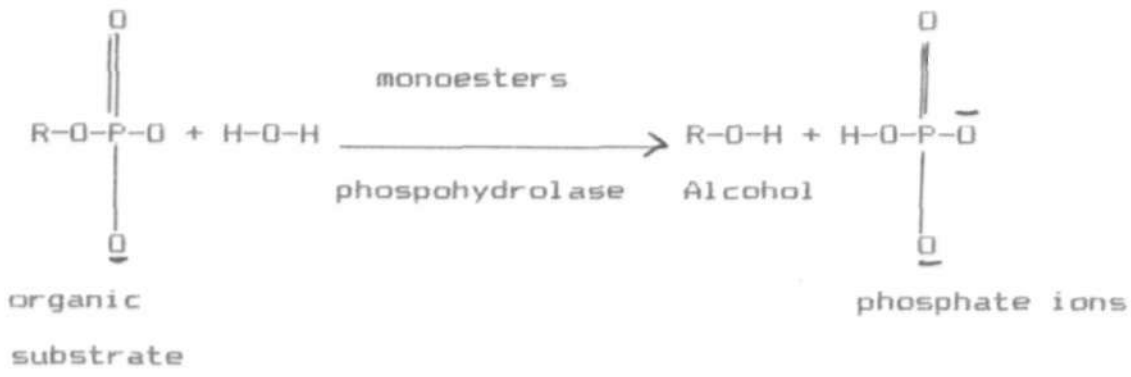
In the early 1950s, it was thought that the heterogeni-city of enzyme was contaminant or partially denatured or degraded enzyme molecules. As a result of these speculations Neilands (1952) used zone electrophoresis to demonstrate any such molecules and was able to show that some enzymes

existed as groups of isoenzymes.

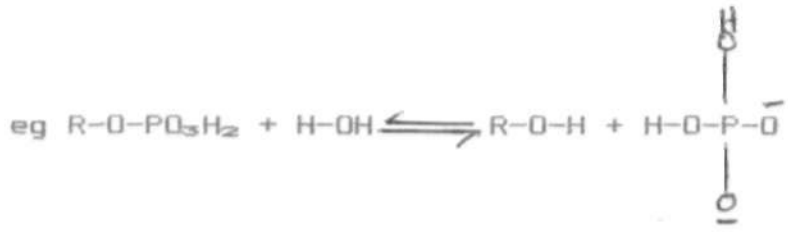
Markert and Moller (1959) were able to show a substantial exponential growth of the isoenzyme and recognise that isoenzymes had formed a fundamental and significant aspect of biological organisation. A given enzyme activity may be present in more than one organelle; when this occur, the individual enzyme protein differ to some degree in their structures. They would exhibit demonstrable differences in their physical, biochemical and immunological properties. It is generally believed that the structure of the "active centres" is identical or at least similar for all enzymes of a given specificity regardless of their origin but their amino-acid sequences in many sections of the peptide chains may differ considerably. The name, isoenzyme, was therefore recommended by the International Union of Biochemistry for such variable (Markert and Moller 1959). The commonest approach to the separation of these iso-enzymes is by electrophoretic migration. They differ also by their stabilities to heat denaturation, resistance to various chemical inhibiting agents and their affinity for substrates and co-enzymes.

2.8.1 The activities of Alkaline phosphatases

Phosphatases belong to the class of enzymes called hydrolases. They catalyse the scission of compounds containing acyl, or phosphoryl (phosphate) ester bounds and also compound containing peptide, amide, hemi-actal and similar bounds. When these copounds are split, a concurrent splitting of an O-H bound in water molecules also takes place



The hydrolytic reactions are exergonic and can proceed easily. They are involved in the breakdown of metabolites and not in their synthesis. They hydrolyse a large variety of organic phosphate esters with the formation of an alcohol and phosphate ions. They also catalyse the transfer of the phosphate group from a donor substrate to an acceptor compound containing an (OH) group (Bassey *et al.*, 1947). If water (HOH) is the acceptor the net effect is hydrolysis.





Phosphatases and esterase activities can be measured in living organisms. Yee-Meiler (1975) was able to measure these activities in Norway sprue needles and leaves to detect hidden injuries due to fire and chemical and was able to use these changes to indicate any type of pathological changes in plants. Hinder and Finch (1966) were able to use the activities of the phosphatases, pyrophosphatases and adenosine triphosphate (ATP) to detect normal bean root from boron deficient roots. Mejnartowiez and Bergamann (1975) used leucine amino-peptidase and phosphatases to study the genetics of the European Larch *Laux decidual mill*. Hudson (1983) was able to measure the molecular mass of alkaline phosphatases from *Escherichia coli* and showed that it has a mass of 80,000 daltons. Hunter (1969) and Lilian *et al*., (1975) in their studies on human were able to show that there were increases in serum alkaline phosphatases during pregnancies while none could be demonstrated in non-pregnant females and males. They showed that these increasing ALP originates from placenta; the enzyme is heat stable and could be used as an index of placenta function.

Agnes (1975) showed no significant change of heat stable alkaline phosphatases in pregnant bovine sera while Parkes and Blackmore (1977) could not demonstrate it in Mare neither Kaantee (1974) in the sera of porcine. Agergaard (1976) was able to relate the plasma alkaline phosphatases activities to growth rate and weight gain in cattle and porcine.

2.9 Haemoglobin, PCV and MCHC

Haemoglobin consists of protein globin united with the pigment haem. The haem is an iron containing porphyrin; the nucleus of phorphyrin consists essentially of four pyrole rings joined together by four methine (= CH –) bridges. As shown in the diagram below (Antonini 1965).

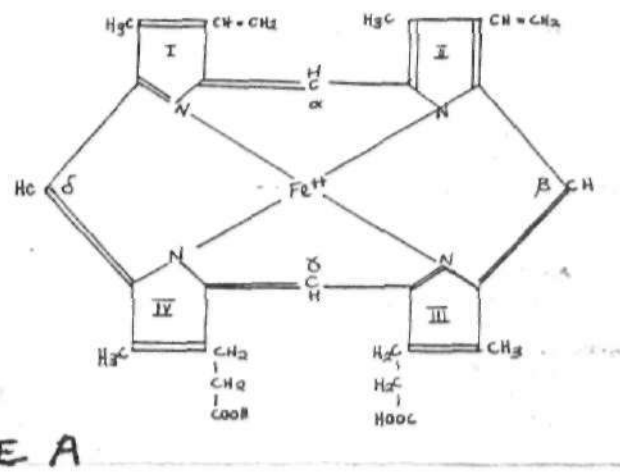


FIGURE A

Figure A. Structure of Haem

The iron in the haem is in the ferrous (Fe++) form and the ferrous is attached to the N^{r} of the imidazole group in the associated globulin. The haem forms a loose union with oxygen (O_2) referred to as oxyhaemoglobin or with carbon dioxide (CO_2) referred to as carboxyhaemoglobin. In the oxidised state the iron is in ferric state and the positions occupied by O_2 or CO_2 are replaced by OH group on the ferric (Fe^{+++}) atom.

The haemoglobin is built from four polypeptide chains of two types each in duplicate; each chain is associated with one haem group, that is, four haem to the molecule of haemoglobin. The combination of O_2 with the Fe^{++} atom is

very loose and depends on the available Fe++ atoms in the haems.

The average human haemoglobin under normal physiological condition in males is 15.80 gm/100ml, in female 13.70 gm/100ml, while the average irrespective of sex is 14.80 gm per 100ml. One gramme of haemoglobin when fully saturated combines with 1.34 mls of oxygen so that the haemoglobin concentration is an index of oxygen-carring power of the blood taken up in the alveoli of the lungs (Keele and Neal, 1971). The haemoglobin concentration is generally expressed as percentage. For example 100 per cent haemoglobin should be equivalent to 14.80 gm/100ml of haemoglobin. Therefore haemoglobin expressed as 50 per cent would be equivalent to 7.4 gm/100ml.

The haemoglobin enclosed in the corpuscles, (red cell) gives certain advantages, when it is dissolved in the plasma it would increase the viscosity and raised the osmotic pressure by about 100 mm Hg. This would derange the mechanism of water interchange between the capillaries and the tissues spaces.

The red cells therefore provide an efficient exchange of gases (CO_2 and O_2) between the target tissue cells and their environments. In this form more haemoglobin carries more oxygen and carbon dioxide per unit volume of blood without any rise in the blood viscosity. The large amount of oxygen carried by the haemoglobin in red corpuscles are needed for the tissues metabolic activities. The carbon dioxide released as a result of the metabolic activities are taken up by the haemoglobin and are then carried to the lungs alveoli where the carbon dioxide is given off and replaced by

oxygen breathed in with air (Keele and Neal, 1971).

The life span of a matured red blood corpuscles is about 120 days, the corpuscles are then destroyed by the reticulo-endothelial system which constitutes part of the lining of the blood sinuses. When the haemoglobins are released from the corpuscles both the iron and globin are conserved to be re-cycled while the bile pigment are excreted from the body through the bile.

Haemoglobin differ from species to species and they have several varieties in man. The haemoglobin moiety is the same while the differences physically and chemically are the globin fractions.

The adult and foetal haemoglobin varieties are physiological while the rest such as haemoglobin "S" (severe sickle cell anaemia), Mediterranean anaemia (Thalassamia), a persistant haemoglobin "F" causing congenital anaemia are pathological conditions.

There are many pathological conditions that give various types of anaemia in animals such as:

1. Simple chemicals, some snake venoms and bacteria toxins
2. Incompatible blood during transfusion.
3. Appearance of abnormal haemolysins that could haemolyse the compatible blood cells and even the patients own blood cells.
4. Sickle cell anaemia, and the Mediterranean anaemia (thalassaemia).
5. Hypersplenism, that is, congestion in the spleen leading to excessive destruction of cellular elements of the blood (pancytopenia).

B. Increased/decreased variations in sizes and shapes of the

red blood cells which can give the following conditions:

1. Haemolytic anaemia giving rise to reduced numbers of red cells and presence of immatured red blood cells.
2. Anisocytosis which could either be macrocytic or microcytic anaemia.
3. Poikilocytosis such as penicious anaemia.

C. Inadequate haemoglobin formation (hypochromasia). This is as a result of iron-defficiency or failure of globin synthesis vital for the haem synthesis.

The above conditions (A, B and C) could cause anaemia. The determinations of the packed cell volume of the blood or haematocrit value (literally blood separation) could be used for early detections of anaemia. In such a condition low/high values of packed cell volume (PCV) are obtained.

The choice of anticoagulants for the collections of the blood is important as these could affect the size of the red blood cells which invariably give lower PCV. The normal values of PCV in animal lies between 28-45 percent of the whole blood.

In some pathological conditions the red blood cells are enlarged and could give higher values of PCV. Here the determination of mean corpuscular haemoglobin concentration (MCHC) is very valuable. MCHC is the amount of haemoglobin as a percentage of the volume of corpuscles. This averages 32.50 ± 2.50 gm/dl.

2.10 Pregnancies in mammalian kingdom and methods for assessing them

Great diversities appear to exist throughout the mammalian kingdom in the mechanisms involved in mother's metabolic recognition of the fact that she is pregnant. Despite these wide differences, it has been recognised that hormones play a major role in the maintenance of pregnancy and the regulation of the reproductive organs throughout the period the offspring is dependent upon the mother (Amoroso, 1960).

Progesterone and oestrogen constantly supplied. maintain the uterus in its correxť physiological condition to allow pregnancy to remain and develop normally in it. Other organs in the body such as adrenal cortical tissues can synthesize progesterone from a variety of steriods precursors, the corpus luteum produce most abundantly hormones of pregnancy. African elephants have been shown to be exceptions (Smith *et al.*, 1969). In addition to the above, luteotrophic complex that are obtained from both pituitary and placenta consist the prolactin and follicle secreting hormone. These two hormones stimulate the production of oestrogen from ovary follicles. The oestrogen in turn prolonged the life span of corpus luteum and stimulation of progestrone by the lutein cells. It also appears that placental protein hormones chorionic gonadotropin (hCG) a glycoprotein with about 30 per cent of the molecule in form of carbohydrate (Morgan *et al.*, 1973) and the placental lactogen (hPL) are also present in the luteotrophic complex. It has been shown by Zarrow (1961) and Diczfalusy (1969) that the synthesis and secretion of oestrogens and their metabolites by pregnant

animals especially in women, depend very much upon the functions of foetal tissues. Thus their measurements in urine provides very satisfactory ways of assessing the health and well-being status of the foetus. The measurement of pregnanediol, metabolite of progesterone in the urine would give useful indications of the placental health. It has been confirmed that as the ages of gestation advance the levels of progesterone, oestrogen and oestriol would increase (Venning, 1955 and Hobson, 1971). The measurement of the levels of these hormones in the blood circulation are therefore indicative of pregnancies in animals. Frieson (1973) showed that placenta lactogen (HPL) is anabolic and in partnership with insulin restricts maternal utilization of protein and thereby increases the flow of amino acids to the foetus. The measurement of the levels of any of the hormones above could be used to identify pregnancy.

There are other protein hormones such as adrenocortico-trophic hormone (ACTH), thyroid stimulating hormone (TSH) melanocyte stimulating hormone (MSH) that are assumed to be produced by placenta. The evidence to support the assumptions is very tenuous. Diczfaluzy and Troen (1961), Brody (1969) reviewed the evidences for and against such claims.

The measurements of some hormones such as progester-one, pregnandiol, oestrogen and oestriol are based on the extractions of these hormone in organic solvents; followed by various other methods for purifications and finally with chemical reactions develop colourations that can be read in a spectrophotometer (Bell and Varley, 1960).

Radioimmunoassay are widely used for the assay of any

the hormones metioned above. Various kits for this assay have been produced in the market for easy assays (Rzasa *et al.*, 1984; Hofman *et al.*, 1985; Vailukaitis, 1985; Gawron, 1986 and Bassett *et al.*, 1986). There are techniques based on antibody-antigen reactions. They are widely in use and there are diferent kits in the market (Der-fong Fan, 1986, Burk *et al.*, 1987; Armbruster, 1987 and Brandi *et al.*, 1987). Monoclonal abtibodies have also been introduced in the antibody-antigen techniques (Gelletlie and Nielsen, 1986); any of these techniques could be used to measure the levels of the hormones and the results would indicate the presence or absence of pregnancy.

There are other techiques in use for the detection of pregnancy. Such techniques are those of Piersion and Ginther (1984) based on the ultrasonography for detecting pregnancy and the embryonic development in heifers. Merilan (1983) technique is based on nuclear magnetic resorance spectra of cervical mucus in pregnant buli. Unfortunately these techniques are very expensive and are not easily available in routine clinical laboratories in Nigeria. They require specially and highly trained personnel to operate them. As such, a search for a simpler method that is less expensive and reliable would be of great advantage to screen out the pregnant from non-pregnant before they are slaughtered in the abattoirs.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Animals - Sixteen Yankasa sheep were used for this project, and they all looked healthy with no apparent signs of any sickness.

There were four rams between nine to twelve months old, one ram and ten ewes between thirteen to fifteen months old while one ewe was above eighteen months old.

The animals were housed in low walled building to provide good ventilation and the house divided into several pens as in Plates II. These animals were fed with acha-hays as the hays are readily available locally from wastes of acha cereals, *Digitaria exillis*. During the rainy season, these animals were fed on green grasses to conform with the feeding pattern of the local ones feeding mainly on acha-hays during the dry season, and green grasses in the rainy season. The experimental animals Plate III were distributed into the pens.

3.1.2 Chemicals and Reagents

Analar grade chemicals and reagents from British Drugs House (BDH) Chemical Limited, Poole, England and Sigma Chemical Company, St. Loius Mo. (USA) were used for this project unless otherwise stated.

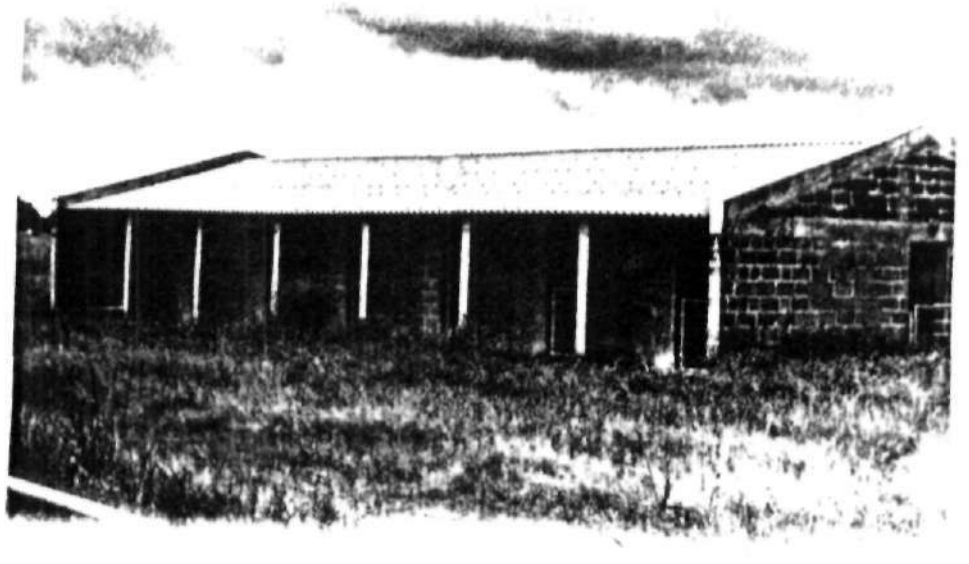


Plate I. The housing of the experimental sheep

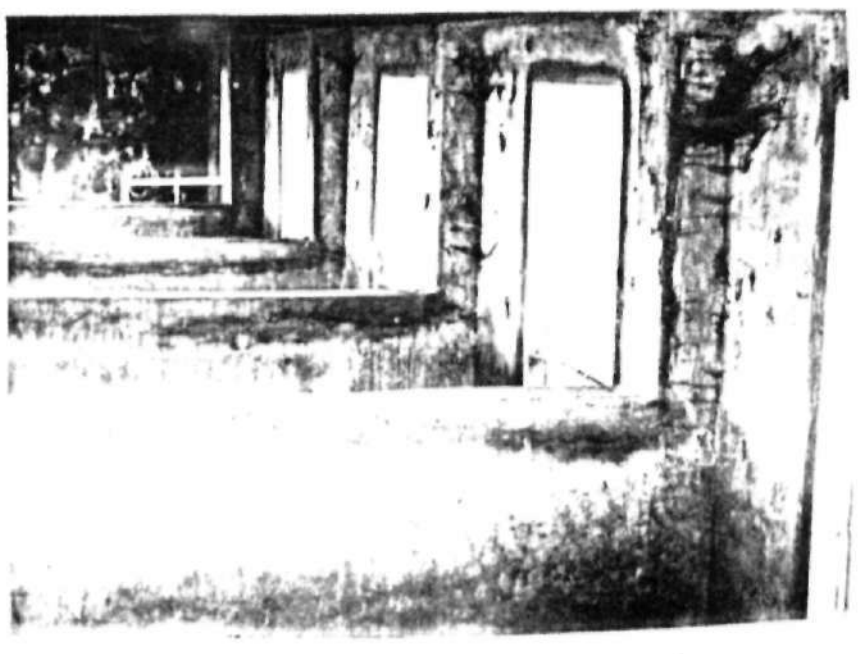


Plate II. The pens in the animals house



Plate III. Some groups of Yankasa sheep from where random selection of the experimental sheep were made

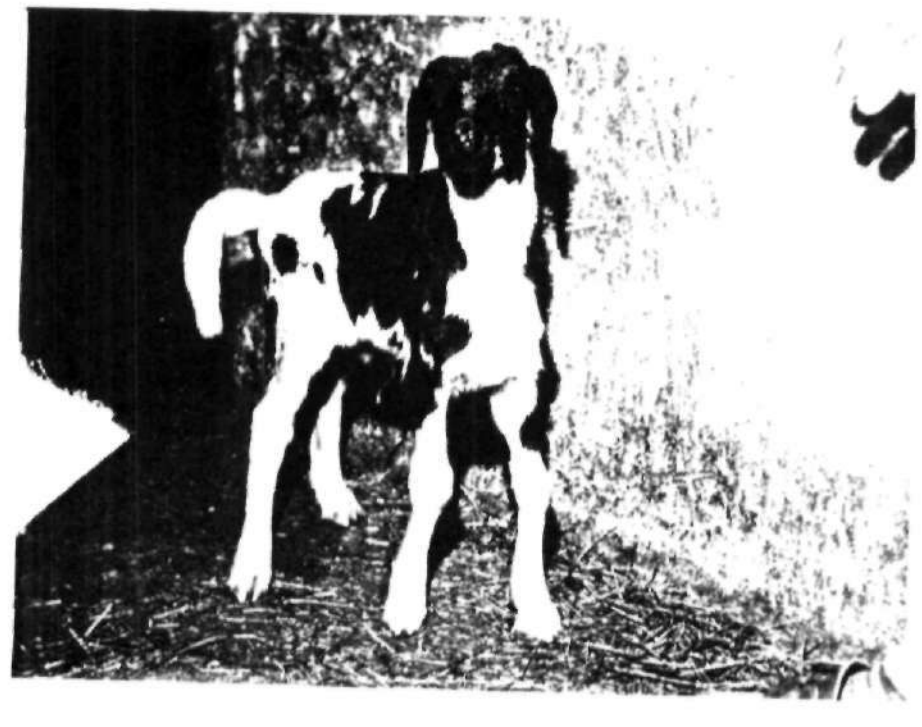


Plate IV. Lamb I from the experimental pregnant ewes between 145 to 150 days (30 hours after lambing)



Plate V. Lamb II from the experimental pregnant ewes between 145 to 150 days

The standard solutions for the various elements measured with atomic absorptiometer spectrometer are supplied by Fisher Scietific Company Fair Lawn, New Jersey, U.S.A. All buffers and reagents were prepared using de-ionized distilled water and kept at about 4°C in the refrigerator. These reagents or buffers were used at room temperature.

3.1.3 Equipments used include:

(a) Spectrophotometer Pye Unicam S.P. 500 Series 2

(b) Flame emission photometer (Cornings 400 Flame photometer)

(c) Atomic absorption spectrophotometer SP 190 series

(d) Haematocrit centrifuge and plain micro-haematocrit tubes

(e) Disc electrophoresis tank and Unicam power pack.

3.2 Methods

3.2.1 Preparation of the animals

The animals were acclamatised in their pens for three weeks and dewormed with anti-helminth drug benminth pyranted tartrate which has a broad spectrum; each animal received a dosage of 25 mg/kg body weight.

After one week, the oestrus cycle of the ewes were all synchronised with lutalyse at a dose of 3 ml (i.e. 15 mg) per sheep injected intramuscularly. The lutalyse used had expired one year thus the increase in its dosage and the animals were closely watched for any changes in their behaviours. After the fourth day, the animals were restless and mounting each other, a sign of their being on heat. The sheep in each pen were then served with an active ram; none of the control rams was used for this servicing. At the end of the twelfth day the rams were removed from the sheep.

3.2.2 The collection of blood samples

Blood samples were collected from each animal every month from December 1984 to November 1985. The hairs around the jugular veins were clipped off and sterilised with 70 per cent absolute alcohol before the veins were punctured with clean sterile hypodermic needles (Luer Record fitting size 1.45 x $2^{3/8}$). Free flowing twenty millilitres of blood were collected into each of two sterile clean universal bottles from each animal. These were allowed to clot undisturbed and the sera separated within two hours and stored at -20° C. Again five millilitres of blood was collected into ammonium oxalate bottles for haematological

analysis. These oxalate bottles were usually mixed immediately to prevent clotting. During the collection of blood no stasis was used and collection was so gentle as to avoid struggling.

3.3 Potassium and sodium estimation by flame emission photometry

In the early years, determinations of sodium and potassium were carried out by chemical treatments and the precipitates obtained re-dissolved. The coloured solutions developed were read in a spectrophotometer (Varley, 1963). With the introductions of flame emission photometer in 1940's, determinations of these elements are carried out with speed and accuracy. There are many improved models of flame photometer in the market but the one used in this work is the corning flame emission photometer model 400.

The atomic absorption spectrophotometer (AAS) was developed and introduced into the market. It is a more specific and most accurate technique for the measurement of elements (Whiteside and Milner, 1983). Unfortunately the AAS is too expensive and outside the reach of most routine clinical laboratories.

3.3.1 For the estimation of potassium and sodium by flame photometer, the method described by Wootton (1974) was adopted in this project.

3.4 The titrimetric methods for the estimations of chlorides and bicarbonates

3.4.1 The serum chloride

The method of Schales and Schales (1941) which has became widely accepted by many laboratories was used in this project

3.4.2 Method of chloride estimation

An aliquot amount of 0.20 mls of plasma/serum/standard was added to 1.80 mls of de-ionised water. Few drops of diphenylcarbazone indicator (about 0.06 ml) were added; mixed and titrated with mercuric nitrate solution. An end point was obtained when a bright - violet blue colour was seen. The results were calculated by Test/standard x concentration of standard.

3.4.3 The serum bicarbonate

The plasma or serum bicarbonats contains three compounds that are equivalent with each other.

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3 + H^+$$

Rappaport *et al.*, (1956) added N/100 H_2SO_4 to neutralise all the carbonate and the excess acid was back titrated with N/100 NaOH. Provided the normal precautions are taken, this procedure is quite simple, convenient and accurate. The N/100 H_2SO_4 and N/100 NaOH were diluted from standardised solutions of N H_2SO_4 and N NaOH prepared by BDH. The method of Rappaport *et al.*, (1956) was used in this project.

3.4.4 Method of bicarbonate estimation

A volume of 0.20 ml of serum/standard/control was added to 5.00 mls carbon dioxide free de-ionized water, and mixed. Then 2.0 ml of N/100 H_2SO_4 and a few drops of indicator were added. They were mixed and back titrated with N/100 NaOH from 2.00 ml burette until end point was reached. The result was therefore calculated as the mol/litre of bicarbonate which is equal to 2 ml of N/100 H_2SO_4 - volume of N/100 NaOH used x 50 (Appendix A 1.4).

3.5 Atomic Absorption Spectrophotometer (AAS)

Methods for the assays of elements: There are many models of AAS in the market but the PYE Unicam 190 model which has a single beam monochromator with wavelengths 210-700 nm was used in this project. The instrument is very sensitive to pick very low concentrations of elements in a solution. The stock standards of the elements are prepared in 1000 ppm in polythene bottles and the standards for calibrations were prepared from these standards with de-ionized water. For the estimations of calcium and magnesium in serum, dilutions of 1:100 were made with 10 per cent lithium chloride solution while for the copper, zinc, cobalt and manganese dilutions with 20 per cent solutions of trichloroacetic acid were used. The mixtures were centrifuged and the clear supernatants solutions were used for the analysis, all analysis were carried out in duplicates. The operational conditions for the elements are given as in the Table 3.5 and the concentrations of the elements in the sera were read from their respective graphs (Appendices A 8.1 - A 8.6).

Table 3.5 The operational precautions for elements examined with atomic absorptiometer spectrophotometer (AAS) (Whiteside and Milner, 1983)

	Ca++	Mg++	Cu++	Co++	Zn++	Mn++
Wavelength (nm)	422.70	285.20	324.80	240.70	213.90	299.50
Bank pass (nm)	0.30	0.40	0.30	0.20	0.40	0.30
Air pressure (psi)	38.00	30.00	38.00	38.00	38.00	38.00
Flame Litre (min)	1.0-1.31	1.0-1.3	0.8-1.1	0.9-1.2	0.9-1.2	0.9-1.2
Air/Acetylene	sp 9 19-22	sp 9 21-25	sp 9 19-22	sp 9 19-22	sp 9 19-22	sp 9 21-24
Lamp current (mA)	6.00	4.00	4.00	11.0	9.00	11.00
Calibration (ppm)	0-3.00	0-2.00	0-2.00	0-5.00	0-2.00	0-5.00
Lamp height (cm)	12.00	12.00	12.00	12.00	-	20.00

(The results are as in Appendix A 2.1,and A 2.4, A 3.1 to A 3.4

3.6 Estimation of inorganic phosphate

The method of Goodwin (1970) was used in this project

3.6.1 Procedures

A volume of 0.20 ml of serum/working standard containing 1 mg to 10 mg of phosphorus per 100 ml of distilled water were pipetted into two test tubes labelled "test" and "blank" Then 1.50 mls of borate solution ($Na_2B_4O_7 \cdot 10H_2$, 38.14 g/l) were added to all the test tubes and mixed thoroughly. An aliquot of 0.5 ml of acid molybdate solution was added to all the test and standard tubes while 0.50 ml of acid solution to their respective blanks and mixed. These were followed by 1.00 ml solution of ascorbic acid reagent (1 g/100ml) freshly prepared to all the tests and standard tubes while 1.00 ml of distilled water to their respective blanks. The tubes were left to stand on the bench for 15 mins and 3.00 ml of carbonate solution (2.0 mol/l) were added to all the tubes, mixed, and allowed to react for 5 minutes. The readings of the absorbance of the test against their respective blanks were carried out at 700 nm. The concentrations of phosphates in the sera were read from the graph (Appendix A 8.7).

Citrate, florides, oxalates, nitrites and manitol were said to interfere with this classical molybdate method but with the method of Goodwin (1970) these effects are not pronounced. Bilirubin at higher concentration was stated to affect this procedure and that the effect could be removed with the use of albumin in the solution/reagent. This effect could be tolerated with the Goodwin method up to concentration of 9 mg of bilirubin per 100 ml and could even

be up to 20 mg per 100 ml if albumin blank is used. Haemolysis of samples would not affect this method so much, as much as 600 mg has been found not to affect this method.

In this project, the only modification that was introduced was the ommision of albumin because all sera samples collected from the animals had less than 10 mg bilirubin per 100 ml of serum. The amount of bilirubin in these sera laid within the tolerable quantity.

3.7 Estimation of blood urea

Most method for the estimation of blood urea rely on the specific hydrolysis of urea to ammonia and carbon dioxide by the urease enzyme. The ammonia or carbon dioxide can be measured directly and this is equivalent to amount of urea present in the sample. The methods of Marsh *et al.*, (1965) depends directly on the chemical reactions of urea with diacetyl monoxime. Urea reacts with diacetyl monoxime in strong acid condition to produce yellow colouration. The addition of ferric ions and thiosemicarbazide gives a red complex solution which is measured in a spectrophotometer at wavelength 520 nm. This is the method of determination employed in this study.

3.7.1 Procedure

(a) A dilution of 1:100 with the sera/standard/controls were made with distilled water in labelled test tubes. From each test tube, a volume of 1.00 ml was pipetted into test tubes similarly numbered containing 1.00 ml of distilled water.

To their respective blanks, 2.00 mls of distilled water was added. Then 2.00 mls of working colour reagents followed by 2.0 ml of mixed working acid reagents were added to all the tubes and mixed thoroughly. They were placed in a boiling water bath for twenty minutes, after which they were transferred to a cold water bath for 5 minutes. The test solutions were read against their respective blanks in a spectrophotometer at wavelegth 520 nm. The concentration of the urea in the samples were calculated as follows:

$$\frac{\text{Reading of the solution}}{\text{Reading of the standard}} \times \text{Concentration of the standard}$$

The results are given in Appendices A 7.2

3.8 Cholesterol

There were various modifications to the Libermann Buchard reagents by many workers (Kenny, 1952; Rappaport, 1955; Henly, 1957). The reagent used for this procedure was a modification of Libermann Buchard with the incorporation of anhydrous sodium sulphate to give a bluish end coloration that is more stable and read at 640 nm. The reagent is made up as follows:

Acetic anhydride	600 ml
Glacial acetic acid	300 ml
Concentrated sulphuric acid	100 ml
Sodium sulphate (anhydrous)	20 gms

They were stored in brown bottle and kept in refrigerator.

3.8.1 Procedure

All the test tubes and pipettes used must be clean and dry. A volume of 0.10 ml of serum/standard/control was added to 0.10 ml of distilled warter in the labelled test tubes and mixed thoroughly. Then 4.00 mls of the cholestrol reagent was added; mixed well and left for 5 minutes in the dark. They were read very quickly to avoid any fading of the colour in a spectrophotometer at 640 nm.

The concentrations of the cholestrol in the sera were calculated from:

$$\frac{\text{Reading of the solution}}{\text{Reading of the standard}} \times \text{Concentration of the standard}$$

The results are given in Appendices A 7.1

3.9 The estimation of total protein in serum and exudates by Biuret method (Kingsley 1972)

Serum proteins form a violet-blue complex with copper ions in alkaline solution. The intensity of the colour produced is proportional to the number of the peptides bound reacting. The absorbance of the complex is measured at 540 nm

3.9.1 Procedure

The method used by WHO (1986) was followed in this project. Sample blanks were used in order to avoid errors due to turbidity. From the results obtained, the concentrations of the protein in the sera samples were read on the calibration graph in gm/100 ml. (Appendise A8.8)

3.9.2 The calibration graph for total protein using bovine serum albumin

It is very important to check the concentration of protein albumin in the bovine serum albumin standard (BSA). This is because, lost in values can occur as a result of poor storage or aging. This is carried out by micro-Kjeldahl technique (King and Wooton, 1964).

The technique as described by WHO (1986) was strictly followed (Appendix A.8.8).

3.10 Estimation of albumin in serum

The method using the bromocresol green (BCG) dye in succinate buffer pH 4.2 in accordance with WHO (1986) was used in this project.

3.10.1 Procedure for albumin estimation

A volume of 5.00 mls of the working dye solution was pipetted into a labelled test tubes and an aliquot of 25 µl of standard/sample/control/blank was added and mixed thoroughly. They were read immediately within seconds in a spectrophotometer at wavelength 632 nm against their respective blanks.

In cases where there might be an extremely lipaemic serum sample, a serum blank is put up using succinate buffer solution instead of the working dye solution.

3.10.2 Calibration of albumin

Preparation of working albumin standard solution and calibration. This is carried out by diluting the albumin standard (5 gm/100ml) in sodium chloride/sodium azide solution as in Table 3.10.2

Table 3.10.2

Test tube Nos.	1	2	3	4	5
Vol.of albumin Std. 5gm/100ml	0.4	0.8	1.2	1.6	1
Sodium chloride/ azide solution	1.6	1.2	0.8	0.4	-
Concentration of working Std gm/100ml	1.0	2.0	3.0	4.0	5.0

From the above Table 3.10.2, preparations 25 µl of solution from each test tubes 1 to 5 was added to their respective 5 ml working dye solution. They were well mixed and read against the blank at 632 nm.

From the graph in Appendix A 8.3 the concentrations of the test samples were read.

3.11 The estimation of globulin in serum

This was carried out by the difference between the values for total protein and the albumin.

3.12 The alkaline phosphatases estimation

Yeh *et al.*, (1984) described their methods using buffer pH 10.3 and magnesium as co-activator. After 15 minutes of reactions at 37°C they quenched the activities with sodium hydroxide. The method is very convenient for the facilities available in this laboratory.

3.12.1 Procedure

A volume of 1.00 ml of reagent substrate in a clean dry test tube was pre-warm at 37°C for 5 minutes. After the temperature equilibrium, 100 µlof the sample was added and mixed quickly in the water bath. When the activities had taken place for 15 minute, 10 mls of 0.05mol/L NaOH was added to terminated the enzyme activities. Blank was set up for each test sample and the serum was added after the addition of the NaOH solution. The test solutions were read against their respective blanks using spectrophotometer Pye Unicam 1900 at 405 nm.

Calculation - The international definition for ALP is that amount of enzyme which hydrolyses 1 mole of the substrate per minute, (Wilkinson *et al*., 1969).

Therefore

$$U = \frac{A}{\text{time}} \times \text{total vol} \times \frac{1}{E} \times \frac{10^4}{\text{sample vol.}}$$

OR

$$U/ml = \frac{A\ 405/min}{18.75} \times \frac{3.00\ (test\ vol)}{0.10\ serum} \times \frac{1}{temp\ factor}$$

OR $$U/L = \frac{A\ 405/min \times 3.00 \times 1000}{18.75 \times 0.10}$$

Where A = absorbance change

Time = Time in minute

Total vol = Volume of reaction mixture in millilitres

E = Molar absorptivity of p-nitro-phenol = 18.75 to 18.80 x 10^3 sq cm per mole at 405 nm in 2A2MIP buffer at pH 10.30

U = International Unit

Sample vol = volume of serum used in millilitre.

3.13 Iso-enzymes of alkaline phosphatases

The modified acrylamide gel technique of Green *et al.*, (1972) was used in this project to compare separations from normal non-pregnant sheep with pregnant one.

For the demonstrations of the visualization of the bands of alkaline phosphatases, the methods of Yeh *et al.*, (1984) was used. The demonstration using the methods of Green *et al.*, (1972) proved most difficult to show the bands of the isoenzymes.

3.13.1 Procedure

A. The sucrose solution (20 per cent) was diluted with equal volume of the serum sample and kept frozen until ready for use. If any serum had alkaline phosphatases above 20 KA (King Armstrong Unit) units it should be diluted to at least 10 KA units with heated serum from non-pregnant subjects

B. Stock reagent electrode reservoir buffer solution was

prepared by dissolving 45.5 gms of Tris in 900 ml of distilled water. The pH was adjusted to 9.5 with boric acid and made up to a litre with distilled water. Before use the buffer solution was diluted 1:4 with distilled water.

C. Stock separating gel buffer solution was prepared as for "B" above, and 1.2 ml of TEMED (NNN'N' tetra methylene diamine) was added and the pH was adjusted to 9.5 with boric acid.

D. Stock reagent ammonia - zinc solution - was prepared weekly by weighing 0.20 gms ammonium persulphate and 0.022 gms zinc acetate dihydrate and then dissolved in 100 mls of distilled water.

E. A gel monomer solution was prepared by weighing 27.60 gm of Acrylamide monomer and 0.40 gms Bis-acrylamide (NN-methyl-ene-bis-acrylamide) and in distilled water. The solution was stored in amber bottle.

F. Preparation of working polyacrylamide separation gel solution was made by addition of one volume of gel monomer "E" with one volume of separating buffer "C" and two volumes of ammonium persulphate - zinc acetate solution "D". This solution now contained 0.50 nM zinc, 0.10 per cent ammonium persulphate, 6.90 per cent acrylamide and 0.10 per cent bisacrylamide in 0.094 Molar tris-borate TEMED buffer pH 9.5.

The mixed solution was de-aerated under vacuum for 30 seconds to remove entrapped gasses.

G. Immediately, the mixture was pipetted into the glass tubes (8.5 x 0.7 mm) capped at the bases until the level was 0.50 cm from the top and standing in perfectly vertical position. Then a volume of 0.10 ml of distilled water was layered on the gel to exclude air and all the tubes were left at room temperature for 30 minutes for the gel to polymerise. They were then stored in the refrigerator under water until they were used. The best results were obtained with one day old gels.

H. Loading the serum - sucrose samples

The acrylamide tubes were carefully arranged on the disc tanks and filled with the buffer (B) at the bottom compartment and the top compartment. A volume of 2 ml concentrated solution of bromo-phenol blue (BPB) indicator was added to the top compartment.

Very carefully a volume of 50 µl of the sample were layered between the gel and the buffer immediately before running the electrophoresis. At this stage, the electrical lines were connected to the Vokan power pack; the lower chamber to the positive lead while the top chamber to the negative lead. Then the electrophoresis was run at temperature between 2°C - 4°C in a cold room or refrigerator. The power pack was at 1 mA per gel tube or 55 volts for the first 5 minutes or until the sample and BPB from the spacer solution entered into the gel. The current then increased to 4 mA per gel tube or 200 volt for the duration of 40 minutes or until the end of the BPB indicator was about 2 mm from the bottom of the gel tube.

I. The visualization dyes

The method of Yeh *et al.*, (1984) was used.

i) Beta-Naphthyl phosphate (1 mg/ml) and 10 mmol/L magnesium chloride (Mgcl) in 0.05 mol/L Tris-borate buffer pH 9.5.

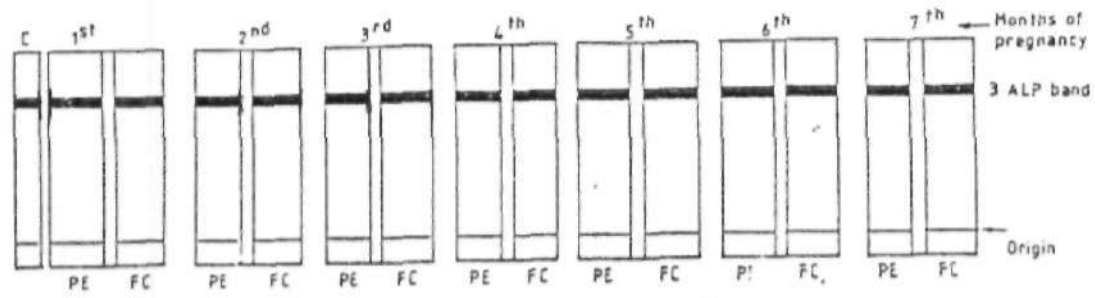
ii) Fast Blue BB (1 mg/ml and 10 mmol/L ($Mgcl_2$) in 0.05mol/L Tris-borate buffer pH 9.5.

iii) De-staining solution containing methanol-acetic acid - water (100:20:100 by volume).

J. Removal of the gels from their tubes and staining

Absolute care must be taken at this stage to avoid the gel from breaking to pieces. To do this, the beta- Naphthul phosphate solution above was layered between the gel and the tube to hypodermic syringe and carefully remove the gel into test tubes containing the substrate Beta-Napthyl phosphate for 30 minutes at 37°C. Then the Beta-naphthyl phosphate was decanted and then the gel were stained in the dark for 30 minutes at 4°C with fast Blue BB. The gels were washed thoroughly in the destaining solution and finally washed in the buffer solution (B) to clear the stains on the gels.

Figure 3.13.1. The diagramatic sketch of the stained iso-enzymes gels



C = ALP control, PE = Preg, Ewes, FC = Female control

ALP = Alkaline phosphatase.

3.14 Measurement of Haemoglobin, Packed Cell Volume (PCV) and Mean Corpuscular Haemoglobin Concentration (MCHC)

3.14.1 Measurement of haemoglobin

The most commonly practised method in clinical laboratories is the Haemoglobin cyanide. The haemoglobin in the blood is oxidised to haemoglobin by the action of potassium ferricyanide. Haemoglobin complexes with cyanide to give haemoglobin cyanide (HiCN). The presence of potassium dihydrogen phosphate keeps the reagent pH at a value at which the reactions are completed within a reasonable time. A detergent is added to enhance haemolysis of blood and to prevent turbidity by plasma proteins. The absorbance of the resulting solution is measured at 540 nm. The procedure used in this project is that method recommended by WHO (1986).

3.14.2 Technique for haemoglobin estimation and calibration graph of the standard

The technique described by WHO (1986) was followed. The haemoglobin concentration were read off the graph plotted.

3.14.3 Measurement of Packed cell volume (PCV)

The method by Coles (1974), was applied for the estimation of PCV using the micro-method with the capillary tubes having no anti-coagulant. The results are given in Table 6 and Appendix A 6.2.

3.14.4 The measurement of absolute values of mean corpuscular haemoglobin concentration (MCHC)

This was calculated from the measure-ment of haemoglobin (3.14.2) and packed cell volume (3.14.3). MCHC which is the concentration of haemoglobin in the average erythrocyte was calculated:

$$\frac{\text{Haemoglobin (Hb) gm/100ml}}{\text{Packed cell Volume}}$$

Example: If Hb = 15 gm/100ml

PCV = 45 gm/100ml or 0.45 gm/ml

Then MCHC = 15/0.45 = 33.3 gm/100ml

Or 15/45 X 100 = 33.3 gm/100ml.

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 Statistical methods used

In this project, samples were selected from several different population. There were variations in the periods of samplings, the state of the animals and ages of the animals. There are also climatic variations from cold dry period when the experiment was started to a hot humid periods when it was terminated. The content of the feeds as well varied throughout the experimental period. It is therefore obvious that these hypotheses would affect the statistical significance. The technique most frequently used to test such hypotheses is the analysis of variance and it is the method used in this project.

4.2 Sodium, Potassium, Chloride and Bicarbonate

The monthly mean serum values for sodium, potassium, chloride and bicarbonate are presented in Appendix A 1.5 Figure 4.2 while the individual monthly values are presented in Appendix A1 and A1.1 to 1.4.

The mean values for sodium in pregnant ewes were between 139.29 ± 4.5 mmol/L and 148.57 ± 4.03 mmol/L. These values were variable periodically when compared with those from the female controls which were between 143.75 ± 3.86 mMol and 149.25 ± 5.38 mmol/L. The lowest mean values 139.29 ± 4.35

mmol/L in pregnant ewes was shown in the first month of pregnancies compared with the female controls 143.75 ± 3.86 mmol/L (Table 4.2). The male controls mean values between 144.33 ± 2.25 mmol/L and 147.80 ± 2.28 mmol/L did not show much differencies from the female controls. There are no noticeable significant differences between the animals while there are observed differences between the periods of samplings. The sodium results show some increases from the second month of pregnacies to the fourth month while there is a drop in the fifth and sixth month of pregnancies.

The potassium mean values for pregnant ewes between, 4.64 ± 0.25 mmol/L and 4.84 ± 0.39 mmol/L, were lower than the female controls between 4.65 ± 0.60 mmol/L and 5.13 ± 0.42 mmol/L except for the second and third month of pregnancies. The male controls were between 4.88 ± 0.29 mmol/L and 5.33 ± 0.74 mmol/L

There is significant difference $P < 0.001$ between the animals and the observed differences are between the male controls and other animals. The pregnant ewes had the least mean values of 4.80 ± 0.10 mmol/L followed very closely by the non-pregnant female controls 4.86 ± 0.10 mmol/L while the male controls was 5.11 ± 0.10 mmol/L. However the periods of samplings did not affect the valuees of potassium significantly

The bicarbonate mean values in pregnant ewes were between 29.86 ± 2.49 mmol/L and 31.71 ± 1.98 mmol/L showed very little variations (see Fig. 4.2). These results are lower than the variable mean values from the female controls which were between 28.75 ± 1.50 mmol/L and 34.50 ± 1.29 mmol/L. There is significant difference ($P < 0.10$) observed

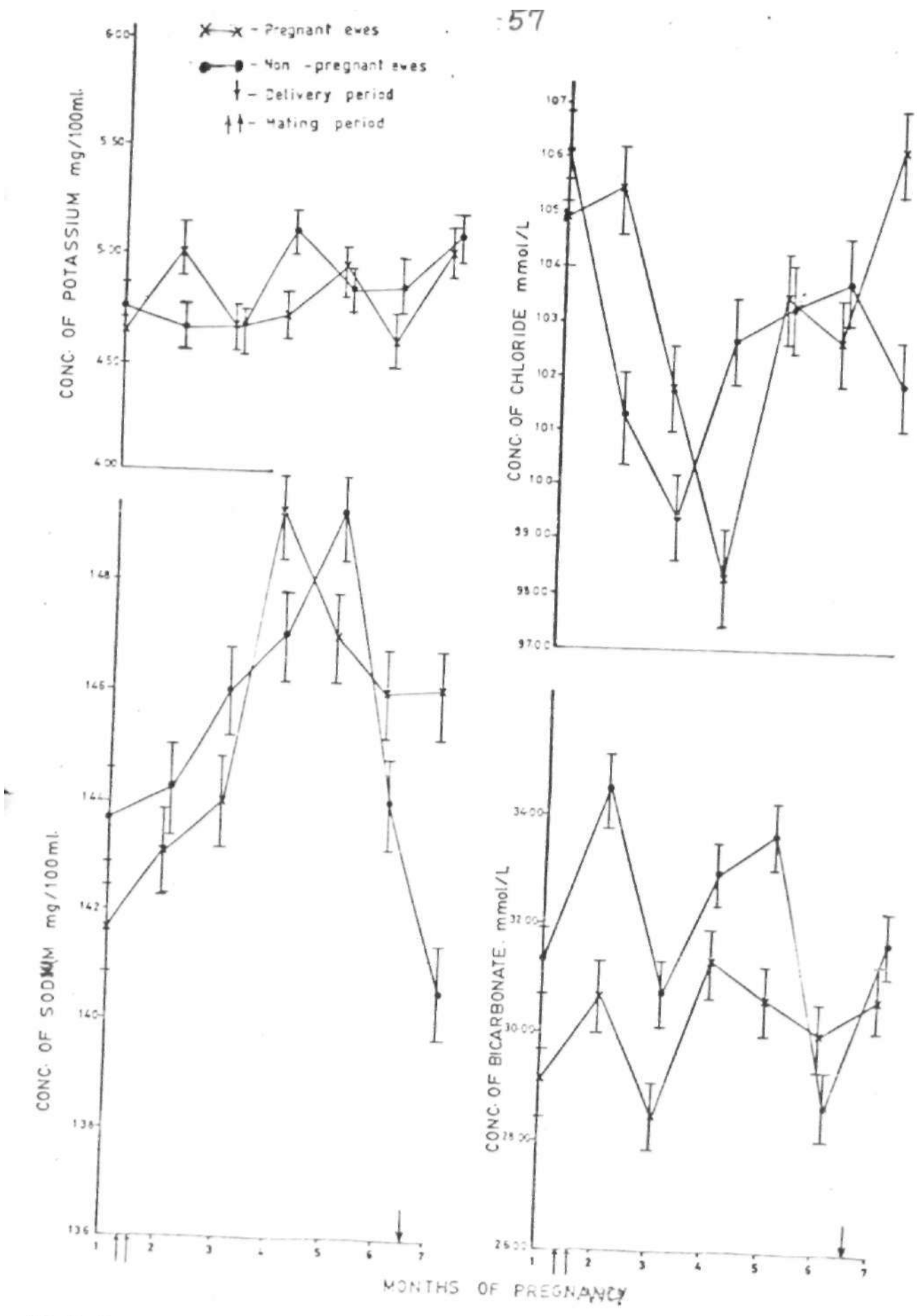


Fig 4.2 Monthly mean concentration of serum K^+, Na^+, Cl^-, HCO_3^- in pregnant ewes, and female controls during the gestation period

between the states of animals and the sampling periods.

The chloride monthly mean values in pregnant ewes were very similar to those in the female control and they were very variable. These values were between 100.95 ± 4.29 mmol/L and 104.76 ± 3.11 mmol/L for pregnant ewes while 99.37 ± 4.96 mmol/L and 106.04 ± 2.09 mmol/L for female control. The male control values between 102.94 ± 4.73 mmol/L and 106.40 ± 2.30 mmol/L did not show different variation from the pregant ewes and female control (histogram Fig 4.2). There are no significant differences observed between the state of the animals and the periods of samplings.

From the results obtained above, the mean values for sodium, potassium and chloride agree very closely to those of Pugh (1966) and Tumbleson *et al.*, (1968) while the values for bicarbonate are higher than those of Spector *et al.*, (1956).

It would be remembered that the experimental animals were under nutritional stress and fed on both stored hays and standing dried grasses; therefore the lower values of sodium, potassium and chloride ions could be due to their losses during the storage of the hays.

4.3 Calcium, phosphorus, magnesium and calcium/phosphorus ratio

The monthly mean values of serum calcium, inorganic phosphorus, magnesium and calcium/phosphorus ratio are presented in Figure 4.3, Appendix A2.5 while individual values are presented in Appendix A2 and A2.1 to 2.4.

4.3.1 Calcium

The lowest calcium values were recorded in the sixth month of pregnancy when the ewes had lambed. The same results were obtained for female and male controls and this could be due to periodic effects. There is signficant difference ($P < 0.01$) between the pregnant ewes and female controls. The specimen mean values were; female ewes 11.45 ± 0.78 mg/100 ml, female controls 11.55 ± 0.78 mg/100ml, male controls 11.72 ± 0.78 mg/100ml.

It would appear from the data that the calcium values from pregnant ewes are lower than those from female controls, although there is no significant difference between them. This could be as a result of calcium demand during pregnancies despite the fact that some calcium ions are re-absorb from the bone reserve and absorbed from the diet under a normal physiological conditions.

The mean values obtained for the pregnant ewes and controls are slightly higher than those given by Hackett *et al.*, (1957) and Kumeresan *et al.*, (1982)

4.3.2 Phosphorus

The phosphorus range in pregnant ewes was between 3.73 ± 0.46 mg/100ml and 5.16 ± 0.52 mg/100ml, in female control between 3.90 ± 0.76 mg/100ml and 4.63 ± 0.29 mg/100ml; while in male control between 4.83 ± 0.43 mg/100ml and 5.42 ± 0.18 mg/100ml.

The means for the first month and second month of pregnancy were above 5.00 mg/100ml, these were followed by lower values till the ewes lambed., after lambing, these values increased above 5.00 mg/100ml. Despite these

differences there is no significant difference between the pregnant ewes and the female controls while there is significance ($p < 0.05$) between the periods of samplings.

The phosphorus means in pregnant ewes generally showed lower values than the female and male controls; this is during the periods from third month to sixth month when the values of phosphorus dropped, (Figure 4·3)

This might suggest that the animals were affected by seasonal nutritional variations and in addition the pregnancies of the ewes.

During the active vegetative growth stages of plants and grasses, phosphorus is used for the active growths and as such phosphorus would diminish with the maturity of the grasses or herbages. The hays used for feeding these animals were bailed from matured grasses or herbages that would therefore contain low phosphorus. In most part of Nigeria, matured grasses and herbages are commonly bailed for hays for feeding the animals during the long dry season. In a more general practise, dry standing grasses and herbages are eaten by a large population of the animals during the same period. Again, these bailed or standing grasses and herbages contain microflora that would deplete the phosphorus contained in them for their own survival as such the presence of microflora would further diminish the phosphorus content in these grasses and herbages. Kapu (1975) reported that phosphorus content in grasses, browses and standing hays in Northern Nigeria are very low and that this might not be adequate to meet the nutritional requirement of the animals without additional supplement. The majority of animals in Nigeria are not nutritionally supplemented and are therefore

Underwood (1966) and Church (1971) reported that the first evidence of phosphorus deficiencies in livestock is a a fall in plasma phosphorus level. The reason being bone phosphorus is less readily mobilised to maintain serum level, as such plasma or serum inorganic phosphorus determination is the most sensitive index for the detection of phosphorus deficiencies in animals.

4.3.3 Magnesium

The magnesium did not show much variations between the pregnant ewes and the female controls. There were higher but more variable results for the male controls.

The means for pregnant ewes were between 3.40 ± 0.86 mg/100ml and 3.85 ± 0.55 mg/100ml while in female and males controls between 3.25 ± 0.50 mg/100ml and 3.87 ± 0.75 mg/100ml; 4.00 ± 0.50 mg/100ml and 5.34 ± 0.85m g/100ml respectively. There was periodic significance ($P < 0.01$) but no significance between the pregnant and female controls. The pattern for all the animals as shown in figure 4.3 is not distinct but fluctuate from period to period.

These values compared very favourably with the normal range quoted by Christians and Williams (1975). Which means that there was no evidence of any deficiencies throughout the pregnancy period of the ewes.

Simenson (1970) pointed out that about 70 per cent of the total body magnesium is in the skeleton while one third of this is available for mobilization to the soft tissues, when dietary intake is inadequate. Skeletal magnesium acts as a labile source for magnesium and therefore this explains why there was little variations in magnesium level in the serum.

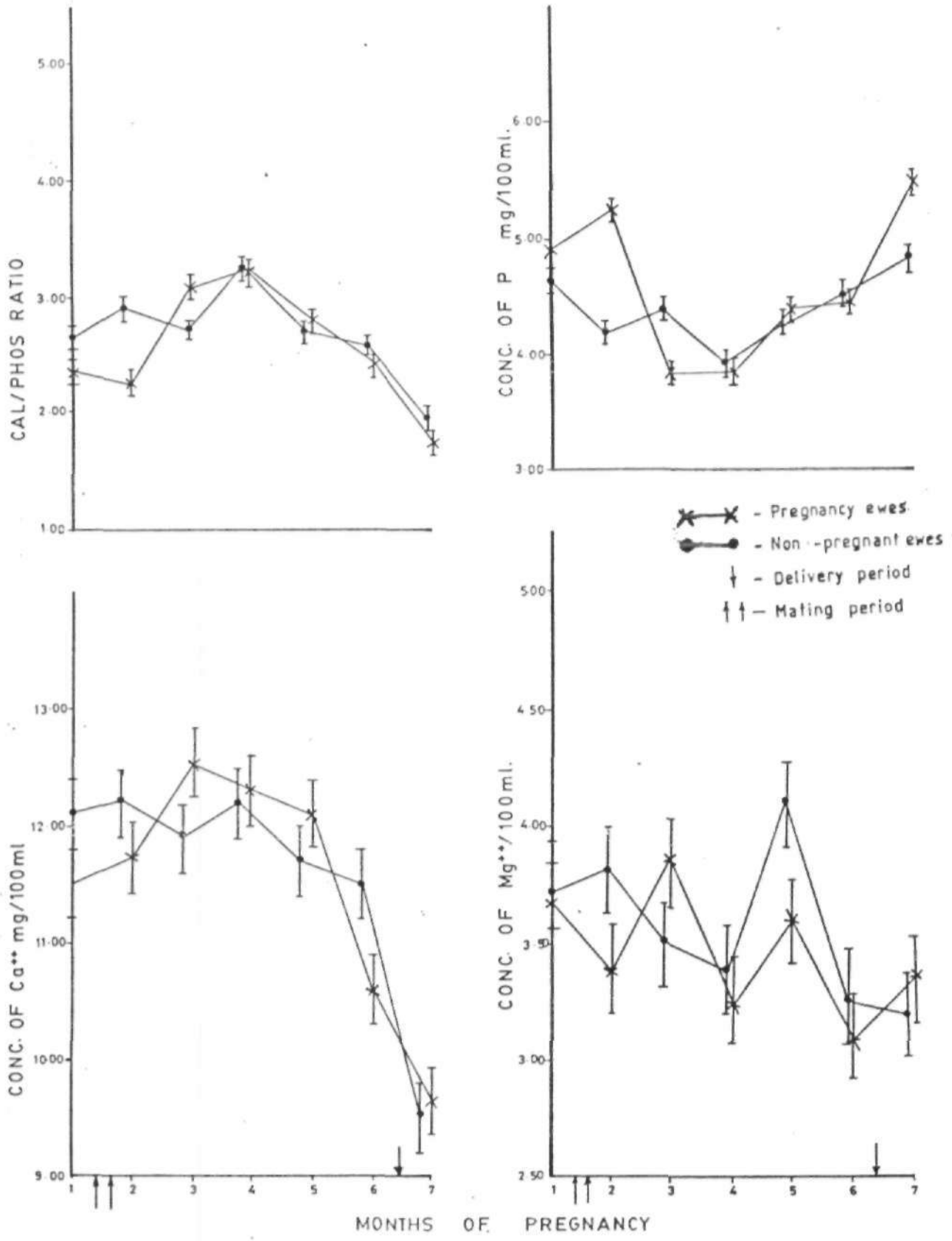


Fig 4.3 Monthly mean concentration of serum calcium, phosphorus, magnesium and calcium/phosphorus ratio in pregnant ewes, and female controls during the gestation period.

4.3.4 The calcium/phosphorus ratio

The ratio in sheep showed general values above 2.00, the pregnant ewes had lower ratios than the female controls. The mean for the pregnant ewes, female controls were 2.58 ± 0.11 and 2.68 ± 0.11 respectively while the male controls was 2.41 ± 0.11. There is a periodic significance ($p < 0.01$) but none between the pregnant ewes and the female controls. It would appear that there was no evidence of interactions between the state of the animals and the periods of samplings.

The general pattern over the periods showed a gradual increase of the ratio with a drop in the sixth month of pregnancy (Figure 4.2b)

4.4 Trace elements - Copper, cobalt, manganese and zinc

The monthly mean values for serum copper, cobalt, manganese and zinc are presented in Figure 4.4, Appendix A3.5 and the individual monthly values in Appendix A3 and A3.1 to A3.4.

4.4.1 Copper

It was observed that although there were generally lower values in pregnant ewes with the exception of the fourth and fifth month of pregnancies, there was no significant difference obtained. This could indicate that pregnancies in ewes had lowing effect on serum copper levels. The periods of samplings from the animals had no effect on the performances of the pregnant ewes and the female control.

The means for pregnant ewes were between 1.25 ± 0.14 µg/ml and 1.42 ± 0.13, for female controls between 1.28 ± o.13 and 1.14 ± 0.09 ug/ml. These ranges compared very

favourably with those of Wiener *et al*., (1969) and the higher values obtained for the fourth and fifth months agreed with those from Anastasiadis *et al*., (1981).

4.4.2 Cobalt

The levels of cobalt in pregnant ewes and female controls showed neither significant differences nor periodic differences; although generally lower values were observed in pregnant ewes. It appeared that pregnancies had lowering effect on cobalt levels in the plasma of the sheep.

The monthly values for pregnant ewes and female controls were between 0.86 ± 0.23 µg/ml and 1.16 ± 0.45 µg/ml 0.97 ± 0.21 µg/ml and 1.30 ± 0.21 µg/ml respectively. The variable values of cobalts in this project conformed with those reported by Underwood (1977).

4.4.3 Manganese

It would appear that manganese levels were higher in pregnant ewes than the female controls. The means in pregnant ewes and female controls were 0.615 ± 0.05 µg/ml and 0.522 ± 0.05 µg/ml respectively. These results suggested that pregnancies had an increasing effects on manganese levels (see Figure 4.4). The levels of manganese were found to drop in the fourth and sixth month of pregnancies. Despite these drops, there was no significant differences between the pregnant ewes and female controls. Significant difference ($p < 0.01$) was noticed between periods of samplings.

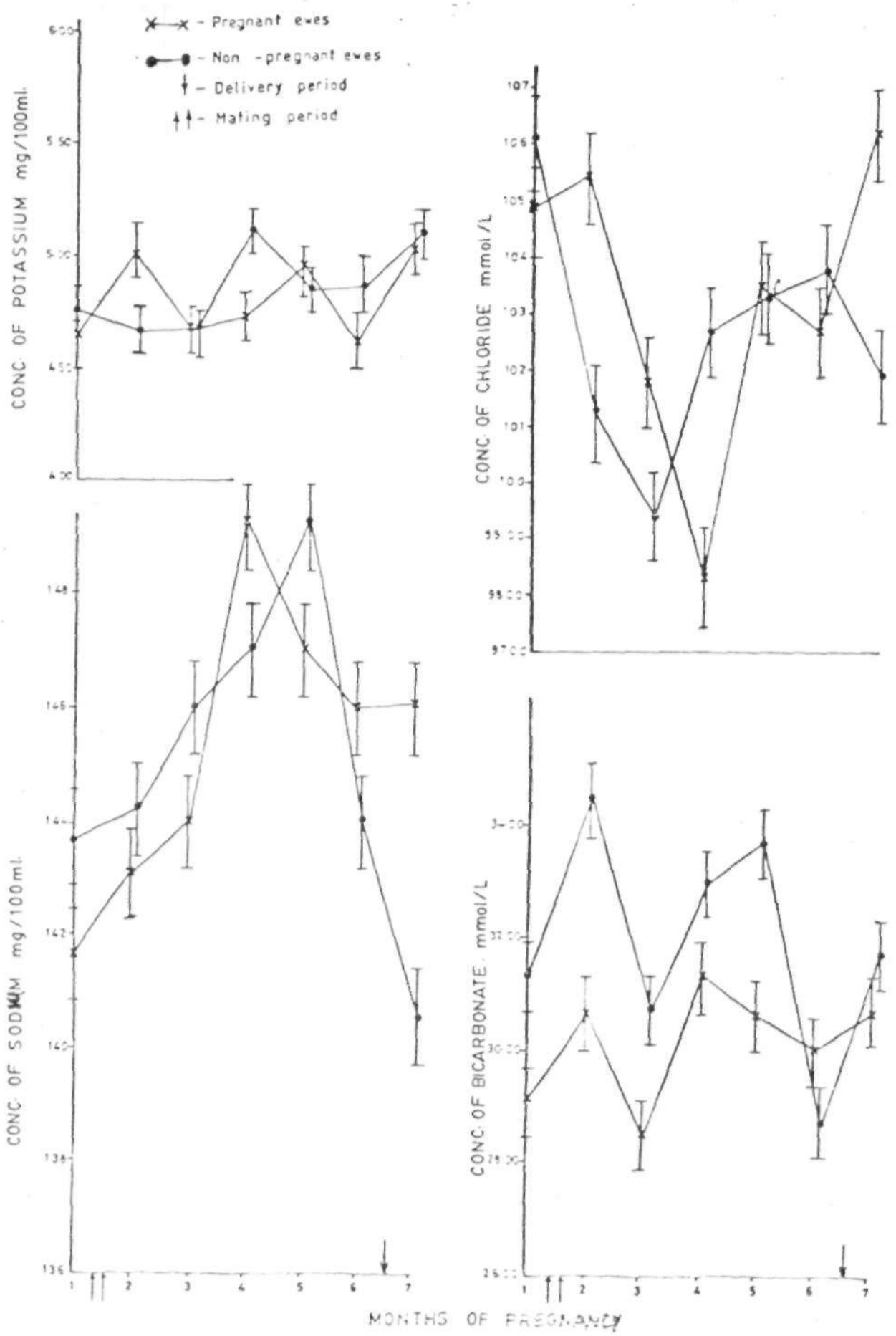


Fig 4.2 Monthly mean concentration of serum K^+, Na^+, Cl^-, HCO_3^- in pregnant ewes, and female controls during the gestation period

4.4.4 Zinc

The zinc values in the pregnant ewes were higher than those of the female control except for the fourth and fifth month of pregnancy. The mean values for the ewes were between 0.70 ± 0.17 µg/ml and 2.18 ± 0.28 while the female controls between 0.57 ± 0.16 µg/ml and 2.65 ± 0.46 µg/ml, showing the means of 1.43 ± 0.10 µg/ml amd 1.28 ± 0.1 µg/ml respectively.

Despite these observed increases in zinc levels throughout the periods of pregnancies, there was no significant differences between them. This would suggest that there might be increasing effects on the levels of zinc during pregnancies. There was significant difference ($P < 0.01$) between the periods of samplings and a possible interactions between the states of the sheep and the sampling periods (see Figure 4.4). The values obtained in this project are within the normal values quoted by Hills *et al.*, (1967) but disagreed with those quoted by Halsted *et al.*, (1968). There was a gradual increase of plasma zinc levels as the ages of pregnancies advanced. The same was not found in female and male controls.

4.5 Total protein, albumin, globulin and albumin/globulin ratio

The monthly mean values of serum total protein, albumin, globulin and albumin/globulin ratio are presented in Figure 4.5, Appendix 4.5 and the individual values in Appendix A4 and A4.1 to A4.4.

4.5.1 Total Protein

The monthly mean values of serum total protein variation was very narrow ranging between 6.03 ± 0.42 gm/100ml and 6.43 ± 0.45 gm/100ml in pregnant ewes, the female controls between 5.89 ± 0.13 gm/100ml and 6.78 ± 0.33 gm/100ml while the male controls were between 6.12 ± 0.71 gm/100ml and 6.74 ± 0.65 gm/100ml. The pregnant ewes and female controls had mean values of 6.18 ± 0.10 gm/100ml and 6.31 ± 0.10 gm/100ml respectively. These gave significant differences ($P < 0.01$) between them while there was no significance between the sampling periods that would not affect the total protein from each group of animals.

There was a general decrease in the total protein concentration in the pregnant ewes throughout the gestation period suggesting the evidence that the protein values in these ewes were extremely on severe demand during the development of the foetus. This agrees with the findings of Larson and Hays (1958). The male control showed higher mean values than the pregnant ewes and female controls.

4.5.2 Albumin

The monthly mean values of serum albumin in pregnant ewes were lower than the female and male controls especially in the first half of pregnancies, the lowest values being in the first and second months of pregnancy and this returns to normalcy at term conformed with the work of Dunlop and Dickson (1955).

The values for pregnant ewes were between 3.87 ± 0.33 gm/100ml and 4.12 ± 0.14 gm/100ml, the female control between 4.01 ± 0.13 gm/100ml and 4.24 ± 0.07 gm while the male control between 3.96 ± 0.23 gm/100ml and 4.42 ± 0.10 gm/100ml

The albumin values for each group of animals were not affected by the periods of samplings and there is no significant differences between them.

4.5.3 Globulin

The pregnant ewes showed lower mean values for globulin concentration for the third to sixth month of pregnancy. The mean values were between 2.19 ± 0.30g m/100ml and 2.39 ± 0.39 gm/100ml, the female control between 1.70 ± 0.39 gm/100ml and 2.59 ± 0.62 gm/100ml while the male control between 2.08 ± 0.95 gm/100ml and 2.77 ± 0.33 gm/100ml. There was significant difference ($P < 0.05$) between the pregnant ewes and female controls.

It could be seen that during the later half of pregnancy period, the concentration of globulin decreased compared with the female control. This agrees with Dimopoullous (1970), but the marked decrease of the globulin concentrations were not achieved in this project. This might be due to nutritional stress the sheep were naturally subjected to during the period of pregnancy.

4.5.4 The Albumin/Globulin Ratio

The Albumin/globulin (A/G) ratios in pregnant ewes were found below 2.00 with their monthly mean values ranging between 1.66 ± 0.26 and 1.89 ± 0.18 while in female controls, the A/G ratio were above 2.00 for the first to fourth month of pregnancy with the mean values between 1.62 ± 0.44 and 2.59 ± 0.81. There was significant difference ($P < 0.05$) between the periods of samplings but no significance between the pregnant ewes and the female control. The ratios showed gradual fall from the third to the fifth month of pregnancy

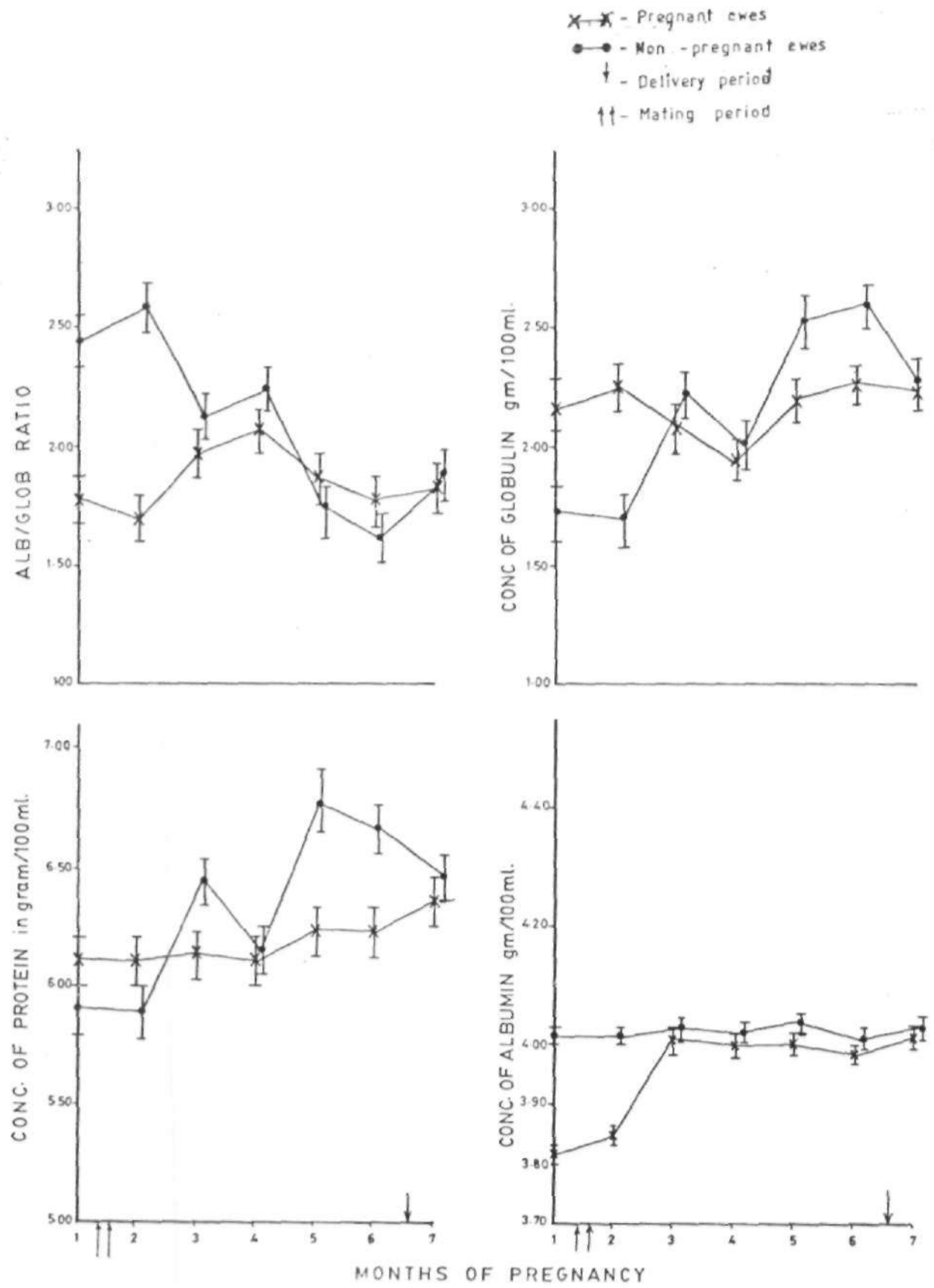


Fig 4.5 Monthly mean concentration of serum total protein, albumin, globulin and albumin/globulin ratio in pregnant ewes, and female controls during th gestation period.

before a rise was obtained in the sixth month (Fig 4.4).

4.6 Alkaline Phosphatases

The monthly mean values of serum alkaline phosphatases are presented in Figure 4.6, Appendix A5.2 while the individual values in Appendix A5 and A5.1.

Lower mean values were obtained in pregnant ewes than the female control while the values in male controls during the same period were highest. These values in ewes gradually fell as the ages of pregnancies advanced (Fig 4.6)

The lowest values were observed when the ewes lambed and this continued for at least one month after lambing. The same pattern was obtained for female and male controls, the difference being higher values in these controls (Appendix A5.2).

Kirchgessner *et al.*, (1976) showed that the activity of alkaline phosphatases, a zinc-metalloenzyme, would be reduced rapidly within two to four days if there is zinc deficiency long before the symptoms appear. The results of zinc levels in this project Figure 4.6 and Table 4.6 showed a continous increase of zinc throughout pregnancy period. Therefore, the falling values of alkaline phosphatases might be due to other nutritional deficiencies especially the phosphorus deficiencies that showed lowest values in June (Fig 4.3 and Table 4.3). The protein adequacy in animals is an important factor for enzymes production. These sheep had no supplements supplied to them and the protein level in the hay diet continuously leached away with long storage as a result of presence of microflora. The same is true for other elements such as phosphorus in the diet. As the protein and phosphorus level drop the maintainance of these animals in general and enzymes (alkaline phosphatases) productions in

particular would be affected. Alkaline phosphatases in animals is continuously excreted from the body through the bile and as such should be replaced in health. The mean values for pregnant ewes were between 67.47 ± 26.41 µM/ml and 116.47 ± 41.89 µM/ml, female control between 81.68 ± 32.36 µM/ml and 125.47 ± 29.91 µM/ml. There was no significant difference between the pregnant ewes and the female controls but there was difference ($P < 0.001$) between the sampling periods. Nevertheless the pattern of each group of animals was not affected by the samping periods.These values in sheep fall within normal level (Ford, 1958). In this project, no elevated values were observed during pregnancy in contrary to the findings of Halsted *et al.*, (1968) and Mills *et al.*, (1967).

Lilian *et al.*, (1975) showed that some animals during pregnancy had iso-enzymes of alkaline phosphatases; these iso-enzymes have different mobilities and molecular weight from the normal alkaline phosphatases in the blood circulation. One of these iso-enzymes (placental iso-enzyme) is heat stable and can be separated into distinct bands by electrophoresis. In this project, some sera from pregnant ewes and female control were subjected to electrophoresis for possible separation of the iso-enzymes. There was only one band of alkaline phosphatases obtained in both the sera of the pregnant ewes and female control. This band had reducing intensity with the ages of pregnancies. The diagrammatic sketch of the stain gels is shown in Figure 3.13.1 above.

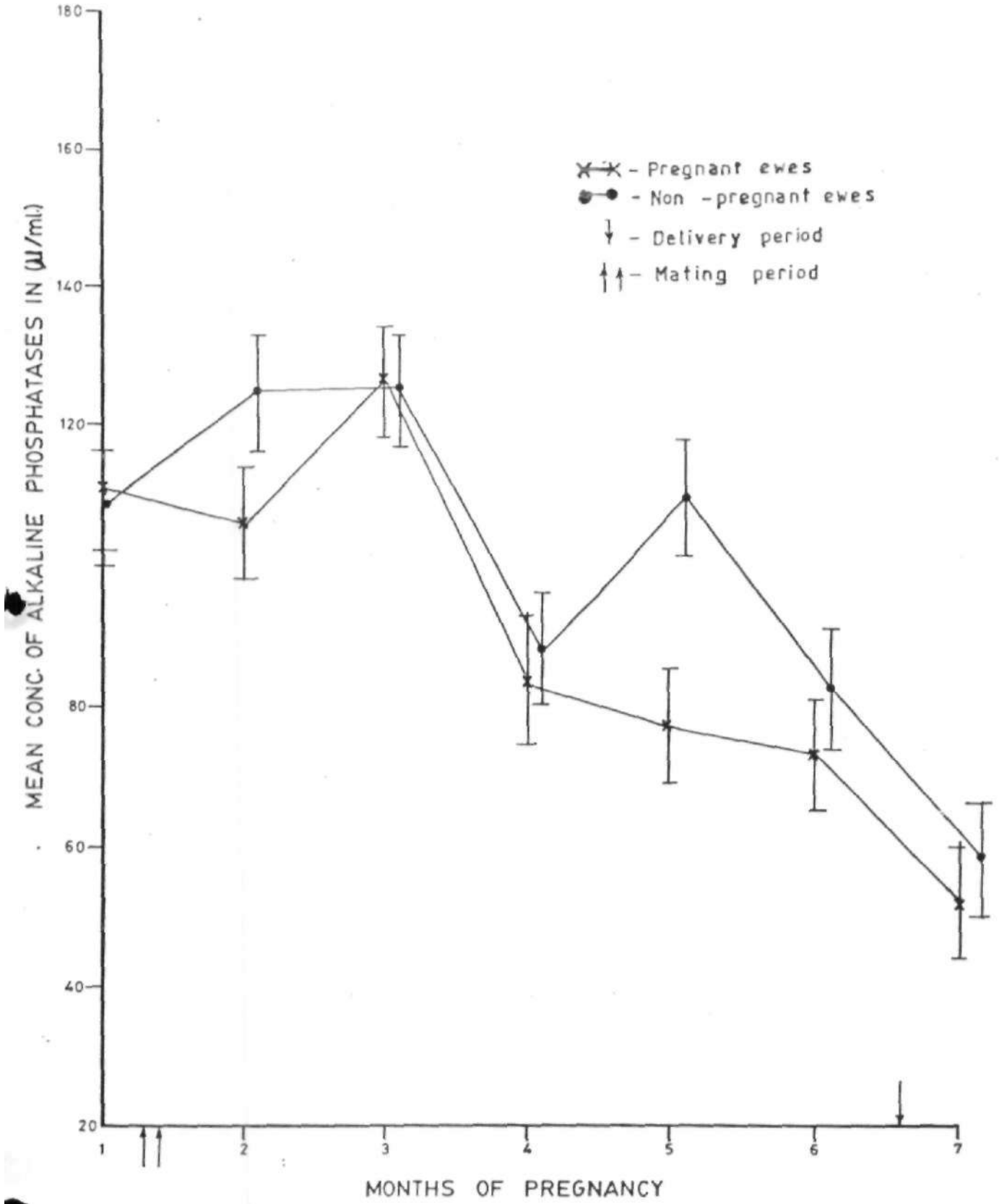


Fig 4.6 Monthly mean concentration of serum alkaline phosphatases in pregnant ewes, and female controls during gestation period.

4.7 Haemoglobin, Packed cell volume (PCV) and Mean corpuscular haemoglobin concentration (MCHC)

The monthly mean values of the blood haemoglobin (Hb), Packed cell volume (PCV) and Mean corpuscular haemoglobin concentration for the pregnant ewes, female and male controls are given in Figure 4.7 and Appendix A6.4. The individual monthly values are shown in Appendix A6 and A6.1 to A6.3.

4.7.1 Haemoglobin (Hb)

The monthly mean haemoglobin values for pregnant ewes were between 10.23 ± 0.38 gm/100ml and 11.07 ± 0.36 gm/100ml. These values were higher than those of the female control between 9.50 ± 0.41 gm/100ml and 10.80 ± 0.41 gm/100ml. There is an evidence that there was a gradual increase in the haemoglobin up to the third month of pregnancy and followed with a gradual drop in the fourth and fifth month of pregnancy. The same dropping patterns were obtained for the female control with mean values in non-pregnant controls lower than the pregnant ewes (Figure 4.7).

4.7.2 Packed cell volume (PCV)

The packed cell volume (PCV) monthly mean values showed similar histogram pattern as for those of haemoglobin (Fig. 4.7). The monthly mean values for the PCV was between 31.00 ± 2.58 and 35.86 ± 2.27 for pregnant ewes, the female control between 30.00 ± 1.41 and 34.75 ± 2.99.
These values were not significant while the periods of samplings was significant ($P < 0.01$)

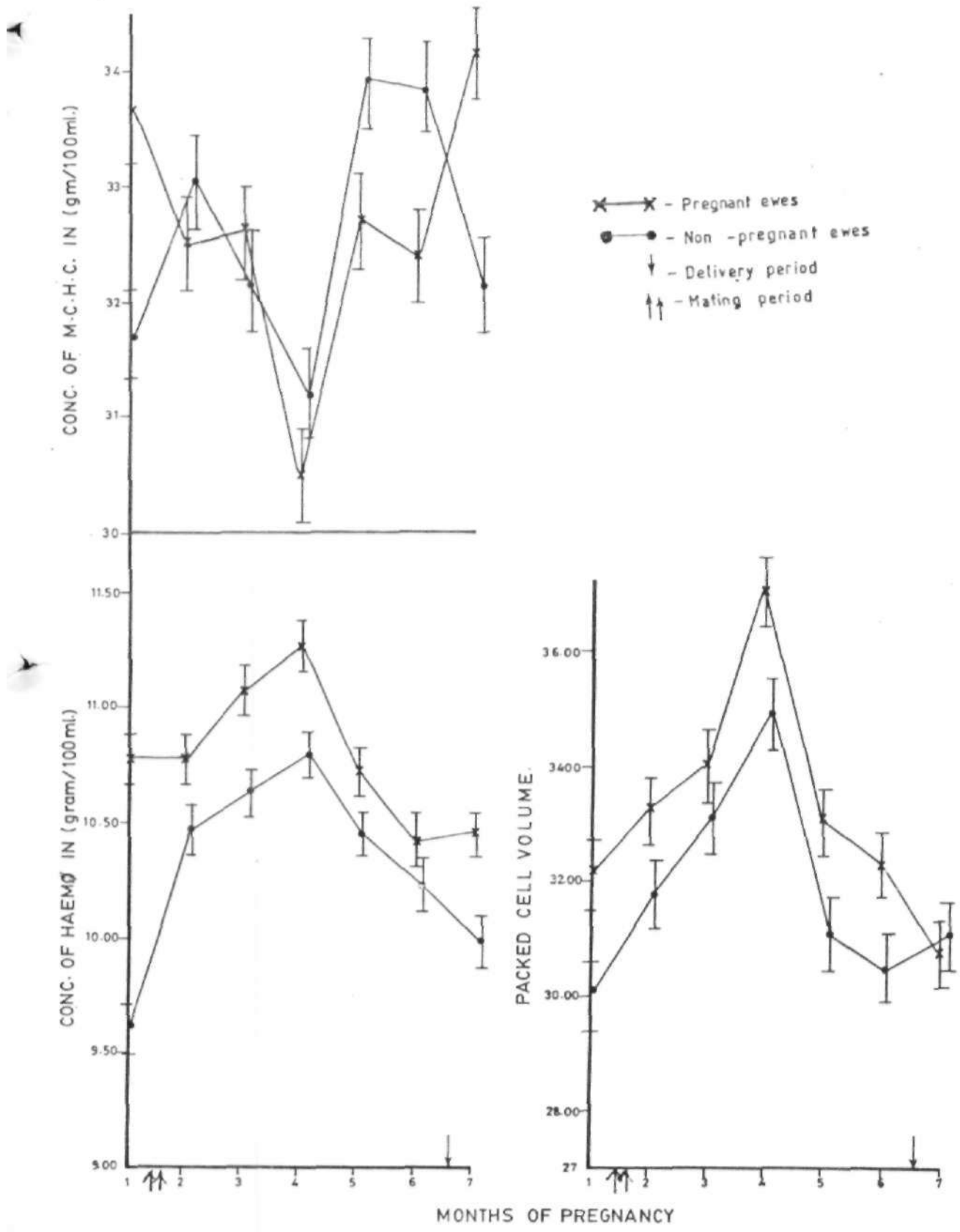


Fig.4.7 Monthly mean values of plasma packed cell, haemoglobin and mean corpuscular haemoglobin concentration in pregnant ewes, and female controls during the gestation period.

4.7.3 The mean corpuscular haemoglobin concentration (MCHC)

The MCHC for pregnant ewes showed little differences from those of the female controls. The male controls had lower values from the first to fourth month of pregnancy while higher values in the fifth and sixth month. The mean values for pregnant ewes and and female controls were 32.65 ± 1.40 and 32.55 ± 1.42 respectively and they were none significant while periods of d samplings was significant (P < 0.10)

4.8 Cholesterol and Urea

The monthly mean values of serum cholesterol and urea are presented in Appendix A7.3, Figure 4.8 and the individual values in Appendix A7 and A7.1 and A7.2.

4.8.1 Cholesterol

The cholesterol monthly mean values in pregnant ewes were generally higher than the female control. These values in pregnant ewes were between 106.03 ± 14.30 mg/100ml and 174.03 ± 32.14 mg/100ml, in female control 103.84 ± 4.09 mg/100ml and 145.71 ± 21.25 mg/100ml while the male control between 128.35 ± 18.92 mg/100ml and 146.82 ± 12.29 mg/100ml.

The mean values in pregnant ewes and female controls were 139.53 ± 5.49 mg/100ml and 125.56 ± 5.49 mg/100ml respectively. In pregnant ewes gradual increases in chlestrol levels were observed with ages of pregnancies; especially there was tendency for sharp increases every two months from 113.86 ± 15.99 mg/100ml in the first month of pregnancies to 120.70 ± 21.39 mg/100ml in the second month, from 123.77 ± 20.55 mg/100ml in the third month to 153.01 ±

34.93 mg/100ml in the fourth month; from 153.01 ± 34.93 mg/100ml in the fifth month to 174.02 ± 32.14 mg/100ml in the sixth month. This same reflection was obtained on the graph on Figure 4.8 It would be noted that this type of variations were not obtained for the female controls and male controls (see Figure 4.8 and Table 4.8).

These high results in pregnant ewes, might be due to the continuous production of some of some steriods hormones (examples progesterone and oestrogen) secreted into the blood circulations to sustain the pregnancies. The cholesterol has the same skeletal ring as most of the steriods produced by the body and as such would give the same chemical reactions with acetic anlydride/ sulphuric acid reagents used for the cholesterol estimations. There were significant differences ($P < 0.05$) between the stages of animals and the periods of samplings ($P < 0.001$)

4.8.2 Urea

The urea values of the pregnant ewes were generally lower than those from the female and male controls. These values in the ewes were between 23.16 ± 4.76mg/100ml and 49.38 ± 3.92mg/100ml, the female control between 29.11 ± 4.69mg/100ml and 58.18 ± 15.15mg/100ml while the male control was between 26.05 ± 8.86mg/100ml and 50.76 ± 15.77mg/100ml. The mean value for the pregnant ewes and the female controls were 29.34 ± 2.36 mg/100ml and 35.77 ± 2.36 mg/100ml of serum. There were significant differences ($P < 0.05$) between the stages of animal and the periods of samplings ($P < 0.01$).

The results obtained in this project conform with those of Benamilih (1985) who explained that sheep on a constant

diet would increase their turnover of urea during pregnancy and lactation while their urine urea excretion reduce when compared to the non-pregnant and non-lactaling state. He further stated that the mechanism of increased urea recycling is not well understood but argued that there is increased re-absorption of urea during pregnancy and lactation by the tubules reabsorption. Also the high demand for protein during this period might encourage the recycling of the urea and/or there might be need for the increase of biosynthesis of protein tus the reduction of deamination to produce urea. This also is an area of close investigation.

It was also realised that the pregnant ewes, the female and male controls had a noticeable increase of urea in the second month of pregnancy. No explanation could be given for this increase but it could be due to nutritional and/or seasonal effect. It could not have been due to a break down in the recycling of the urea by the pregnant ewes because the controls gave the same high results for that month.

A further studies would be necessary to find out why the sudden rise in the blood urea at this period.

There might be need for the increase of biosynthesis of protein thus the reduction of deamination to produce urea by the liver.

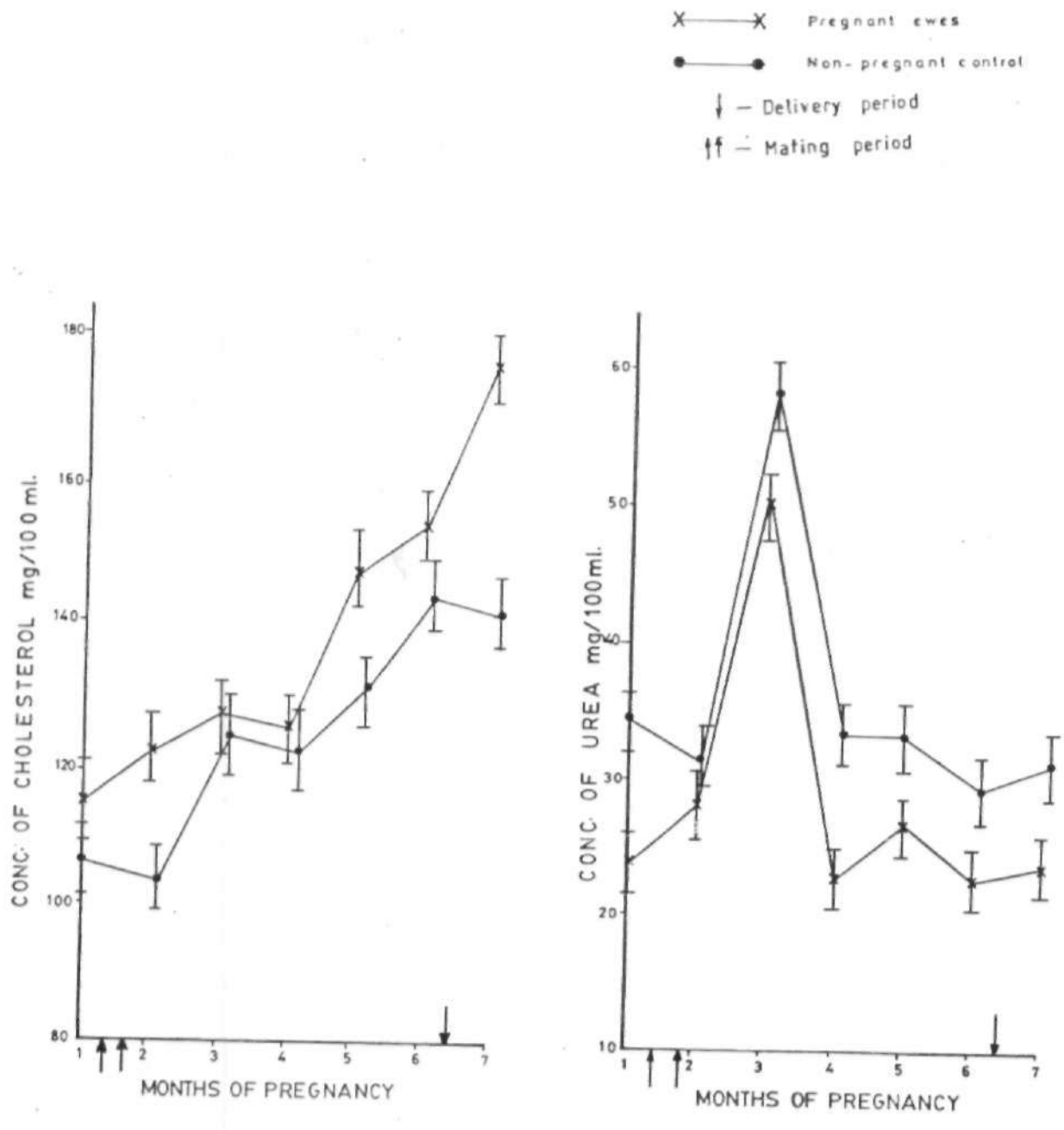


Fig.4.8 Monthly mean concentration of serum urea and cholesterol in pregnant ewes, and female controls during the gestation period

CHAPTER FIVE

CONCLUSION

The sodium mean values in pregnant ewes were slightly lower than those from non-pregnant controls. The potassium values during the same period were lower except for thee first and second month of pregnancy when the results were inconsistent.

The results for magnesium did not show much variations while bicarbonate mean values were lower than the female controls except for the fifth month of pregnancy.

The findings for calcium and phosphorus levels in pregnant ewes showed some noticeable differences from those of non-pregnant controls. The calcium showed lower levels in the pregnant females than the non-pregnant controls for the first and last month of pregnancy (Fig 4.3). The phosphorus mean values were higher during the first month of pregnancy, dropped sharply in the second month and remained lower than the non-pregnant controls throughout the gestation period. The calcium/phosphorus ratio in pregnant ewes above 3.25 the second and third month of pregnancy but below 3.25 during the same period for non-pregnant controls.

Copper and cobalt in pregnancy had lower mean values than the non-pregnant controls. Cobalt particularly, had values below 1.00 mg/ml in pregnant ewes whilee the values in the non-pregnant controls were above 1.00 mg/ml during the same period.

The zinc mean values in pregnancy were higher than the non-pregnant controls throughout the gestation period except

for the fourth month of pregnancy. The manganese levels were also higher than the non-pregnant controls throughout the same period except for the fourth month. It would therefore appear that in pregnancy, the zinc and manganese blood levels in Yankasa sheep are enhanced.

Cholesterol has a very wide range of normal values; the monthly means between the second and third, the fourth and fifth month of pregnancy seemed to have a plateua as shown on the graphs in Figure 4.4. It might be necessary to carry out further studies on this as it might be of significant values.

The mean urea values during the gestation period in pregnancy were depressed. It was also observed that during the second month of pregnancy there was a sharp rise in the urea levels; despite this increase, lower mean values were still recorded for the pregnant ewes. No explanation could be rendered other than the drastic changes in the climate from harmattan, a cold period; to a warmer temperature. It might be necessary to carry out further studies on these changes.

The mean values of total protein and albumin in pregnant ewes were lower than the non-pregnant controls throughout the gestation period but the globin mean values were only lower from the second to the fifth month; this is as a result of colostrum formation in preparation for the lambing. The albumin/globulin ration in pregnant ewes was below 2 while the non-pregnant controls had variable results during the same period.

Many workers (Hundricks *et al*., 1970; Lilian *et al*., 1972; Kaantee, 1974; Agness, 1975; Parkes and Black-more, 1977) studied the changes of alkaline phosphatases and

placental iso-enzyme of alkaline phosphatases in different animals during pregnancy and variable findings were obtained. In this project, it would appear that the values of alkaline phosphatases were on a continual decline during the gestation period; the lowest being the month after lambing.

The placental iso-enzyme of alkaline phosphatase did not show any separation after electrophoresis and staining. It would appear that placental isoenzyme did not filter through placental cell membranes into the maternal circulation. It would be very necessary to carry our further studies to confirm the absence of placental isoenzyme in the maternal circulations.

The ratio of albumin/phosphorus mean values of each monthly results during the gestation period were used to compare the pregnant ewes with the non-pregnant controls. The results are shown in Table 5a. It appears that if the ratio equal to or below 80 could indicate early pregnancy; the ratio between 100 and 110 indicates pregnancies between three and four months old, while ratio between 85 to 95 are for pregnancies at term and non-pregnant ewes.

An extensive field work must be carried out on the levels of phosphorus in this country. These levels vary in plants, water and soil from place to place (Kapu, 1975); such varied levels would definitely reflect on their levels in the sera. If these findings are proved conclusive both in experimental animals and the field survey, the determination of albumin/phosphorus ratio can be used as a measure for determining pregnancy and ages of pregnancy in ewes and thus reeduce the colossal losses of foetus during slaughter. This

would consequently increase the population of sheep in meat industries and improve the economy of the country.

The estimation of albumin and phosphorus are routinely carried out in clinical laboratories and such laboratories could easily be established and attached to abattoirs. The albumin/phosphorus ratio can then replace pregnancies determinations by Cunningham *et al.*, 1983; Merilan, 1983; Pierson and Ginther, 1984 and Langford *et al.*, 1984) which are very expensive and are not available in routine laboratory set up.

The overall conclusion of the results are summarised as in Table 5a and 5b.

Table 5a. The monthly mean ratio of albumin/phosphorus of pregnant ewes and female controls

No	Dec	Jan	Feb	Mar	Apr	May	Jun
1	66.70	106.58	126.56	95.35	95.35	67.86	58.86
2	87.50	95.43	97.67	100.00	90.87	99.50	85.00
3	68.52	61.40	111.11	97.59	79.17	94.85	70.83
4	88.37	74.00	95.24	101.00	102.05	100.00	85.71
Mean	76.10	76.12	109.85	104.37	95.85	90.60	81.61
5	97.62	118.82	102.63	140.65	108.93	106.33	98.41
6	88.54	92.22	90.63	111.76	103.17	92.56	84.00
7	86.17	95.35	87.61	95.56	89.58	79.59	75.00
8	89.58	94.57	102.22	87.17	91.06	82.29	90.00
Mean	90.48	100.24	95.77	108.79	98.19	90.19	86.85

S.E between two means for control and treated animals = 2.541

Conclusion:

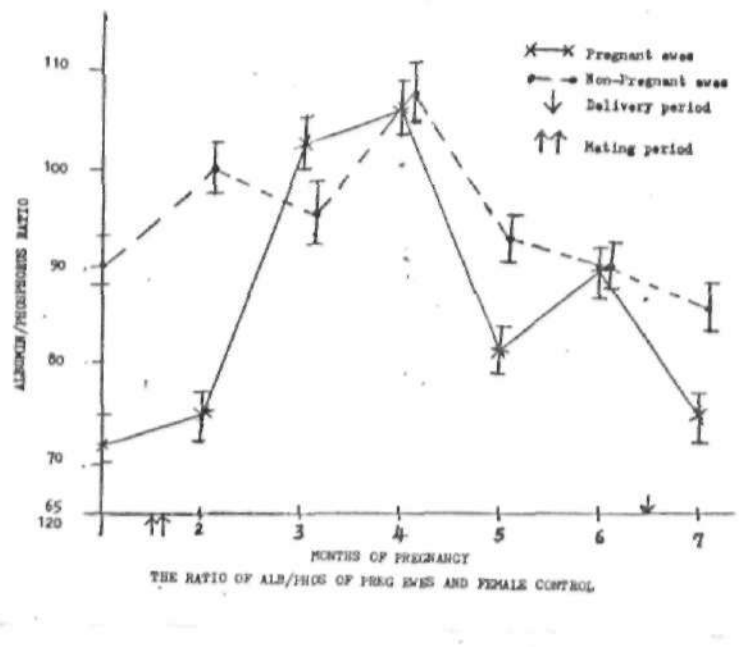
A. In the first month of pregnancy, the monthly mean ratio was below 80 that is 76.10 to 676.12.

B. In the second and month after pregnancy the ratio lied between 104.37 and 109.85.

C. In the 4th and 5th month after pregnancies the ratio was between 90.60 and 95.85.

D. In the female control there was no such pattern as the pregnant ewes bu they have varied monthly mean ratio between 90.19 and 108.79.

E. There is significant difference $P < 0.01$ between the controls and pregnant ewes; also between the periods of their samplings.



THE RATIO OF ALB/PHOS OF PREG EWES AND FEMALE CONTROL

Table 9. Comparing the results of all parameters between the pregnant ewes and the female control

Electrolytes	Dec (84)	JAN	FEB	MAR	APRIL	MAY
Na+	+NS	+NS	+NS	+NS	+NS	+NS
K+	↓NS	↓↑NS	↓↑NS	↓NS	↓NS	↓NS
Cl'	≠NS	≠NS	≠NS	≠NS	≠NS	≠NS
HCO3'	≠NS	≠S	≠NS	≠NS	≠NS	≠NS
Ca++	↓NS	↓NS	↓↑NS	↓NS	↓NS	↓NS
Mg++	≠NS	≠NS	≠NS	≠NS	≠NS	≠NS
P+++	↑NS	↑S	↓S	↓NS	↓NS	↓NS
Ca/P ratio						
Trace elements						
Cu++	↓NS	↓NS	↓NS	↓NS	↓NS	↓NS
Co++	↓NS	↓NS	↓NS	↓NS	↓NS	↓NS
Mn++	↓NS	↑NS	↑NS	↑NS	↓NS	↑NS
Zn++	↑NS	↑S	↑S	↑NS	↑S	↑NS
Cholesterol	↑NS	↑NS	↑NS	↑NS	↑NS	↑NS
Urea	↓S	↓NS	↓S	↓S	↓NS	↓NS
Protein and Enzymes						
T/Pr	↓NS	↓NS	↓NS	↓NS	↓NS	↓NS
Albumin	↓NS	↓NS	↓NS	↑NS	↑NS	↑NS
Globulin	↑NS	↑S	↑NS	↓NS	↓NS	↓NS
ALP	↓NS	↓NS	↓NS	↓NS	↓NS	↓NS
A/G ratio						
Hb	↑S	↑NS	↑NS	↑NS	↓↑NS	↓NS
PCV	↑NS	↑NS	↑NS	↑NS	↑NS	↓NS
MCHC	↑NS	↓NS	↓↑NS	↓NS	↓NS	↓NS

Keys:
+ = Slightly lower than
↑ = Higher than
↓ = lower result
≠ = Indifferent result
↓↑ = doubtful - lower/higher

Table 5.b Comparing the results of all parameters between the pregnant ewes and the female control

Electrolytes	Dec (84)	JAN	FEB	MAR	APRIL	MAY
Na+	+NS	+NS	+NS	+NS	+NS	+NS
K+	°NS	**NS	**NS	°NS	°NS	°NS
Cl'	*NS	*NS	*NS	*NS	*NS	*NS
HCO3'	*NS	*NS	*NS	*NS	*NS	*NS
Ca++	°NS	°NS	**NS	°NS	°NS	°NS
Mg++	*NS	*NS	*NS	*NS	*NS	*NS
P+++	++NS	++NS	*NS	*NS	*NS	*NS
Ca/P ratio						
Trace elements						
Cu++	°NS	°NS	°NS	°NS	°NS	°NS
Co++	°NS	°NS	°NS	°NS	°NS	°NS
Mn++	°NS	++NS	++NS	++NS	°NS	++NS
Zn++	++NS	++NS	++NS	++NS	++NS	++NS
Cholesterol	++NS	++NS	++NS	++NS	++NS	++NS
Urea	°NS	°NS	°NS	°NS	°NS	°NS
Protein and Enzymes						
T/Pr	°NS	°NS	°NS	°NS	°NS	°NS
Albumin	++NS	++NS	++NS	++NS	++NS	++NS
Globulin	++NS	++NS	++NS	°NS	°NS	°NS
ALP	°NS	°NS	°NS	°NS	°NS	°NS
A/G ratio						
Hb	++NS	++NS	++NS	++NS	++NS	++NS
PCV	++NS	++NS	++NS	++NS	++NS	++NS
MCHC	++NS	°NS	**NS	°NS	°NS	°NS

Keys:
+ = Slightly lower than
++ = Higher than
° = lower result
* = Indifferent result
** = doubtful - lower/higher

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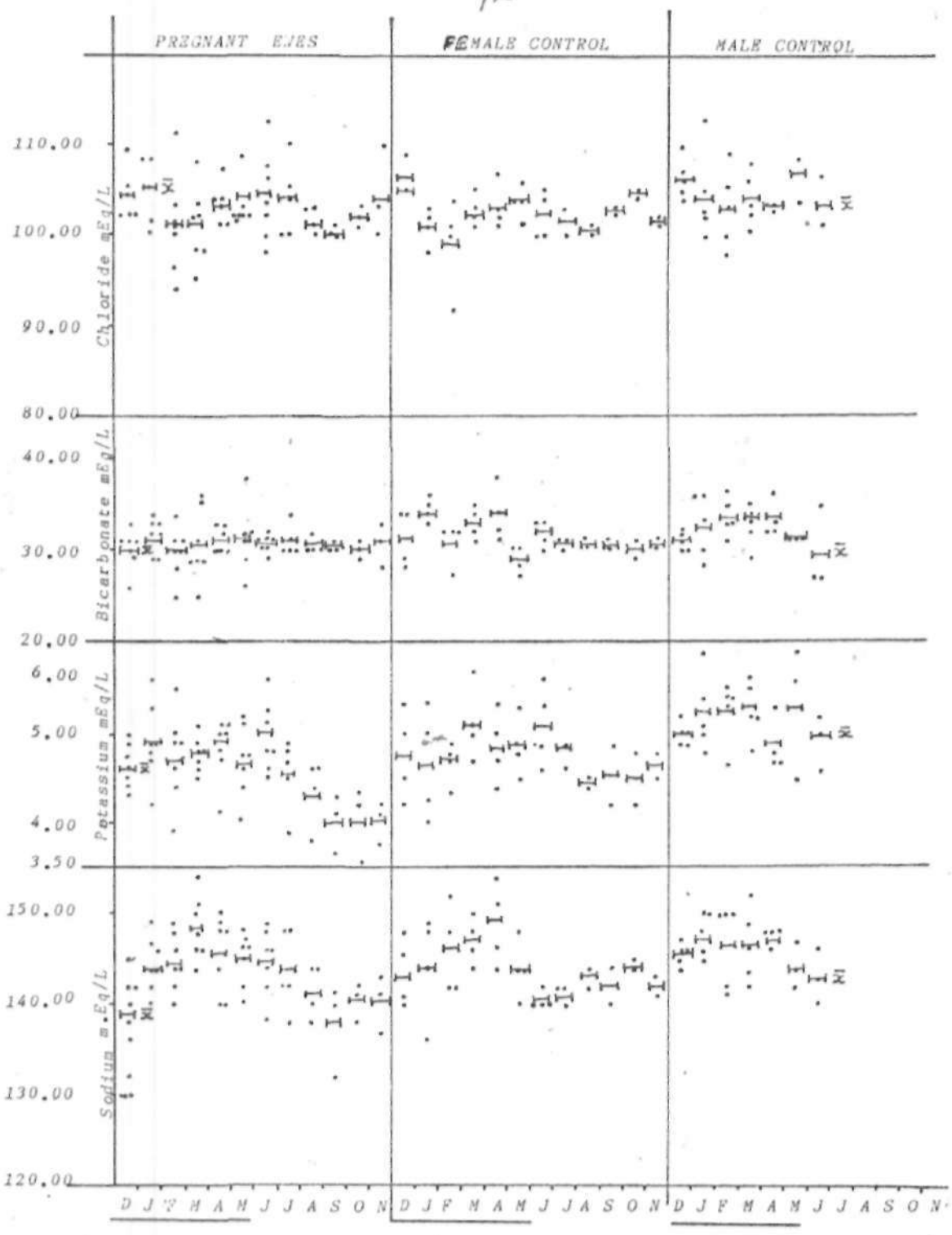
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APPENDIX



A1 Monthly concentration of serum Sodium, potassium Chloride and bicarbonate in pregnant ewes, male and female control.

The solid horizontal lines mark the mean $\bar{x}$ of each monthly group.

A 1.1. Analysis of potasium in mMol/litre from pregnant ewes, female and male controls

Sample No.	Dec 1984	Jan 1985	Feb 1985	Mar 1985	Apr 1985	May 1985	June 1985
			Pregnant ewes				
1	4.90	5.60	4.40	4.50	5.30	4.70	4.60
2	4.40	4.70	3.90	4.70	4.70	4.40	5.10
3	4.50	4.90	5.50	4.60	4.80	4.60	4.80
4	4.60	4.90	4.90	5.10	5.00	4.70	5.60
Range	4.40 to 4.90	4.70 to 5.60	3.90 to 5.50	4.50 to 5.10	4.70 to 5.30	4.40 to 4.70	4.60 to 5.60
Mean	4.60	5.03	4.68	4.73	4.95	4.60	5.03
			Female control				
8	4.50	4.30	4.70	4.70	4.40	4.50	4.60
9	5.00	4.00	4.30	5.10	5.30	5.30	5.30
10	5.30	5.30	4.90	5.00	5.00	4.80	4.90
11	4.20	5.00	4.80	5.70	4.70	4.90	5.60
Range	4.20 to 5.30	4.00 to 5.30	4.30 to 4.90	4.70 to 5.70	4.40 to 5.30	4.50 to 5.30	4.60 to 5.60
Mean	4.75	4.65	4.68	5.13	4.85	4.88	5.10
			Male control				
12	5.00	4.80	5.30	5.20	5.30	5.60	4.80
14	5.00	5.10	5.40	4.80	4.70	4.50	5.20
15	5.20	5.40	5.50	5.50	4.70	5.90	5.00
16	4.90	5.00	4.60	5.60	4.80	5.33	5.00
Range	4.90 to 5.20	4.80 to 5.40	4.60 to 5.50	4.80 to 5.60	4.70 to 5.30	4.50 to 5.90	4.80 to 5.20
Mean	5.03	5.08	5.20	5.28	4.88	5.33	5.00

S.E for the specimens = 0.058

A 1.2. Analysis of sodium in mMol/litre from pregnant ewes, female and male controls

Sample No.	Dec 1984	Jan 1985	Feb 1985	Mar 1985	Apr 1985	May 1985	June 1985
				Pregnant ewes			
1	142.00	140.00	144.00	146.00	150.00	145.00	146.00
2	142.00	142.00	142.00	146.00	144.00	146.00	148.56
3	145.00	146.50	146.00	151.00	148.00	146.00	146.00
4	138.00	144.00	144.00	154.00	148.00	147.00	144.00
Range	138.00 to 145.00	140.00 to 146.50	142.00 to 146.00	146.00 to 154.00	144.00 to 150.00	145.00 to 147.00	144.00 to 148.56
Mean	141.75	143.13	144.00	149.25	147.50	146.00	146.14
				Female control			
8	141.00	144.00	142.00	144.00	151.00	148.00	142.00
9	140.00	136.00	142.00	146.00	154.00	140.00	140.00
10	148.00	148.00	148.00	150.00	146.00	144.00	140.00
11	146.00	149.00	152.00	148.00	144.00	144.00	140.00
Range	140.00 to 148.00	136.00 to 149.00	142.00 to 152.00	144.00 to 150.00	144.00 to 156.00	140.00 to 148.00	140.00 to 142.00
Mean	143.75	144.25	146.00	147.00	149.25	144.00	140.50
				Male control			
12	144.00	146.00	150.00	149.00	146.00	147.00	143.00
14	145.00	150.00	142.00	142.00	148.00	142.00	146.00
15	147.00	148.00	150.00	144.00	148.00	144.00	140.00
16	146.00	145.00	141.00	146.00	148.00	144.2	145.20
Range	144.00 to 147.00	145.00 to 150.00	141.00 to 150.00	142.00 to 149.00	146.00 to 148.00	142.00 to 147.00	140.00 to 146.00
Mean	145.50	147.25	145.75	145.25	147.50	144.33	143.00

S.E. for the specimens = 0.474

A 1.3. Analysis of chloride in mMol/litre from pregnant ewes, female and male controls

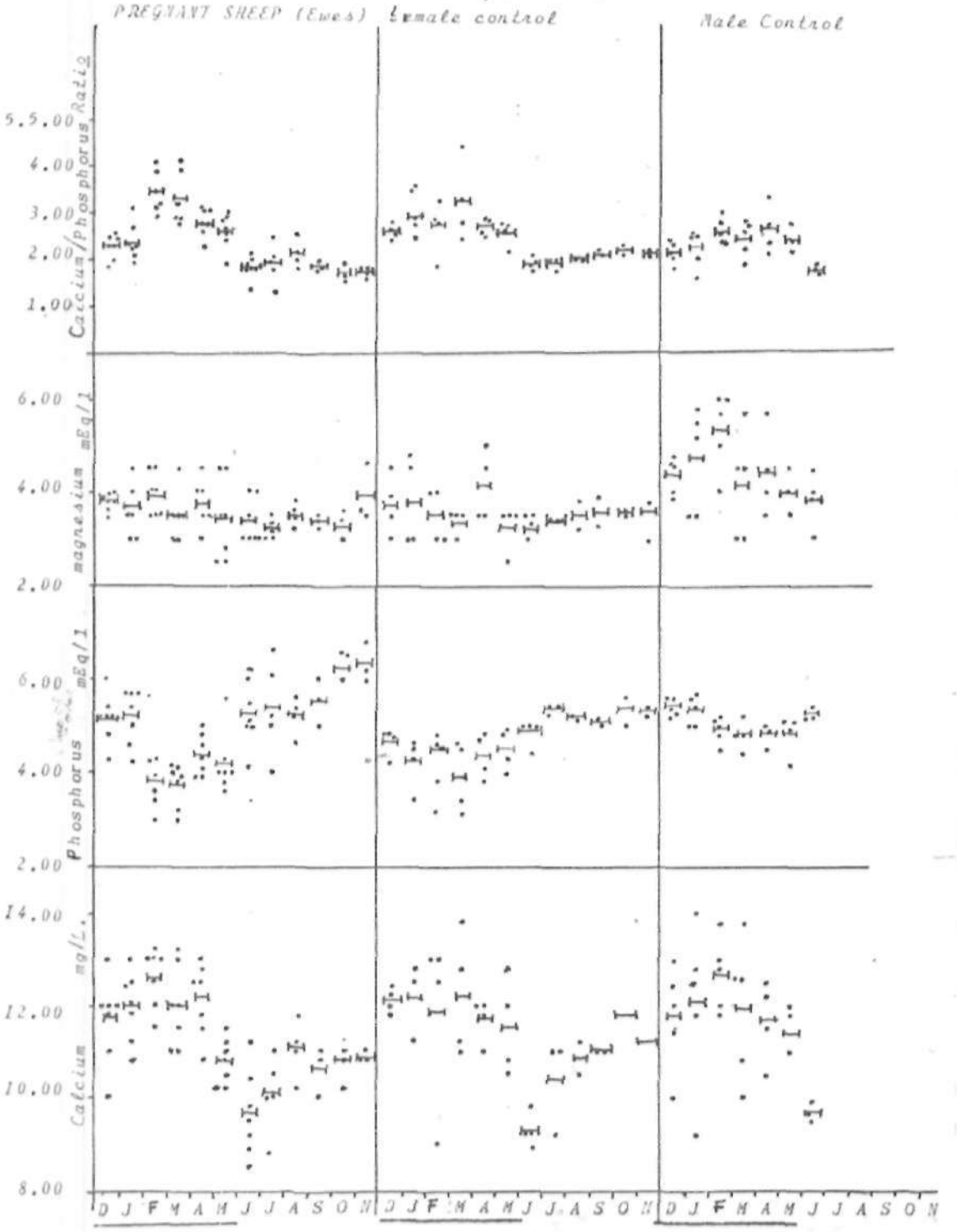
Sample No.	Dec 1984	Jan 1985	Feb 1985	Mar 1985	Apr 1985	May 1985	June 1985
				Pregnant ewes			
1	109.00	108.33	94.12	95.00	106.67	102.52	112.61
2	104.00	105.00	100.00	98.33	103.33	102.52	107.16
3	105.00	108.33	112.00	101.67	103.33	102.52	105.88
4	101.67	100.00	101.00	98.33	100.84	103.52	99.16
Range	101.67 to 109.00	100.00 to 108.33	94.12 to 112.00	95.00 to 101.67	100.84 to 106.67	102.52 to 103.51	99.16 to 112.61
Mean	104.92	105.42	101.78	98.33	103.54	102.77	106.20
				Female control			
8	105.00	101.67	92.44	100.84	103.30	100.84	105.00
9	109.17	103.33	104.20	105.00	106.67	105.89	99.16
10	105.00	98.33	100.00	101.67	100.84	104.20	99.16
11	105.00	101.67	100.84	103.33	102.50	104.20	104.20
Range	105.00 to 109.17	98.33 to 103.33	92.44 to 104.20	100.84 to 105.00	100.84 to 106.67	100.84 to 105.89	99.16 to 105.00
Mean	106.04	101.25	99.37	102.71	103.33	103.78	101.88
				Male control			
12	105.00	101.67	103.48	105.83	103.33	105.00	104.84
14	107.00	102.50	97.48	100.00	103.33	103.36	100.84
15	104.00	100.33	105.44	108.33	102.50	109.24	105.88
16	106.00	105.00	99.16	102.50	103.33	105.87	103.85
Range	104.00 to 107.00	100.33 to 105.00	96.16 to 105.44	100.00 to 108.33	102.50 to 103.33	103.36 to 109.24	100.84 to 105.88
Mean	105.50	102.38	101.99	104.17	103.12	105.87	103.85

S.E For the specimens = 0.509

A 1.4. Analysis of bicarbonate in mMol/litre from pregnant ewes, female and male controls

Sample No.	Dec 1984	Jan 1985	Feb 1985	Mar 1985	Apr 1985	May 1985	June 1985
				Pregnant ewes			
1	26.00	29.00	25.00	25.00	30.00	26.00	30.00
2	30.00	33.00	30.00	29.00	30.00	32.00	32.00
3	29.60	29.00	28.00	35.50	30.00	31.00	30.00
4	31.00	32.00	31.00	36.00	33.00	31.00	31.00
Range	26.00 to 31.00	29.00 to 33.00	25.00 to 31.00	25.00 to 36.00	30.00 to 33.00	26.00 to 32.00	30.00 to 32.00
Mean	29.15	30.75	28.50	31.38	30.75	30.00	30.75
				Female control			
8	28.00	35.00	27.00	31.00	34.00	27.00	31.00
9	29.00	34.00	32.00	32.00	38,00	30.00	33.00
10	34.00	36.00	32.00	35.00	31.00	28.00	33.00
11	34.00	33.00	32.00	34.00	32,00	30.00	30.00
Range	28.00 to 34.00	33.00 to 36.00	27.00 to 32.00	31.00 to 35.00	31.00 to 38.00	27.00 to 30.00	30.00 to 33.00
Mean	31.25	34.50	30.75	33.00	33.75	28.75	31.75
				Male control			
12	31.50	33.00	31.00	34.00	33.00	31.00	27.00
14	30.00	36.00	35.00	32.00	32.00	31.00	35.00
15	32.00	36.00	36.00	29.00	32.00	31.00	27.00
16	31.00	30.00	33.00	35.00	36.00	31.00	29.67
Range	30.00 to 32.00	30.00 to 36.00	31.00 to 36.00	29.00 to 35.00	32.00 to 36.00	31.00 to 31.00	27.00 to 35.00
Mean	31.13	33.75	33.75	32.50	33.25	31.00	29.67

S.E for the specimens = 0.347



A2 Monthly concentration of serum calcium, phosphorus, magnesium, and calcium/phosphorus ratio in pregnant ewes; male and female controls.

The solid horizontal lines mark the mean ($\bar{x}$) of each monthly groups.

A 2.1. Calcium analysis in mg/100ml from pregnant ewes, female and male controls

Sample No.	Dec (1984)	Jan '85	Feb '85	Mar '85	April '85	May '85	June '85
				Pregnant ewes			
1	13.00	12.50	13.00	13.20	13.00	10.20	8.50
2	12.00	12.40	12.50	13.00	12.00	11.50	9.20
3	10.00	10.80	11.50	12.00	12.50	10.50	11.20
4	11.00	11.20	13.00	11.00	10.80	10.20	8.90
Range	10.00 to 13.00	10.80 to 12.50	11.50 to 13.00	11.00 to 13.20	10.80 to 13.00	10.20 to 11.50	8.50 to 11.20
Mean	11.50	11.73	12.50	12.30	12.08	10.60	9.45
				Female control			
8	12.00	12.20	12.50	13.80	11.00	10.80	9.20
9	11.80	11.20	9.00	11.20	11.80	12.00	8.90
10	12.40	12.50	13.00	11.00	12.00	12.80	9.20
11	12.20	12.80	13.00	12.80	12.00	10.50	9.80
Range	11.80 to 12.40	11.20 to 12.80	9.00 to 13.00	11.00 to 13.80	11.00 to 12.00	10.80 to 12.80	8.90 to 9.80
Mean	12.10	12.18	11.88	12.20	11.70	11.53	9.28
				Male control			
12	11.40	11.80	12.80	12.60	12.20	12.00	9.50
14	10.00	9.20	11.80	12.60	12.50	11.80	9.60
15	12.40	12.50	12.00	10.80	11.50	11.00	9.90
16	13.00	14.00	15.80	13.80	10.50	11.60	9.67
Range	10.00 to 13.00	9.20 to 14.00	11.80 to 15.80	10.00 to 13.80	10.50 to 12.50	11.00 to 12.00	9.50 to 9.90
Mean	11.70	11.88	13.10	12.45	11.68	11.60	9.67
S.E	for the specimens= 0.296						

A 2.2. Phosphorus analysis in mg/100ml from pregnant ewes, female and male controls

Sample No.	Dec (1984)	Jan 1985	Feb	Mar	Apr	May	June
				Pregnant ewes			
1	5.20	5.70	3.80	3.20	4.30	5.60	6.20
2	4.80	4.60	4.30	4.10	4.60	4.00	5.00
3	5.40	5.70	3.60	4.15	4.80	4.30	6.00
4	4.30	5.00	4.20	4.00	3.90	4.00	4.90
Range	4.30 to 5.40	4.60 to 5.70	3.60 to 4.30	3.20 to 4.15	3.90 to 4.80	4.00 to 5.60	4.90 to 6.20
Mean	4.93	5.25	3.83	3.86	4.40	4.48	5.53
				Female control			
8	4.20	3.40	3.80	3.10	3.80	3.95	4.40
9	4.80	4.50	4.80	3.40	4.10	4.30	5.00
10	4.70	4.30	4.60	4.50	4.80	4.90	5.00
11	4.80	4.60	4.50	4.60	4.70	4.80	5.00
Range	4.20 to 4.80	3.40 to 4.60	3.80 to 4.80	3.10 to 4.60	3.80 to 4.80	3.95 to 4.90	4.40 to 5.00
Mean	4.63	4.20	4.43	3.90	4.35	4.48	4.85
				Male control			
12	5.60	5.70	4.50	4.80	5.00	4.90	5.20
14	5.40	5.60	4.80	4.40	4.50	4.20	5.45
15	5.20	5.00	5.10	4.80	4.90	5.10	5.20
16	5.30	5.40	5.20	5.00	4.90	5.10	5.28
Range	5.20 to 5.60	5.00 to 5.70	4.50 to 5.20	4.40 to 5.20	4.50 to 5.00	4.20 to 5.10	5.20 to 5.45
Mean	5.38	5.43	4.90	4.75	4.83	4.83	5.28

S.E for the specimens = 0.118

A 2.3. Calcium/Phosphorus ratio from pregnant ewes, female and male controls

Sample No.	Dec (1984)	Jan 1985	Feb	Mar	Apr	May	June
			Pregnant ewes				
1	2.50	2.19	3.42	4.13	3.02	1.82	1.37
2	2.50	2.70	2.90	3.17	2.78	2.88	1.84
3	1.85	1.89	3.19	2.89	2.60	2.44	1.87
4	2.56	2.24	3.10	2.73	2.77	2.55	1.82
Range	1.85 to 2.56	1.89 to 3.10	2.90 to 4.07	2.75 to 4.13	2.30 to 3.05	1.82 to 3.00	1.37 to 2.14
Mean	2.29	2.34	3.41	3.27	2.79	2.60	1.84
			Female control				
8	2.86	3.59	3.29	4.45	2.89	2.73	2.09
9	2.46	2.49	1.88	3.29	2.88	2.79	1.78
10	2.64	2.91	2.83	2.44	2.50	2.61	1.84
11	2.54	2.78	2.89	2.78	2.55	2.19	1.96
Range	2.46 to 2.86	2.49 to 3.59	1.88 to 3.29	2.44 to 4.45	2.50 to 2.89	2.19 to 2.79	1.78 to 2.09
Mean	2.63	2.94	2.72	3.24	2.71	2.58	1.92
			Male control				
12	2.04	2.07	2.84	2.63	2.44	2.45	1.83
14	1.85	1.64	2.46	2.86	2.78	2.81	1.76
15	2.38	2.50	2.35	2.25	3.35	2.16	1.90
16	2.45	2.59	3.04	2.76	2.14	2.47	1.83
Range	1.85 to 2.45	1.64 to 2.59	2.35 to 3.04	2.25 to 2.86	2.14 to 3.35	2.16 to 2.81	1.76 to 1.90
Mean	2.18	2.20	2.67	2.63	2.68	2.47	2.02

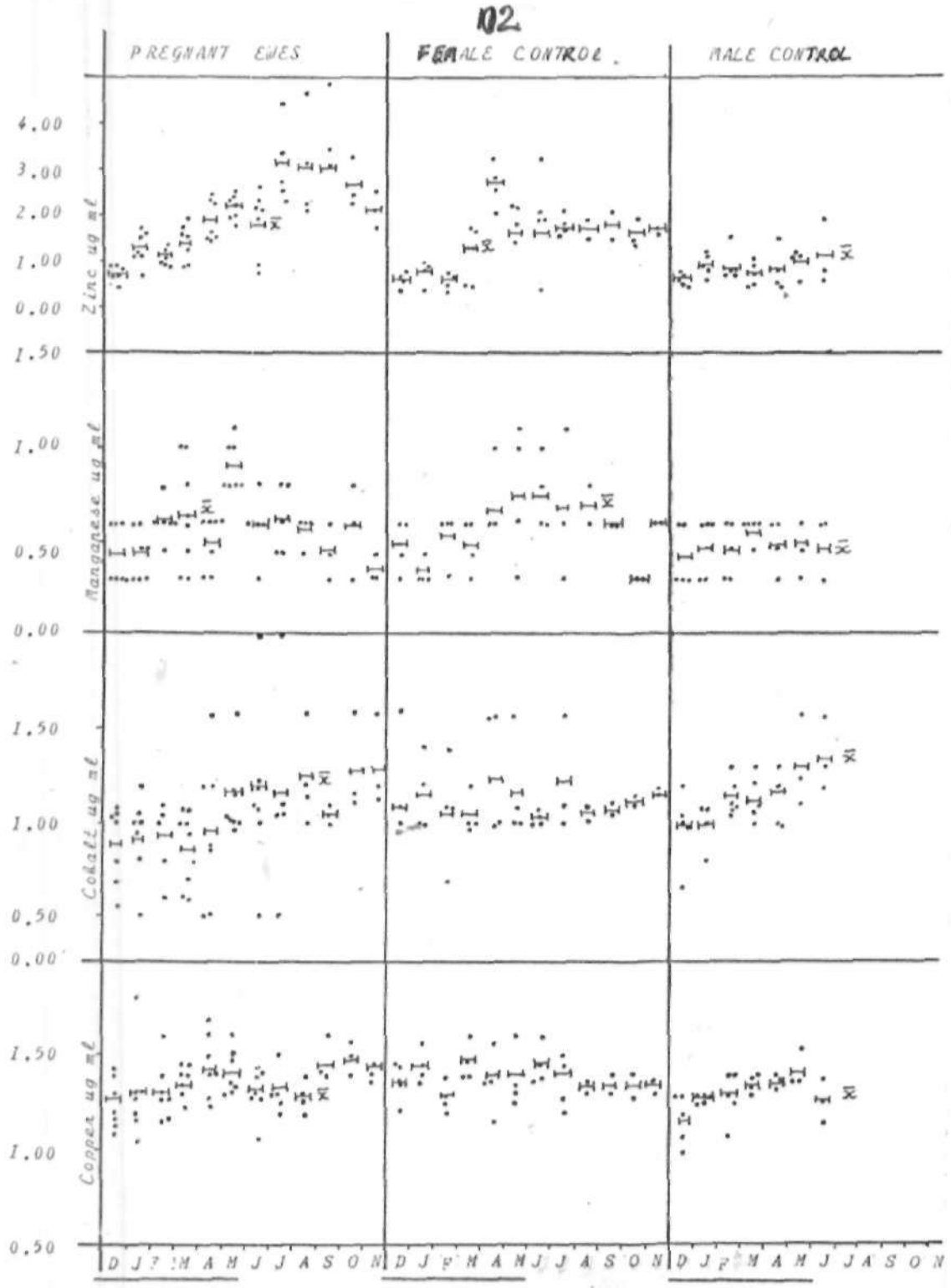
S.E for the specimens = 0.106

A 2.4. Magnesium analysis in mg/100ml from pregnant ewes, female and male controls

Sample No.	Dec (1984)	Jan	Feb	Mar	Apr	May	June
			Pregnant ewes				
1	3.80	3.50	3.50	3.00	4.00	2.80	3.00
2	3.85	4.00	4.00	3.50	3.50	2.50	4.00
3	3.46	3.00	3.50	3.50	4.00	3.50	3.00
4	3.60	3.00	4.50	3.00	3.00	3.50	3.50
Range	3.46 to 3.85	3.00 to 4.00	3.50 to 4.50	3.00 to 3.50	3.00 to 4.00	2.50 to 3.50	3.00 to 4.00
Mean	3.68	3.38	3.88	3.25	3.63	3.08	3.38
			Female control				
8	3.00	3.00	3.00	3.50	4.50	2.50	3.50
9	4.50	4.50	4.00	3.50	5.00	3.50	3.50
10	3.50	3.00	4.00	3.50	3.50	3.50	3.00
11	3.90	4.80	3.00	3.00	3.50	3.50	2.80
Range	3.00 to 4.50	3.00 to 4.80	3.00 to 4.00	3.00 to 3.50	3.50 to 5.00	2.45 to 3.50	2.80 to 3.50
Mean	3.73	3.75	3.50	3.38	4.13	3.25	3.20
			Male control				
12	4.60	5.50	5.70	4.50	5.70	4.50	4.00
14	4.75	5.80	4.00	3.00	3.50	3.50	3.00
15	4.00	3.50	6.00	5.70	4.00	4.00	4.50
16	3.85	3.50	6.00	4.50	4.50	4.00	3.83
Range	3.85 to 4.75	3.50 to 5.80	4.00 to 6.00	3.00 to 5.70	3.50 to 5.70	3.50 to 4.50	3.00 to 4.50
Mean	4.30	4.58	5.43	4.43	4.43	4.00	3.83

S.E for the specimens = 0.18





A3 Monthly concentration of some serum trace elements (copper, cobalt, manganese and zinc) in pregnant ewes, male and female controls.

The solid horizontal lines marks the mean ($\bar{x}$) of each monthly groups.

A 3.1. Analysis of copper in µg/ml from pregnant ewes, female and male controls

Sample No.	Dec (1984)	Jan 1985	Feb	Mar	Apr	May	June
			Pregnant ewes				
1	1.30	1.16	1.26	1.36	1.60	1.30	1.36
2	1.12	1.30	1.28	1.20	1.24	1.28	1.30
3	1.44	1.80	1.60	1.46	1.50	1.60	1.30
4	1.10	1.16	1.40	1.38	1.28	1.34	1.40
Range	1.10 to 1.44	1.16 to 1.80	1.26 to 1.60	1.20 to 1.46	1.24 to 1.50	1.28 to 1.60	1.30 to 1.40
Mean	1.24	1.36	1.39	1.35	1.41	1.38	1.34
			Female control				
8	1.44	1.56	1.20	1.40	1.56	1.60	1.58
9	1.18	1.36	1.40	1.60	1.16	1.30	1.40
10	1.45	1.38	1.28	1.40	1.38	1.26	1.46
11	1.36	1.42	1.24	1.46	1.38	1.32	1.40
Range	1.18 to 1.45	1.36 to 1.56	1.20 to 1.40	1.40 to 1.66	1.16 to 1.56	1.26 to 1.60	1.40 to 1.58
Mean	1.36	1.43	1.28	1.48	1.37	1.37	1.46
			Male control				
12	1.28	1.30	1.40	1.34	1.38	1.36	1.40
14	1.32	1.30	1.10	1.30	1.36	1.52	1.16
15	1.20	1.28	1.30	1.36	1.32	1.36	1.26
16	1.08	1.26	1.28	1.30	1.28	1.41	1.24
Range	1.08 to 1.32	1.26 to 1.30	1.10 to 1.42	1.30 to 1.38	1.28 to 1.38	1.36 to 1.52	1.16 to 1.40
Mean	1.22	1.29	1.27	1.31	1.34	1.41	1.27

S.E for the specimens = 0.033

A 3.2. Analysis of cobalt in µg/ml from pregnant ewes, female and male controls

Sample No.	Dec (1984)	Jan 1985	Feb	Mar	Apr	May	June
				Pregnant ewes			
1	1.08	1.18	1.04	1.00	1.56	1.00	2.00
2	1.06	1.04	1.02	1.08	1.20	1.14	1.06
3	0.54	0.80	0.60	0.58	0.50	1.56	1.28
4		0.80	1.00	1.02	1.00	0.86	1.02 1.08
Range	0.54 to 1.08	0.80 to 1.18	0.60 to 1.04	0.58 to 1.08	0.50 to 1.56	1.00 to 1.56	1.06 to 2.00
Mean	0.87	1.01	0.92	0.92	1.03	1.18	1.11
				Female control			
8	1.08	1.20	1.06	1.00	1.56	1.00	-
9	1.56	1.40	1.38	1.20	1.56	1.00	1.00
10	1.00	0.98	1.06	1.00	0.80	1.00	1.02
11	0.68	1.00	0.68	0.98	1.00	1.04	1.06
Range	0.68 to 1.56	0.98 to 1.40	0.68 to 1.38	0.98 to 1.20	0.80 to 1.56	1.00 to 1.56	1.00 to 1.06
Mean	1.08	1.15	1.05	1.05	1.23	1.15	1.02
				Male control			
12	1.00	1.06	1.08	1.00	0.98	1.10	1.30
14	0.66	0.80	1.06	1.08	1.00	1.56	1.56
15	1.02	1.00	1.20	1.18	1.30	1.24	1.18
16	1.20	1.08	1.30	1.24	1.40	1.34	1.35
Range	0.66 to 1.20	0.88 to 1.08	1.02 to 1.30	1.00 to 1.24	1.98 to 1.40	1.10 to 1.56	1.18 to 1.56
Mean	0.97	0.99	1.16	1.13	1.17	1.31	1.35

S.E for the specimen =0.076

A 3.3. Analysis of manganese in µg/ml from pregnant ewes, female and male controls

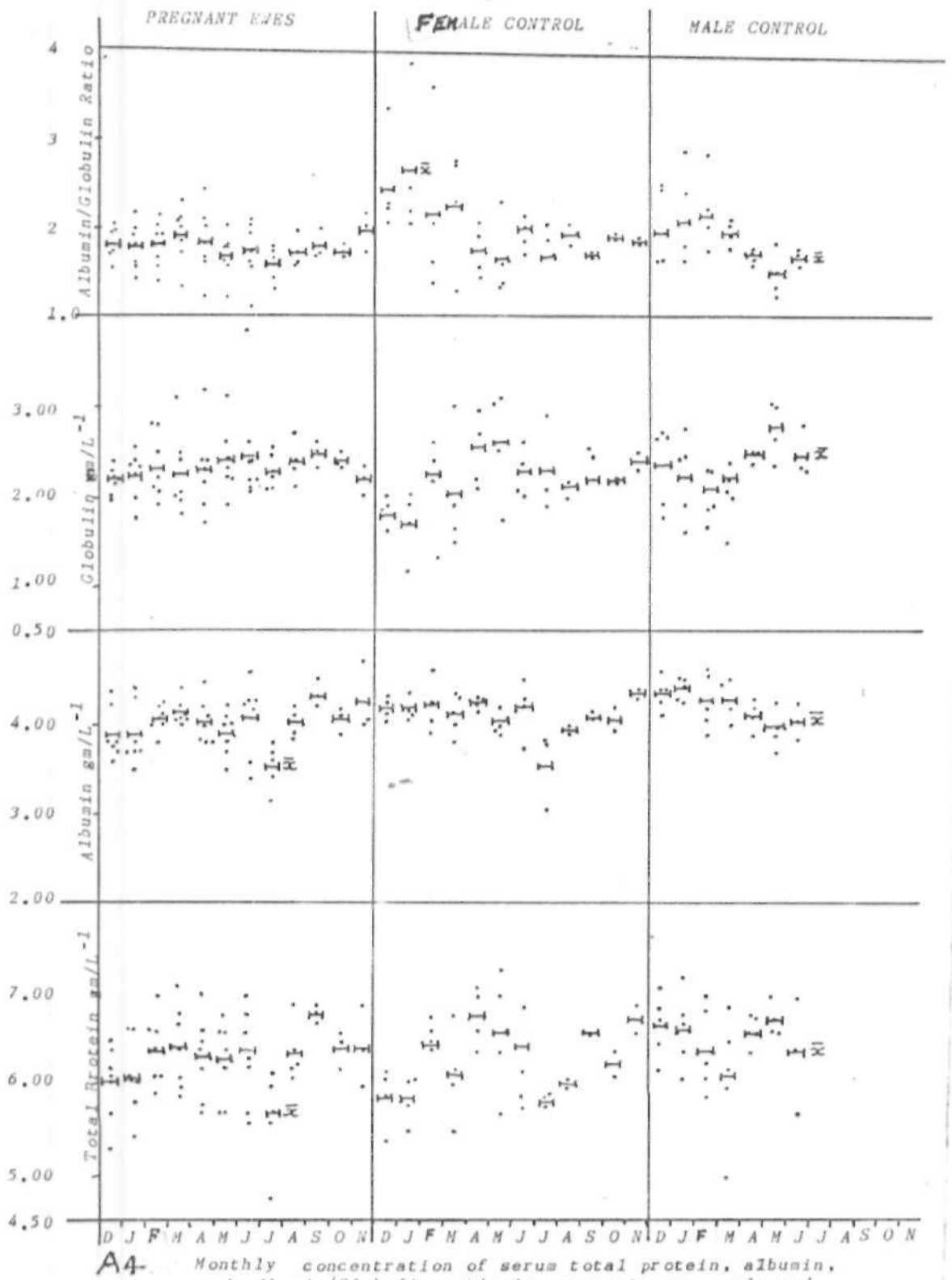
Sample No.	Dec (1984)	Jan 1985	Feb	Mar	Apr	May	June
				Pregnant ewes			
1	0.30	0.46	0.60	1.00	0.60	0.80	0.30
2	0.60	0.30	0.60	1.00	0.30	1.10	0.60
3	0.60	0.60	0.46	0.30	0.60	1.00	0.60
4	0.60	0.30	0.80	0.80	0.60	0.80	0.60
Range	0.30 to 0.60	0.30 to 0.60	0.46 to 0.80	0.30 to 1.00	0.30 to 0.60	0.80 to 1.10	0.30 to 0.80
Mean	0.43	0.44	0.62	0.78	0.53	0.93	0.53
				Female control			
8	0.60	0.30	0.60	0.30	1.00	1.10	0.60
9	0.60	0.30	0.30	0.60	0.60	1.00	0.60
10	0.46	0.30	0.60	0.60	0.45	0.60	1.00
11	0.30	0.46	0.60	0.46	0.60	0.30	0.80
Range	0.30 to 0.60	0.30 to 0.46	0.30 to 0.60	0.30 to 0.60	0.45 to 1.00	0.30 to 1.10	0.60 to 1.00
Mean	0.49	0.34	0.53	0.49	0.66	0.75	0.75
				Male control			
12	0.30	0.60	0.60	0.60	0.46	0.30	0.30
14	0.30	0.30	0.30	0.60	0.60	0.60	0.60
15	0.30	0.30	0.60	0.60	0.30	0.46	0.60
16	0.60	0.60	0.30	0.46	0.60	-	-
Range	0.30 to 0.60	0.30 to 0.60	0.30 to 0.60	0.46 to 0.60	0.30 to 0.60	0.30 to 0.60	0.30 to 0.60
Mean	0.38	0.45	0.45	0.52	0.49	0.45	0.50

S.E for the specimen = 0.05

A 3.4. Analysis of zinc in µg/ml from pregnant ewes, female and male controls

Sample No.	Dec (1984)	Jan 1985	Feb	Mar	Apr	May	June
			Pregnant ewes				
1	0.90	1.48	0.94	1.76	1.44	2.00	2.60
2	0.72	1.58	0.96	1.26	1.62	2.24	1.96
3	0.80	0.66	1.02	1.52	1.48	1.76	2.36
4	0.64	1.06	0.88	0.90	1.46	1.94	2.16
Range	0.64 to 0.90	0.66 to 1.58	0.88 to 1.06	0.90 to 1.76	1.44 to 1.62	1.76 to 2.24	1.96 to 2.60
Mean	0.77	1.11	0.95	1.36	1.56	1.99	2.22
			Female control				
8	0.66	0.78	0.72	0.44	2.80	2.16	0.32
9	0.36	0.36	0.36	1.76	3.20	0.32	2.04
10	0.54	0.98	0.64	1.62	2.08	1.68	1.92
11	0.74	0.88	0.48	1.46	2.52	2.14	1.94
Range	0.36 to 0.74	0.36 to 0.98	0.36 to 0.72	0.44 to 1.76	2.08 to 3.20	0.32 to 2.16	0.32 to 2.04
Mean	0.57	0.75	0.57	1.32	2.65	1.57	1.55
			Male control				
12	0.52	1.22	1.46	0.93	0.84	0.56	0.60
14	0.68	0.60	0.68	0.48	0.56	1.16	1.92
15	0.74	0.90	0.67	0.74	0.46	1.06	0.84
16	0.48	0.82	0.75	1.10	1.50	1.24	1.12
Range	0.48 to 0.74	0.60 to 1.22	0.67 to 1.46	0.48 to 1.10	0.46 to 1.50	0.56 to 1.24	0.60 to 1.92
Mean	0.61	0.89	0.89	0.81	0.84	1.00	1.12

S.E for the specimens = 0.11



A4 Monthly concentration of serum total protein, albumin, and albuain/Globulin ratio in pregnant ewes, male and female controls.
The solid horizontal lines mark the mean ($\bar{x}$) of each monthly group.

A 4.1. Analysis of total protein in gm/100ml from pregnant ewes, female and male controls

Sample No.	Dec (1984)	Jan 1985	Feb	Mar	Apr	May	June
			Pregnant ewes				
1	5.70	5.80	6.10	6.10	5.80	5.70	5.70
2	6.40	6.62	6.40	5.90	6.50	6.20	6.60
3	6.20	6.05	5.90	6.00	6.20	6.40	6.30
4	6.10	6.05	6.10	6.40	6.40	6.60	6.80
Range	5.70 to 6.40	5.80 to 6.62	5.90 to 6.40	5.90 to 6.40	5.80 to 6.50	5.70 to 6.60	5.70 to 6.80
Mean	6.10	6.10	6.13	6.10	6.23	6.23	6.35
			Female control				
8	6.10	6.05	6.80	6.00	7.10	7.30	6.90
9	6.15	6.08	6.50	6.80	7.00	7.00	6.20
10	5.90	5.80	6.60	6.20	6.60	6.40	5.80
11	5.40	5.50	5.90	5.50	6.40	5.70	6.90
Range	5.40 to 6.15	5.50 to 6.08	5.90 to 6.80	5.50 to 6.80	6.40 to 7.10	5.70 to 7.30	5.80 to 6.90
Mean	5.89	5.86	6.45	6.13	6.78	6.60	6.45
			Male control				
12	7.10	7.20	7.00	6.00	6.80	7.00	7.00
14	6.90	6.80	6.85	6.90	6.40	6.60	5.70
15	6.20	6.10	6.25	6.20	6.60	6.60	6.40
16	6.75	6.70	6.10	6.50	6.60	6.75	6.36
Range	6.20 to 7.10	6.10 to 7.20	6.10 to 7.00	6.00 to 6.90	6.40 to 6.80	6.60 to 7.00	5.70 to 7.00
Mean	6.74	6.70	6.55	6.40	6.60	6.74	6.37

S.E for the specimens = 0.103

A 4.2. Analysis of albumin in gm/100ml from pregnant ewes, female and male controls

Sample No.	Dec (1984)	Jan 1985	Feb	Mar	Apr	May	June
			Pregnant ewes				
1	3.75	3.80	4.05	4.05	4.10	3.80	3.60
2	4.20	4.38	4.20	4.10	4.18	3.98	4.25
3	3.70	3.50	4.00	4.05	3.80	4.08	4.25
4	3.80	3.70	4.00	4.04	3.98	4.00	4.20
Range	3.60 to 4.20	3.50 to 4.38	3.80 to 4.20	4.05 to 4.10	3.80 to 4.18	3.50 to 4.08	3.40 to 4.25
Mean	3.80	3.84	4.06	4.06	4.02	3.92	4.08
			Female control				
8	4.10	4.04	3.90	4.36	4.14	4.20	4.33
9	4.25	4.15	4.35	3.80	4.23	3.98	4.20
10	4.05	4.10	4.03	4.30	4.30	3.90	3.75
11	4.30	4.35	4.60	4.01	4.28	3.95	4.50
Range	4.05 to 4.30	4.04 to 4.35	3.90 to 4.60	3.80 to 4.36	4.14 to 4.30	3.90 to 4.20	3.75 to 4.50
Mean	4.18	4.16	4.22	4.12	4.24	4.01	4.20
			Male control				
12	4.40	4.44	3.91	4.45	4.30	3.98	4.25
14	4.25	4.35	4.55	4.50	3.89	3.94	3.84
15	4.41	4.51	4.60	4.18	4.20	4.25	4.05
16	4.10	4.28	4.20	4.30	4.10	3.70	4.05
Range	4.10 to 4.40	4.28 to 4.51	3.91 to 4.60	4.18 to 4.50	3.89 to 4.30	3.70 to 4.25	3.84 to 4.25
Mean	4.29	4.40	4.32	4.36	4.12	3.97	4.05

S.E for the specimens = 0.057

A 4.3. Analysis of globulin in gm/100ml from pregnant ewes, female and male controls

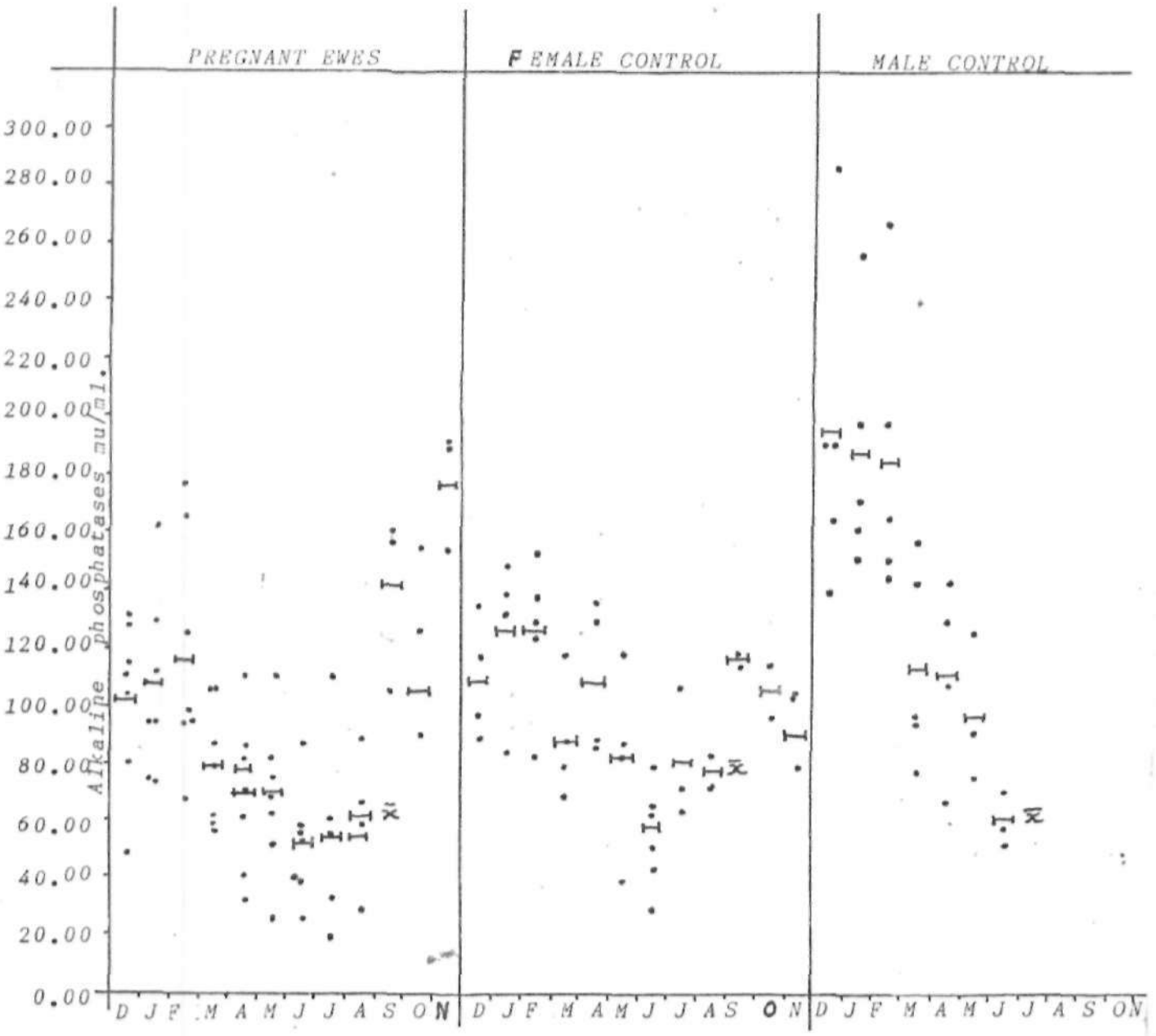
Sample No.	Dec (1984)	Jan 1985	Feb	Mar	Apr	May	June
			Pregnant ewes				
1	1.95	2.00	2.05	2.05	1.70	1.90	2.10
2	2.20	2.24	2.20	1.80	2.32	2.22	2.20
3	2.20	2.55	1.90	1.95	2.40	2.32	2.05
4	2.30	2.35	2.10	2.00	2.42	2.60	2.60
Range	1.95 to 2.30	2.00 to 2.55	1.90 to 2.20	1.80 to 2.05	1.70 to 2.42	1.90 to 2.60	2.05 to 2.60
Mean	2.16	2.24	2.06	1.95	2.30	2.26	2.24
			Female control				
8	2.10	2.01	2.90	1.64	2.96	3.10	2.57
9	1.90	1.93	2.15	3.00	2.77	3.02	2.00
10	1.85	1.70	2.57	1.90	2.30	2.50	2.05
11	1.10	1.15	1.30	1.49	2.12	1.75	2.40
Range	1.10 to 2.00	1.15 to 2.01	1.30 to 2.90	1.49 to 3.00	2.12 to 2.96	1.75 to 3.10	2.00 to 2.57
Mean	1.71	1.70	2.23	2.01	2.54	2.59	2.26
			Male control				
12	2.70	2.76	2.29	2.55	2.50	3.02	2.75
14	2.65	2.45	2.30	2.40	2.51	2.66	2.30
15	1.79	1.59	1.65	2.02	2.40	2.35	2.35
16	2.65	2.42	2.30	2.20	2.50	3.05	2.47
Range	1.79 to 2.70	1.59 to 2.76	1.65 to 2.29	2.02 to 2.55	2.40 to 2.51	2.35 to 3.85	2.30 to 2.75
Mean	2.45	2.31	2.20	2.20	2.48	2.77	2.47

S.E for the specimens = 0.098

A 4.4. Analysis of albumin/globulin ratio from pregnant ewes, female and male controls

Sample No.	Dec (1984)	Jan 1985	Feb	Mar	Apr	May	June
				Pregnant ewes			
1	1.92	1.90	1.98	1.98	2.41	2.00	1.71
2	1.91	1.96	1.91	2.28	1.80	1.79	1.93
3	1.68	1.37	2.11	2.08	1.58	1.76	2.07
4	1.65	1.57	1.91	2.02	1.64	1.54	1.62
Range	1.65 to 1.92	1.37 to 1.96	1.90 to 2.11	1.98 to 2.28	1.58 to 2.41	1.54 to 2.00	1.62 to 2.07
Mean	1.79	1.70	1.98	2.09	1.86	1.77	1.83
				Female control			
8	2.05	2.01	1.34	2.66	1.40	1.35	1.68
9	2.24	2.15	2.02	1.27	1.53	1.32	2.10
10	2.19	2.41	1.57	2.26	1.87	1.56	1.82
11	3.30	3.78	3.54	2.69	2.02	2.26	1.88
Range	2.05 to 2.45	2.01 to 3.78	1.34 to 3.54	1.27 to 2.69	1.40 to 2.02	1.32 to 2.26	1.68 to 2.10
Mean	2.45	2.59	2.12	2.22	1.71	1.62	1.87
				Male control			
12	1.63	1.61	1.71	1.75	1.72	1.32	1.55
14	1.60	1.78	1.98	1.88	1.55	1.48	1.67
15	2.46	2.84	2.79	2.07	1.75	1.81	1.72
16	1.55	1.77	1.83	1.95	1.64	1.21	1.65
Range	1.55 to 2.46	1.61 to 2.84	1.71 to 2.79	1.75 to 2.07	1.55 to 1.75	1.21 to 1.81	1.55 to 1.72
Mean	1.81	2.00	2.08	1.91	1.67	1.46	1.65

S.E for the specimens = 0.113



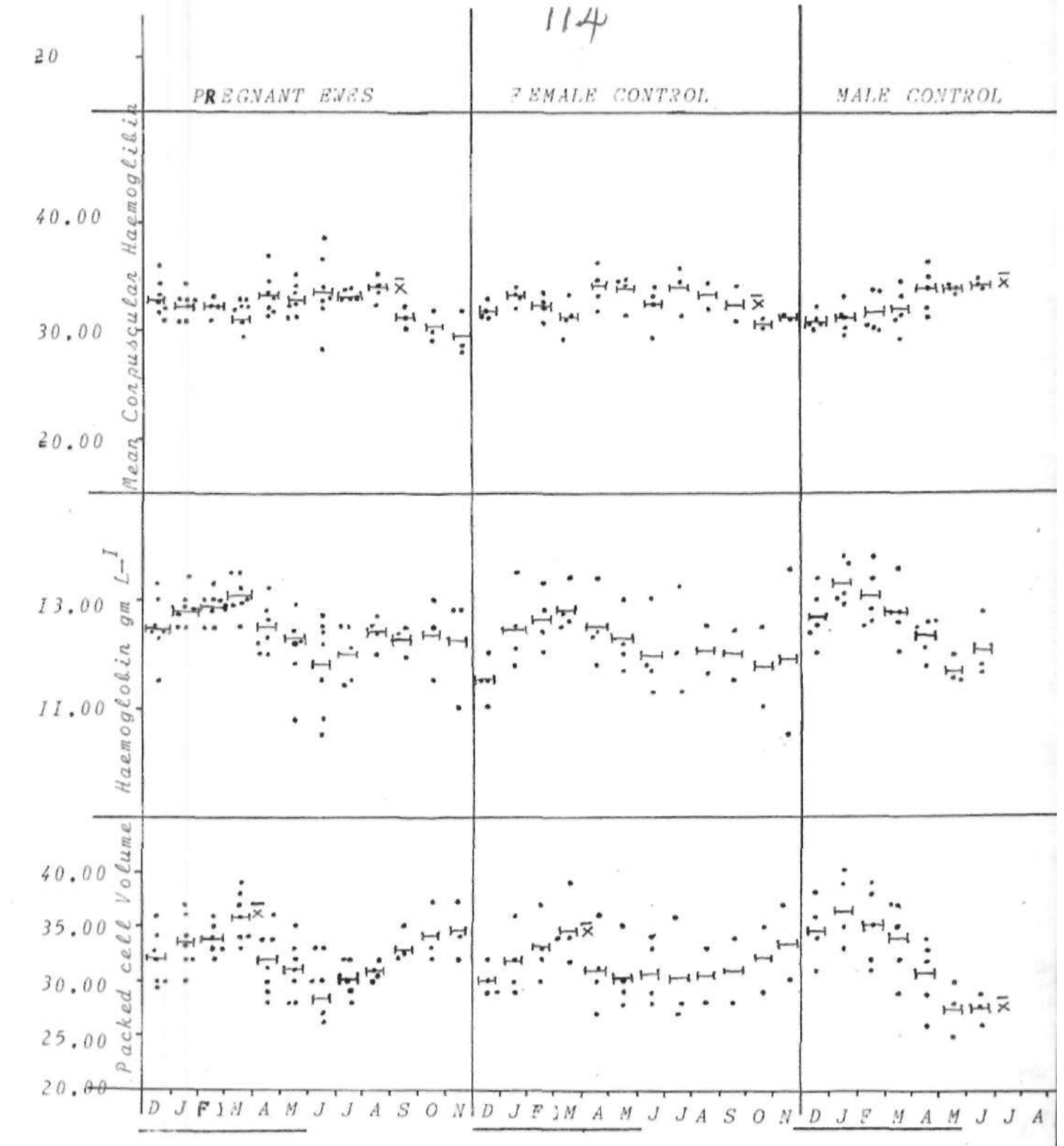
A5 Monthly concentration of serum alkaline phosphateses in pregnant ewes, male and female controls.

The solid horizontal lines mark the mean ($\bar{X}$) of each monthly groups.

A 5.1. Analysis of alkaline phosphatases in mμ/ml from pregnant ewes, female and male controls

Sample No.	Dec (1984)	Jan	Feb	March	April	May	June
				Pregnant ewes			
1	80.04	74.00	66.92	61.01	40.15	25.19	25.19
2	128.07	161.38	167.29	106.28	86.60	75.58	38.57
3	105.08	110.21	177.13	106.28	111.00	110.21	87.38
4	129.89	129.89	94.47	58.26	70.06	82.27	57.07
Range	80.04 to 129.89	74.00 to 161.38	66.92 to 177.13	61.01 to 106.28	40.15 to 111.60	25.19 to 110.21	25.19 to 87.38
Mean	110.77	105.93	126.45	82.96	76.95	93.31	52.55
				Female control			
8	89.52	83.46	82.66	88.56	86.60	39.36	50.00
9	98.41	147.61	129.89	78.72	135.80	86.60	61.01
10	118.09	138.94	137.77	118.09	129.89	118.09	78.72
11	133.83	131.86	151.54	66.92	86.60	82.66	43.30
Range	89.52 to 133.83	83.46 to 138.94	82.66 to 151.54	66.94 to 118.09	86.60 to 135.80	39.36 to 118.69	27.55 to 78.72
Mean	109.96	125.46	125.47	88.07	109.72	81.68	58.26
				Male control			
12	189.81	198.21	196.81	94.47	141.70	125.96	70.06
14	139.06	149.58	143.67	78.72	65.40	74.79	51.17
15	286.21	256.26	267.66	141.70	129.89	90.53	57.08
16	190.11	160.11	161.21	156.52	108.60	-	-
Range	139.06 to 286.21	149.58 to 256.26	143.67 to 267.66	78.72 to 156.21	65.40 to 141.70	74.79 to 125.96	51.17 to 70.06
Mean	201.30	191.04	192.34	117.85	111.40	97.09	59.44

S.E for the specimens = 8.11



A·6 Monthly values of plasma packed cell volume (PCV), haemoglobin and Mean Corpuscular haemoglobin concentration (M.C.H.C.)
The solid horizontal lines mark the mean (x̄) of each monthly group.

A 6.1. Haemoglobin estimation in gm/100ml in pregnant ewes, female and male controls

Sample No.	Dec 1984	Jan 1985	Feb 1985	Mar 1985	Apr 1985	May 1985	June 1985
				Pregnant ewes			
1	10.50	10.30	11.00	10.90	10.30	10.20	10.40
2	11.30	11.40	11.00	11.50	11.20	10.40	10.20
3	10.30	10.50	11.30	11.20	10.347	10.90	10.50
4	11.00	10.90	11.00	11.50	10.80	10.20	10.70
Range	10.30 to 11.30	10.30 to 11.40	11.00 to 11.30	10.90 to 11.50	10.30 to 11.20	10.20 to 10.90	10.20 to 10.70
Mean	10.77	10.78	11.08	11.28	10.73	10.43	10.45
				Female control			
8	9.00	10.10	10.80	10.347	10.40	10.20	11.00
9	9.50	9.80	10.00	10.50	9.80	9.70	9.30
10	10.00	11.50	11.30	11.40	11.40	11.00	9.70
11	9.50	10.50	10.40	10.70	10.30	10.00	9.80
Range	9.00 to 10.00	9.80 to 11.50	10.00 to 11.30	10.50 to 11.40	9.80 to 11.40	9.70 to 11.00	9.30 to 11.00
Mean	9.50	10.45	10.63	10.80	10.48	10.23	9.95
				Male control			
12	10.50	10.90	10.347	10.00	10.10	10.00	9.70
14	10.00	11.00	10.50	10.60	9.80	9.50	10.80
15	11.40	11.70	11.80	11.60	10.60	9.50	9.80
16	10.40	11.10	10.80	10.80	10.50	-	-
Range	10.00 to 11.40	10.90 to 11.80	10.50 to 11.80	10.00 to 11.60	9.80 to 10.60	9.50 to 10.00	9.70 to 10.80
Mean	10.58	11.18	10.03	10.75	10.25	9.67	10.10
S.E	for the specimens = 0.068						

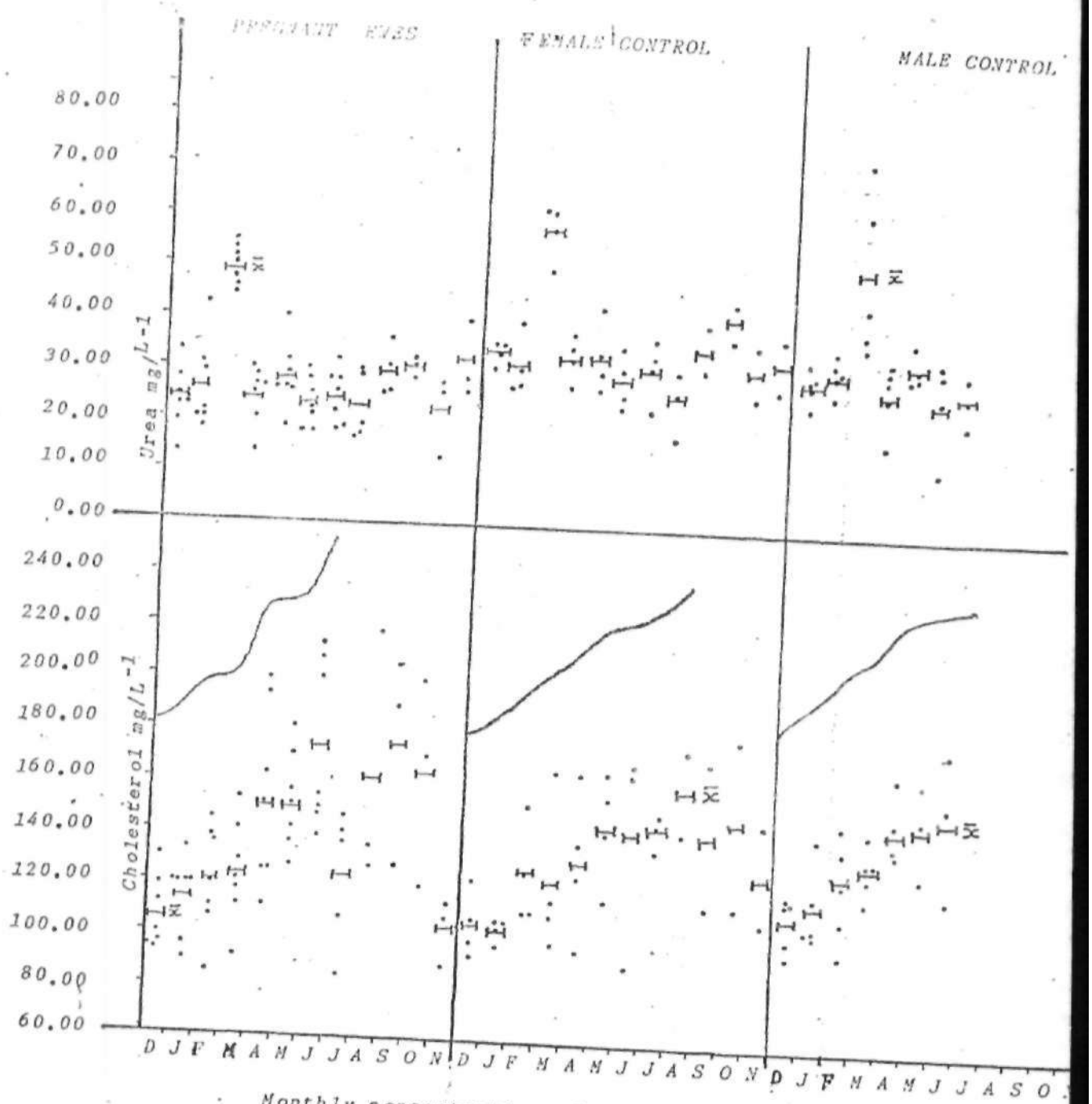
A 6.2. Packed cell volume estimation in mm/100ml in pregnant ewes, female and male controls

Sample No.	Dec 1984	Jan 1985	Feb 1985	Mar 1985	Apr 1985	May 1985	June 1985
				Pregnant ewes			
1	29.50	30.00	33.00	34.00	28.00	30.00	27.00
2	36.00	37.00	34.00	39.00	36.00	31.00	30.00
3	30.00	32.00	35.00	37.00	34.00	35.00	33.00
4	33.00	34.00	34.00	38.00	34.00	33.00	33.00
Range	29.50 to 36.00	30.00 to 37.00	33.00 to 35.00	34.00 to 39.00	28.00 to 36.00	30.00 to 35.00	27.00 to 33.00
Mean	32.13	33.25	34.00	37.00	33.00	32.25	30.75
				Female control			
8	29.00	30.00	33.00	34.00	30.00	30.00	34.00
9	30.00	29.00	30.00	34.00	27.00	28.00	28.00
10	32.00	36.00	37.00	39.00	36.00	35.00	33.00
11	29.00	32.00	32.50	32.00	31.00	29.00	29.00
Range	29.00 to 32.00	29.00 to 36.00	30.00 to 37.00	32.00 to 39.00	27.00 to 36.00	28.00 to 35.00	28.00 to 34.00
Mean	30.00	31.75	33.13	34.75	31.00	30.50	31.00
				Male control			
12	34.00	35.00	35.00	29.00	29.00	30.00	28.00
14	31.00	33.00	31.00	32.00	29.00	28.00	32.00
15	38.00	40.00	39.00	37.00	32.00	28.00	29.00
16	34.00	35.00	32.00	35.00	33.00	28.87	29.67
Range	31.00 to 38.00	33.00 to 40.00	31.00 to 39.00	29.00 to 37.00	29.00 to 33.00	28.00 to 30.00	29.00 to 32.00
Mean	34.25	35.75	34.25	33.25	30.75	28.66	29.67

S.E for the specimens = 0.360

A 6.3. Mean corpuscular haemoglobin concentration in gram per cent

Sample No.	Dec 1984	Jan 1985	Feb 1985	Mar 1985	Apr 1985	May 1985	June 1985
				Pregnant ewes			
1	35.59	34.33	33.33	32.06	36.79	34.00	38.52
2	31.39	30.81	32.35	29.49	31.11	33.55	34.00
3	34.33	32.81	32.29	30.27	31.18	31.14	31.82
4	33.33	32.06	32.35	30.26	31.77	30.91	32.42
Range	31.39 to 35.59	30.81 to 34.33	32.29 to 33.33	29.49 to 32.06	31.11 to 36.79	30.91 to 34.00	31.82 to 38.52
Mean	33.66	32.50	32.58	32.52	32.71	32.40	34.17
S.E between two means for specimens = 0.39							
				Female control			
8	31.03	33.67	32.73	31.18	34.67	34.00	32.35
9	31.67	33.79	33.33	30.88	36.30	34.64	33.21
10	31.25	31.94	30.54	29.23	31.67	31.43	29.39
11	32.76	32.81	32.00	33.44	33.23	34.48	33.79
Range	31.03 to 32.72	31.94 to 33.79	30.54 to 33.33	29.23 to 33.44	31.67 to 36.30	31.43 to 34.64	29.39 to 33.79
Mean	31.68	33.05	32.15	31.18	33.97	33.64	32.19
				Male control			
12	30.88	31.14	30.29	34.48	34.83	33.33	34.64
14	32.20	33.33	33.87	33.13	33.79	33.93	33.75
15	30.00	29.25	30.26	31.35	36.25	33.93	33.77
16	30.59	31.43	33.75	30.80	31.82	-	-
Range	30.00 to 32.20	29.25 to 33.33	30.26 to 33.87	30.80 to 34.48	31.82 to 36.25	33.33 to 33.93	33.75 to 34.64
Mean	30.92	31.29	32.04	32.41	34.17	33.73	34.05
S.E : for the specimens = 0.39							



A7

Monthly concentration of serum urea and cholesterol in pregnant ewes, male and female control.

The solid horizontal lines mark the mean ($\bar{x}$) of each monthly groups.

A 7.1. Analysis of cholestrol in mg/100ml from pregnant ewes, female and male controls

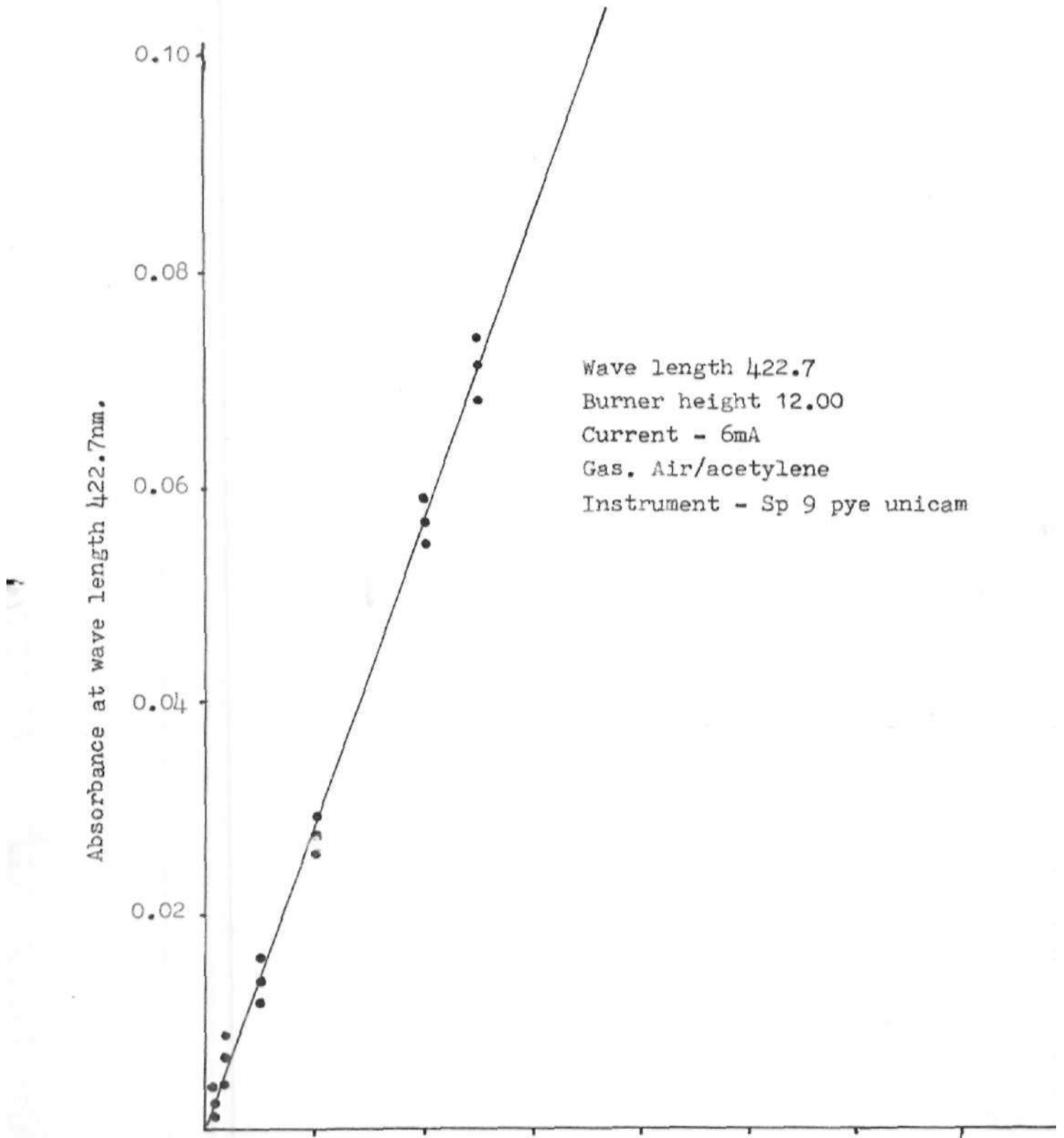
Sample No.	Dec (1984)	Jan	Feb	Mar	April	May	June
			Pregnant ewes				
1	130.00	120.00	119.30	118.30	125.00	171.42	208.57
2	119.00	133.33	111.10	121.20	125.00	142.86	200.00
3	99.62	120.00	138.80	111.00	194.00	157.14	150.29
4	111.10	120.00	144.40	154.00	152.70	151.43	157.14
Range	99.62 to 130.00	120.00 to 133.33	111.10 to 144.40	111.00 to 154.00	125.00 to 192.00	192.86 to 171.42	150.00 to 208.57
Mean	114.93	123.33	128.40	126.13	149.18	155.71	179.00
			Female control				
8	94.00	98.00	111.10	110.00	138.90	165.71	170.29
9	99.40	104.00	153.53	166.70	166.80	142.86	91.43
10	108.40	106.67	111.10	98.90	125.00	117.14	165.71
11	124.50	106.67	125.00	116.70	97.20	157.14	142.86
Range	94.00 to 124.50	98.00 to 106.67	111.10 to 153.53	98.90 to 166.70	97.20 to 166.80	117.14 to 165.71	91.43 to 170.29
Mean	106.58	103.84	125.18	123.08	131.98	145.71	142.57
			Male control				
12	102.50	106.67	130.60	128.00	167.00	165.71	177.14
14	118.00	105.33	125.00	134.00	138.30	151.43	120.00
15	120.00	143.33	148.80	145.00	138.90	128.57	157.14
16	119.00	120.00	138.80	134.00	139.90	148.57	151.43
Range	102.50 to 120.00	105.33 to 143.33	130.60 to 148.80	128.00 to 145.00	138.30 to 167.00	128.57 to 165.71	120.00 to 177.14
Mean	114.88	118.33	135.50	135.25	146.03	148.57	151.43

S.E for the specimens =5.49

A 7.2. Analysis of blood urea in mg/100ml from pregnant ewes, female and male controls

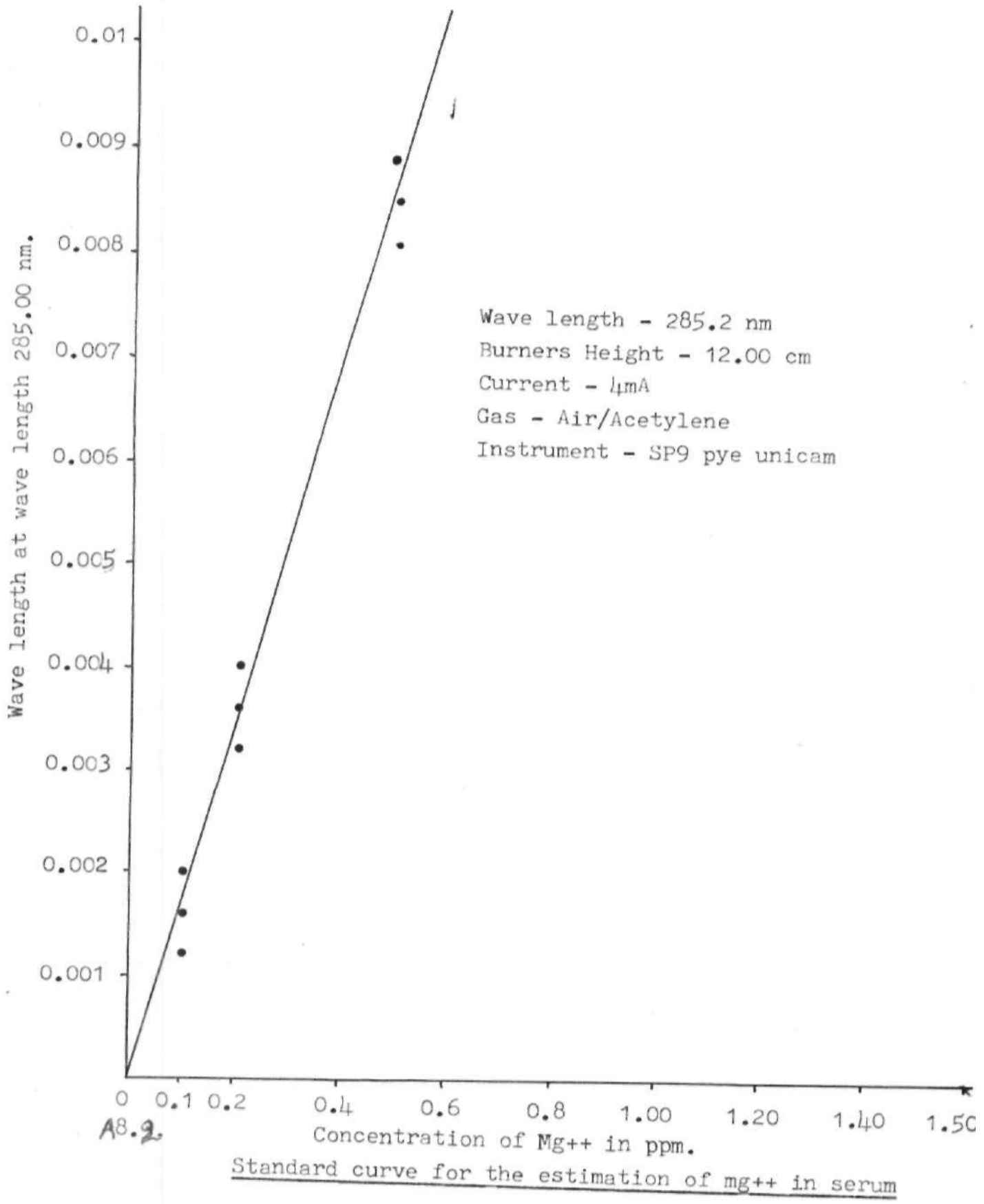
Sample No.	Dec (1984)	Jan 1985	Feb	Mar	Apr	May	June
			Pregnant ewes				
1	28.00	28.89	50.00	27.80	31.11	30.00	32.00
2	19.08	20.00	51.62	20.00	18.67	17.78	17.78
3	24.50	42.22	54.55	28.89	29.00	25.00	27.00
4	22.22	20.56	44.00	13.33	26.67	17.78	18.02
Range	19.08 to 28.00	20.00 to 42.22	44.00 to 54.55	13.33 to 28.89	18.67 to 31.11	17.78 to 30.00	17.78 to 32.00
Mean	23.45	27.92	50.00	22.51	26.36	22.64	23.00
			Female control				
8	30.70	26.67	58.18	32.20	30.00	30.20	31.11
9	35.50	40.00	50.90	35.00	34.20	35.11	33.00
10	35.56	31.11	61.82	37.78	42.22	26.89	37.78
11	34.67	27.08	61.82	28.89	26.67	24.22	22.22
Range	30.70 to 35.56	26.67 to 40.00	50.90 to 61.82	28.89 to 37.78	26.67 to 42.22	24.22 to 35.11	22.22 to 37.78
Mean	34.11	31.22	58.18	33.47	33.27	29.11	31.03
			Male control				
12	28.56	35.56	39.27	33.33	37.78	13.33	22.22
14	34.00	30.60	72.73	32.11	33.00	31.80	32.00
15	30.11	31.11	61.82	17.78	32.00	26.67	28.89
16	31.11	33.40	36.36	26.67	31.11	32.40	27.70
Range	28.56 to 34.00	30.60 to 35.56	36.36 to 72.73	17.78 to 33.33	31.11 to 37.78	13.33 to 32.40	22.22 to 32.00
Mean	30.95	32.67	52.53	27.47	33.47	26.05	27.70

S.E for the specimens = 2.36



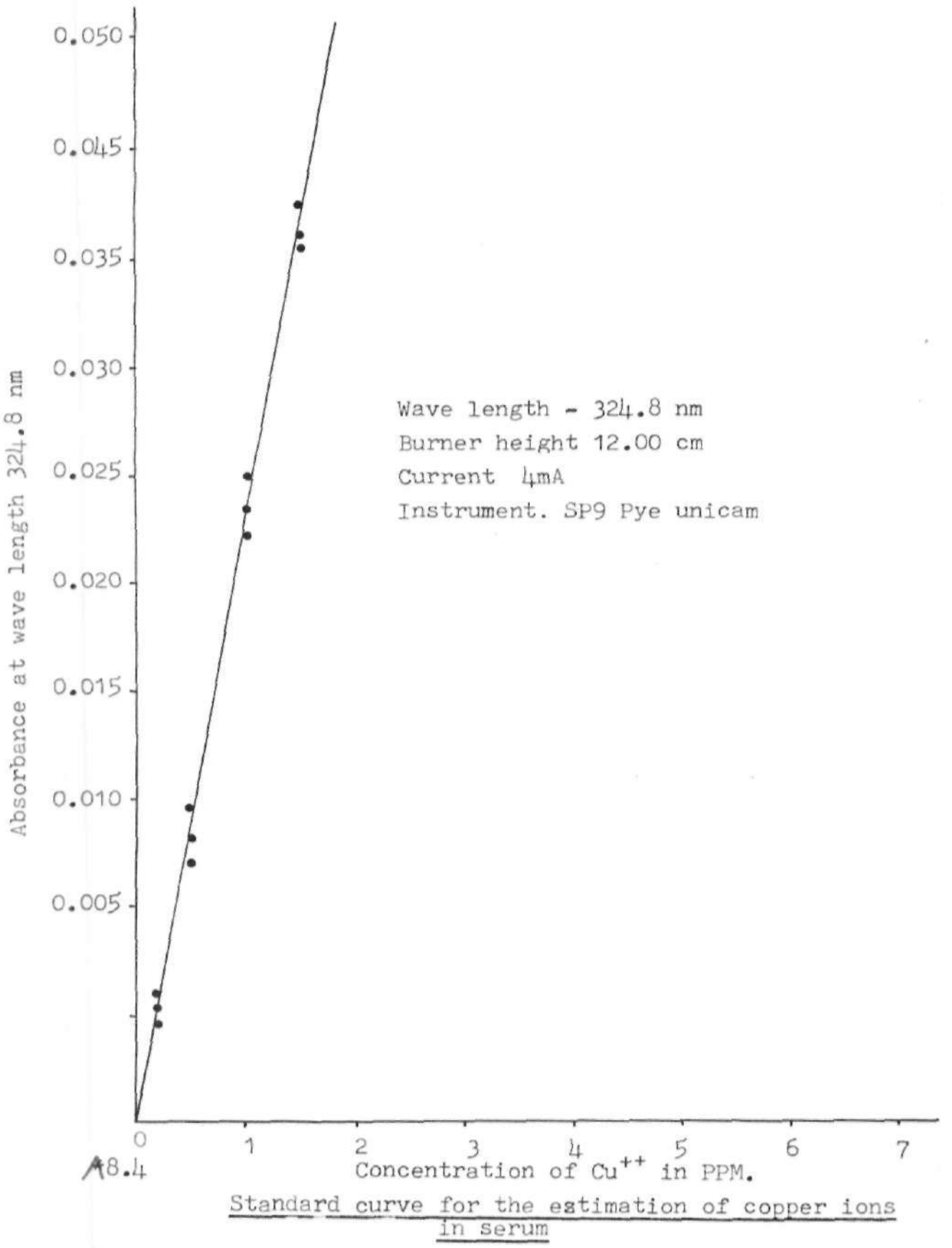
A8.1

Standard curve for the estimation of calcium in serum



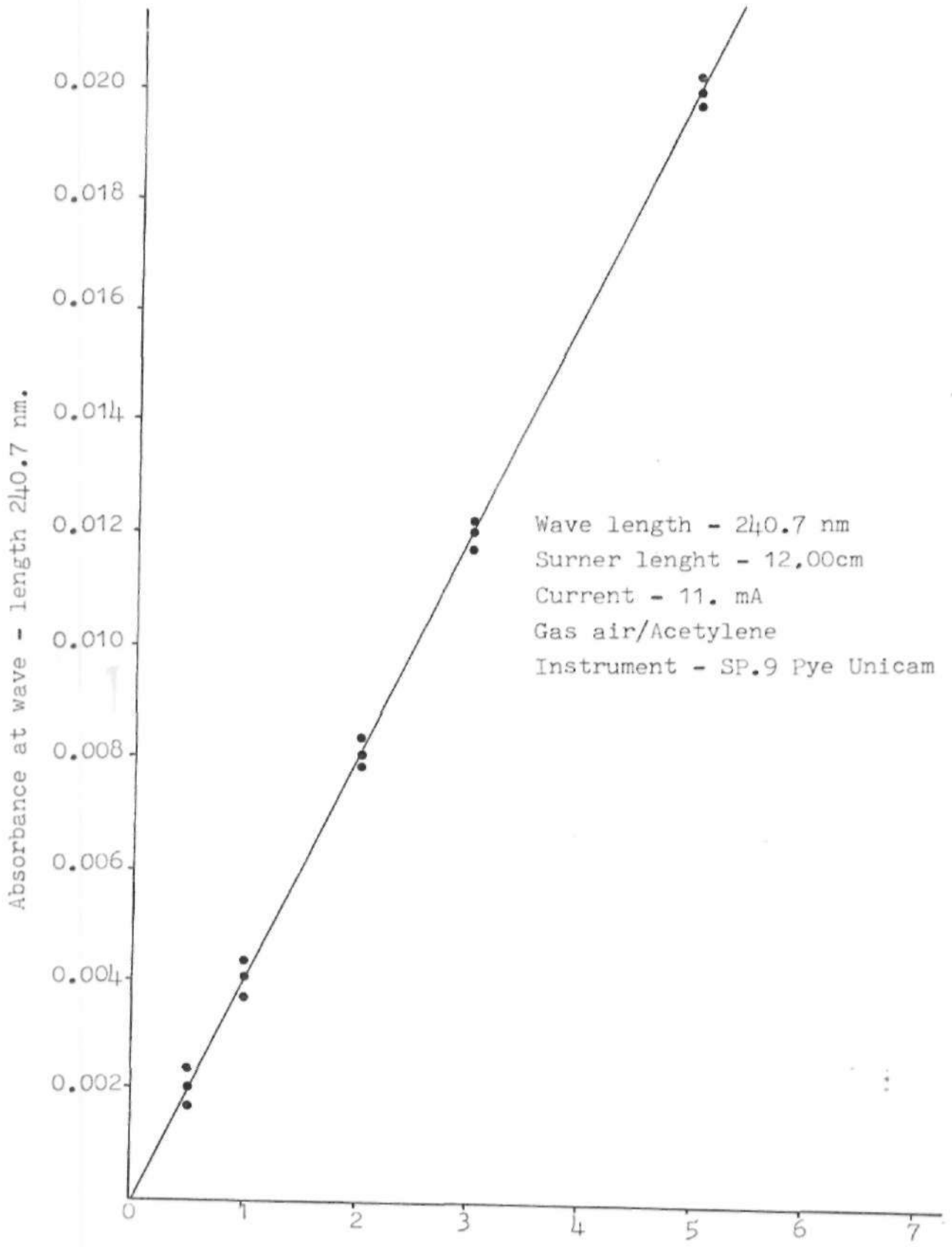
A8.2.

Standard curve for the estimation of mg++ in serum

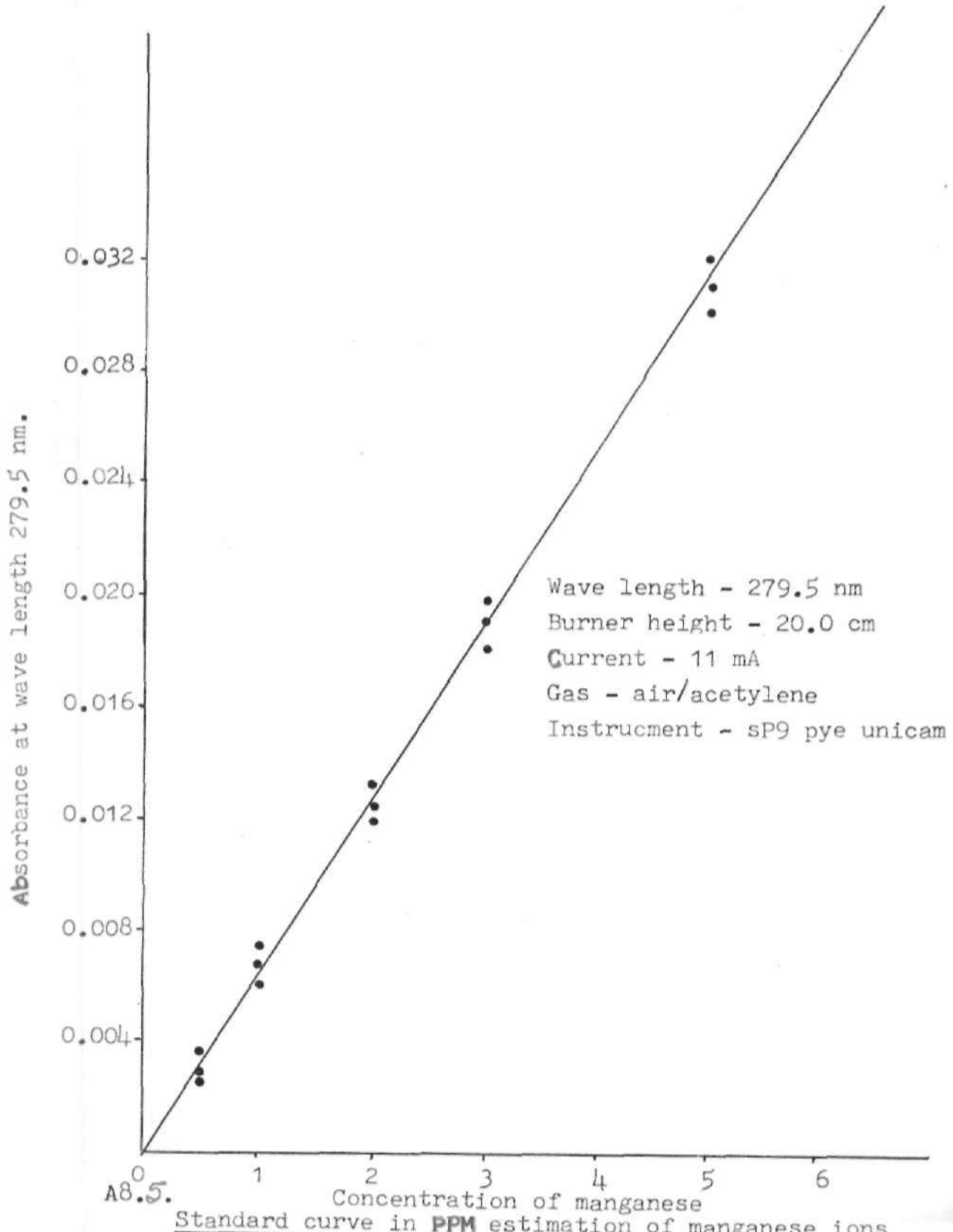


A8.4

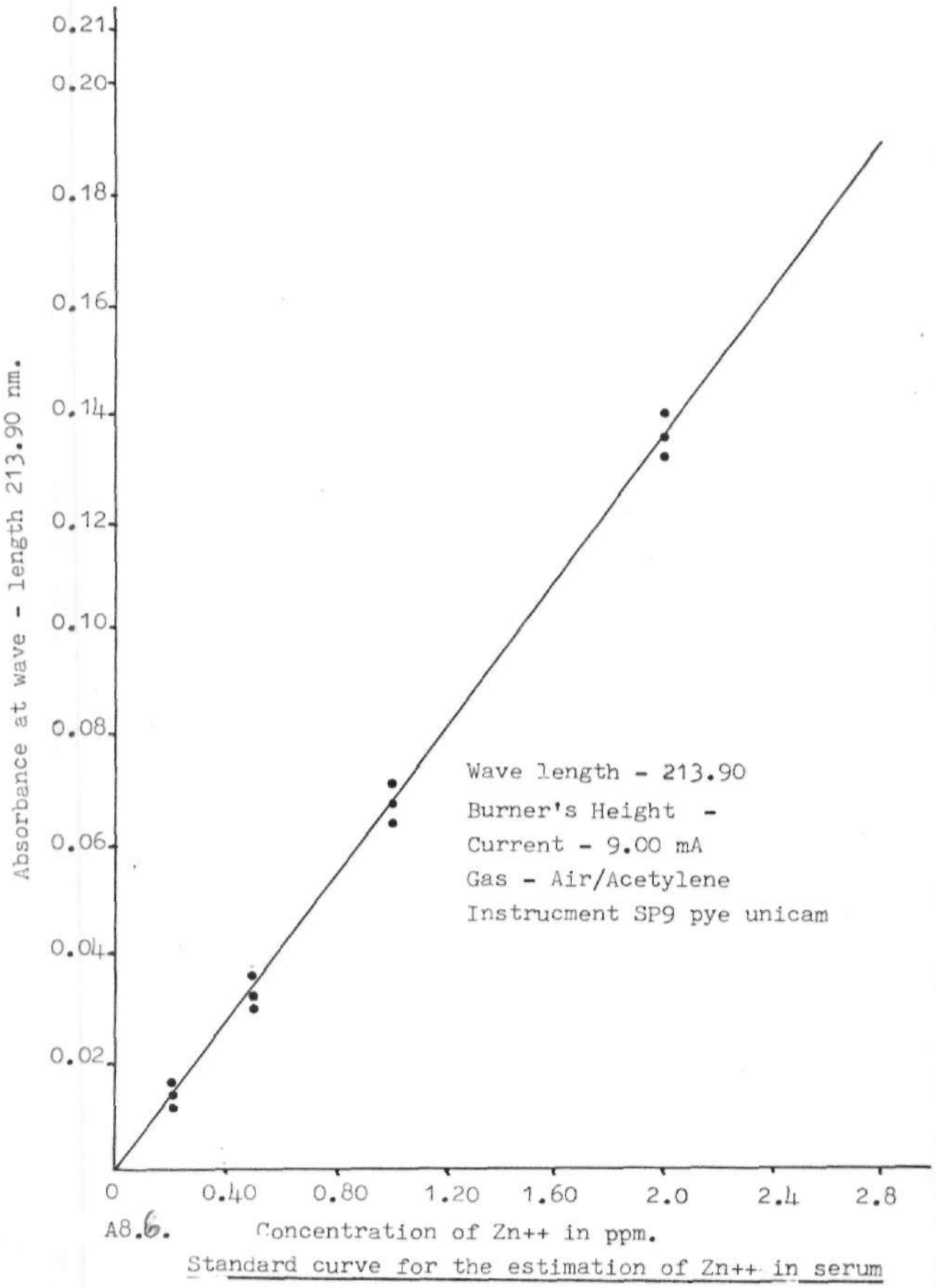
Standard curve for the estimation of copper ions in serum



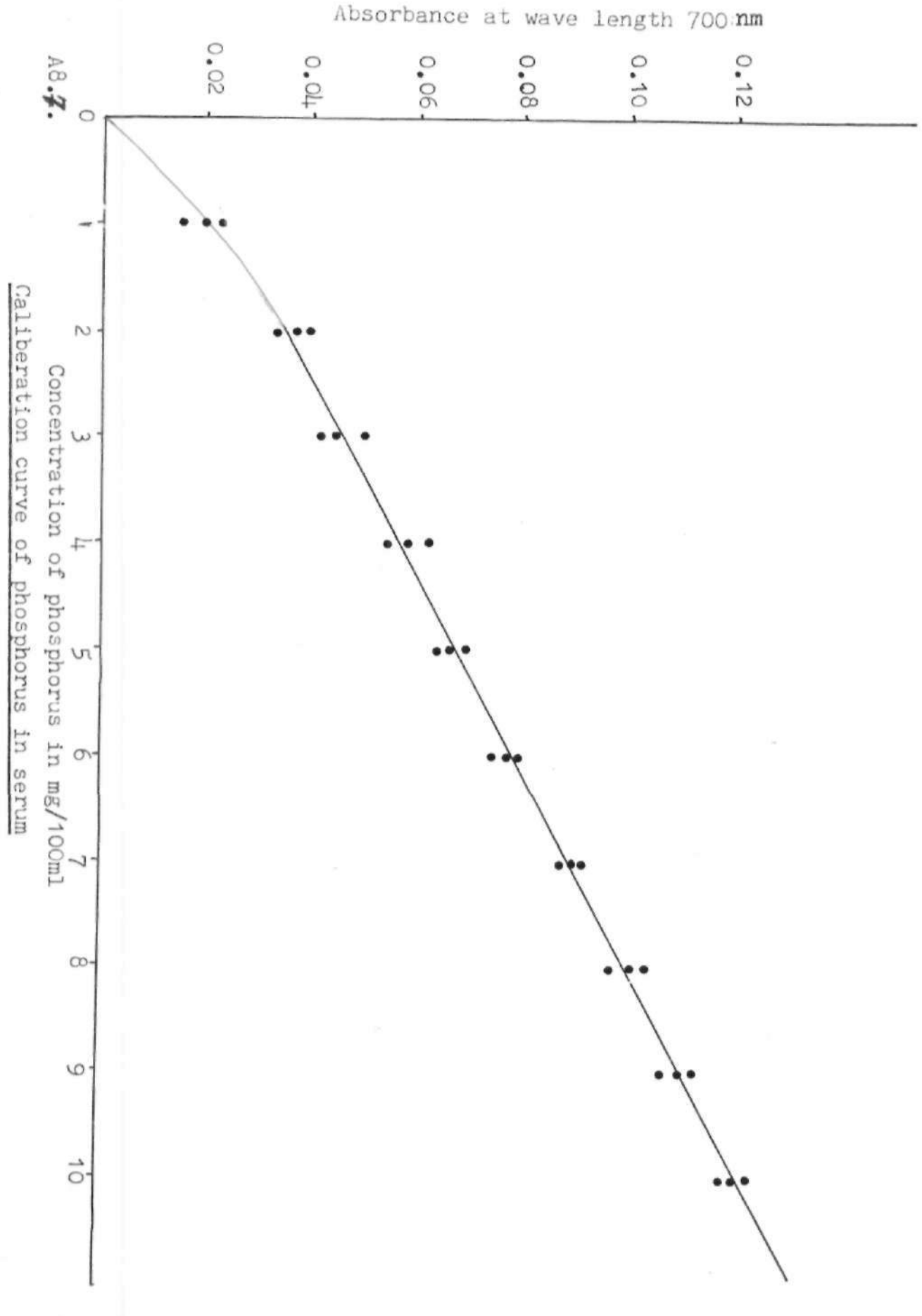
A8.4
Caliberation of standard curve of cobalt in serum



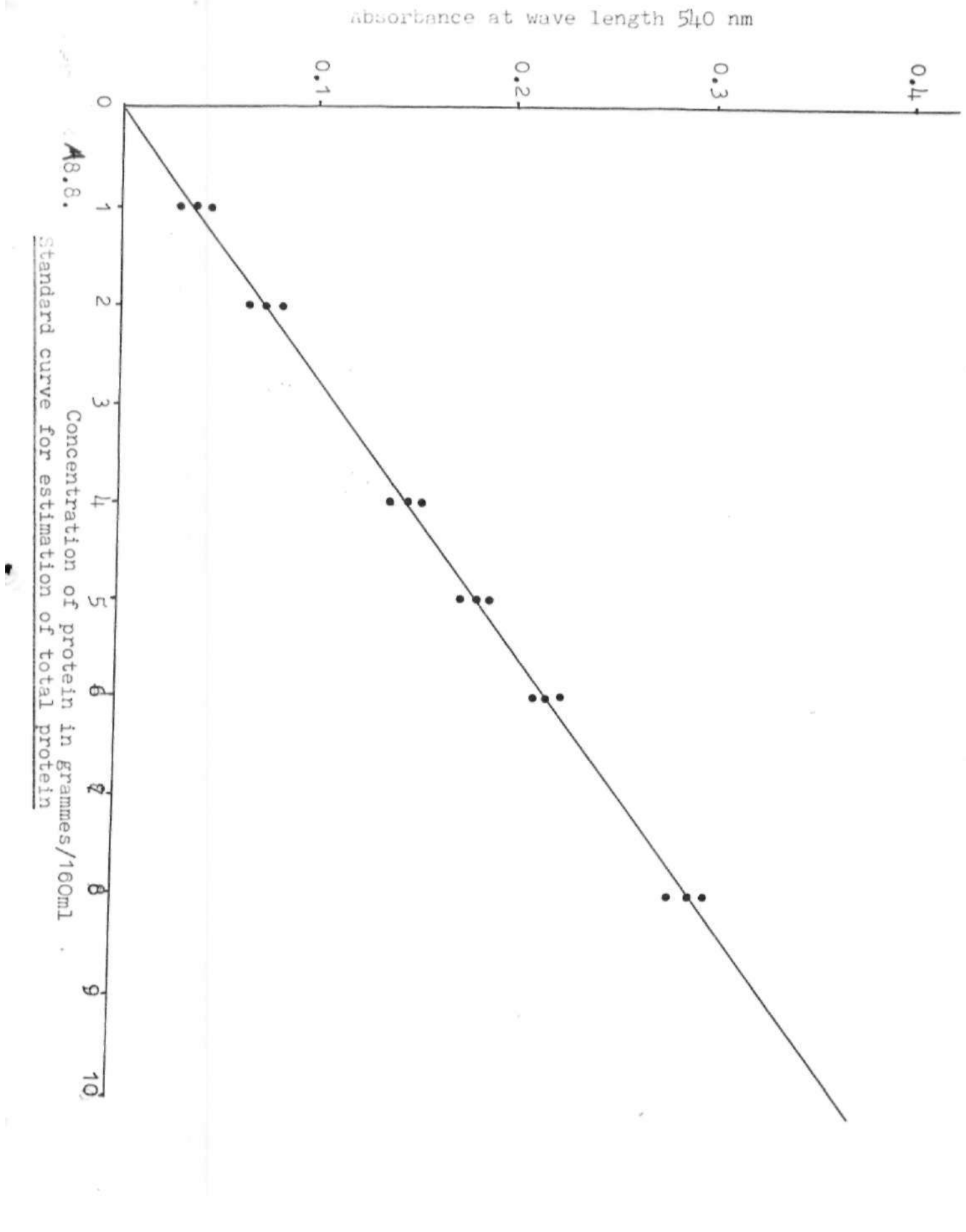
A8.5. Standard curve in PPM estimation of manganese ions in serum



A8.6. Standard curve for the estimation of Zn++ in serum

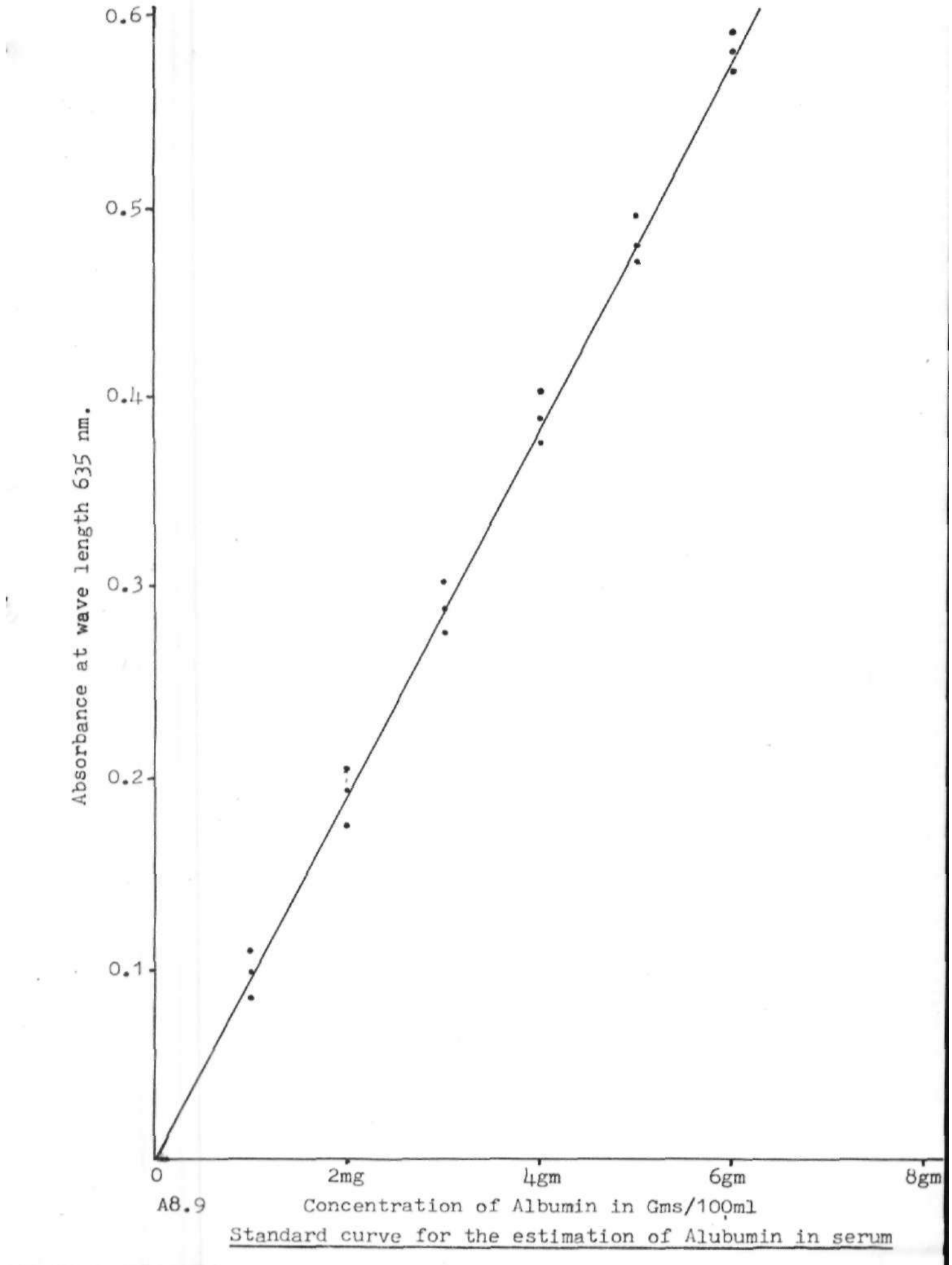


Caliberation curve of phosphorus in serum



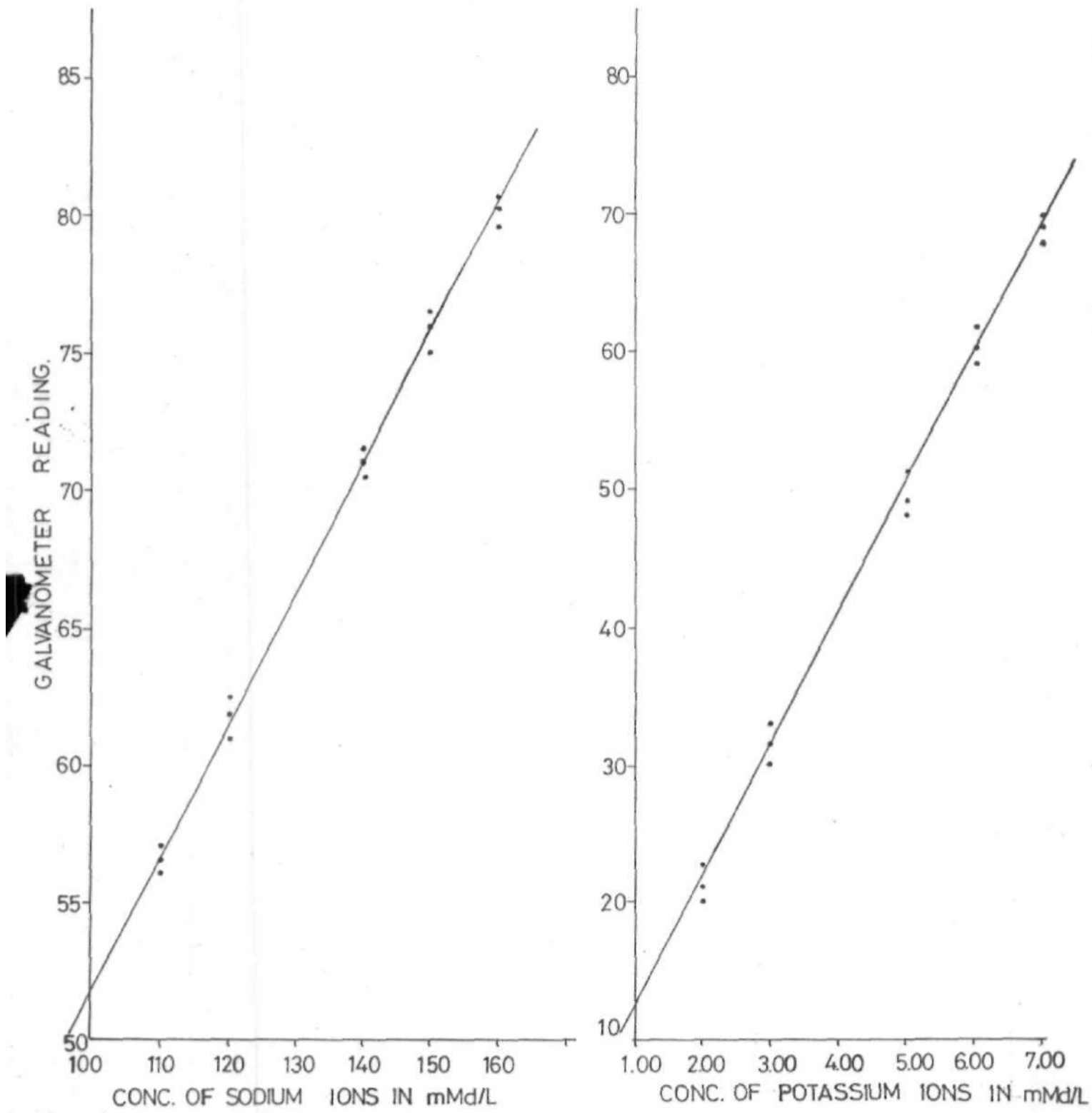
A8.8.

Standard curve for estimation of total protein



A8.9

Standard curve for the estimation of Alubumin in serum



APPENDIX A8-10 THE CALIBERATION OF SODIUM IONS AND POTASSIUM IONS FOR THEIR ESTIMATIONS IN SERUM USING CORNING FLAME EMISSION PHOTOMETER MODEL 400.

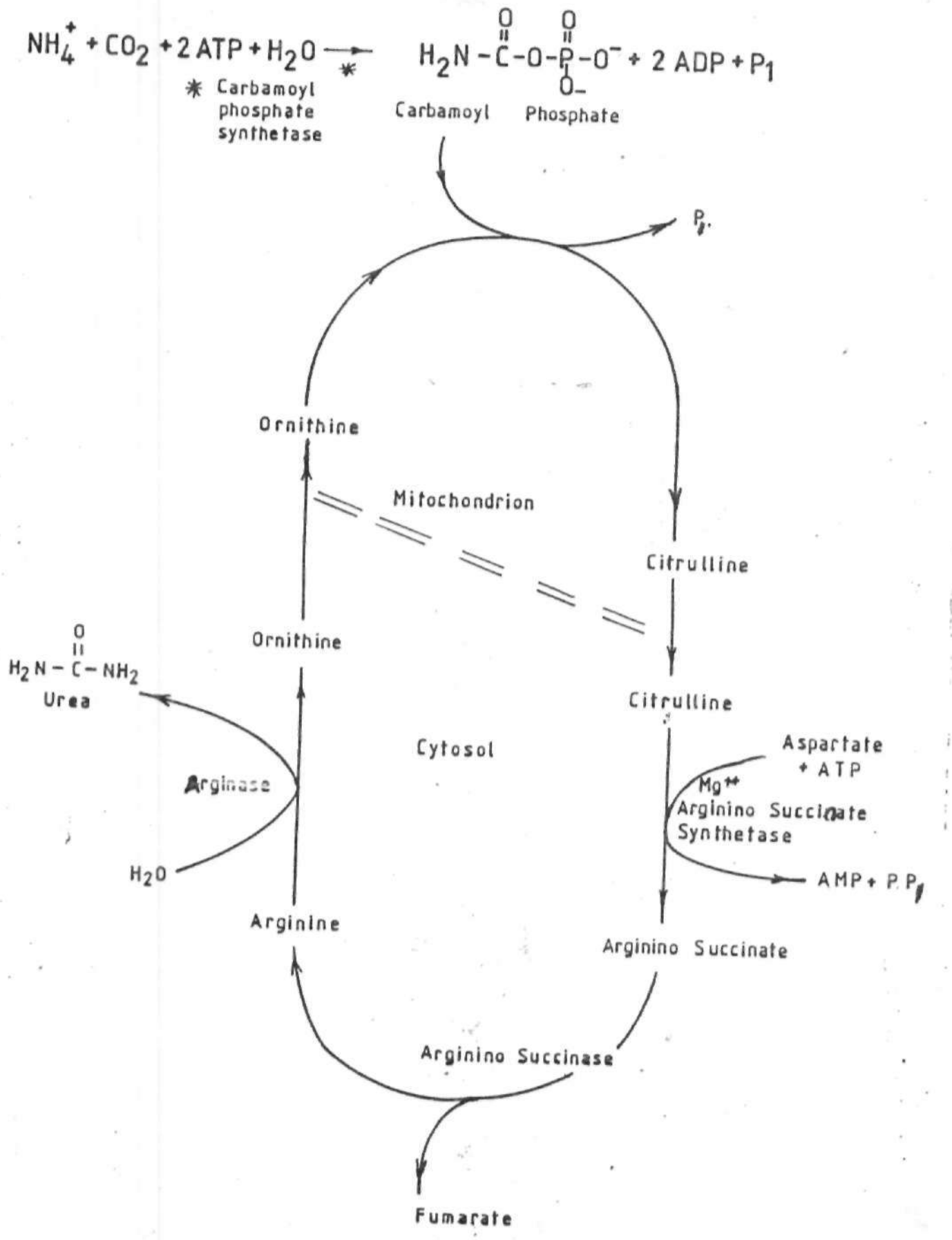


Figure A The ornithine / urea cycle