PATHOGENESIS OF BOVINE PEDICULOSIS ON CALVES: LINOGNATHUS VITULI (LINN), THE LONG-NOSED SUCKING LOUSE

A thesis submitted for the
Degree of Doctor of Philosophy
in the
Faculty of Science
of the
University of Glasgow

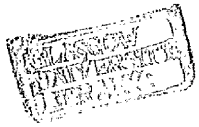
by

LORNA MARGARET BURNS B.Sc. Hons. (Aberdeen)

Biological Sciences Section,
Environmental Sciences Department,
The West of Scotland College
(for Agricultural, Horticultural and Food Studies),
Auchincruive, Ayr.

September 1989

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346



WITH MUCH LOVE

TO MY

MOTHER AND FATHER,

JANE AND GEORGE BURNS

TO A LOUSE

ON SEEING ONE ON A LADY'S BONNET AT CHURCH

Ha! whar ye gaun, ye crowlin ferlie?
Your impudence protects you sairly;
I canna say but ye strunt rarely,
Ower gauze and lace;
Tho', faith! I fear ye dine but sparely
On sic a place.

Ye ugly, creepin, blastit wonner,
Detested, shunn'd by saunt an' sinner,
How daur ye set your fit upon her-
Sae fine a lady?
Gae somewhere else and seek your dinner
On some poor body.

Swith! in some beggar's baffet squattle,
There ye may creep, and sprawl, and sprattle,
Wi'ither kindered, jumping cattle,
In shoals and nations;
Whaur horn nor bane ne'er daur unsettle
Your thick plantations.

Now haud you there, ye're out o'sight,
Below the fatt'rels, snug and tight;
Na, faith ye yet! ye'll no be right,
Till ye've got on it-
The verra tapmost, tow'rin height
O' Miss's bonnet.

My sooth! right bauld ye set your nose out,
As plump an' grey as ony groset;
O for some rank, mercurial rozet,
Or fell, red smeddum,
I'd vie you sic a hearty dose o't,
Wad dress your droddum.

I wad na been surpris'd to spy
You on an auld wife's flainen toy;
Or aiblins some bit duddie boy,
On's wyliecoat;
But Miss's fine Lundari! fye!
How daur ye do't?

O Jeany, dinna toss your head,
An' set your beauties a' abread!
Ye little ken what cursed speed
The blastie's makin;
Thae winks an' finger-ends, I dread,
Are notice takin.

O wad some Power the giftie gie us
To see oursels as ithers see us!
It wad frae mony a blunder free us,
An' foolish notion;
What airs in dress an' gait wad lea'e us,
An' ev'n devotion!

By Robert Burns (1759-1796)

ACKNOWLEDGEMENTS

The author wishes to express her appreciation to the following people for their help during the course of the present studies.

Foremost to Dr. R. N. Titchener under whose supervision these studies were performed, for his guidance, support and enthusiasm during the last three years.

I also wish to thank the staff of the Brickrow Farm Unit for their practical assistance with the experimental herd and the staff of the Veterinary Investigation Centre, Auchincruive for allowing me to carry out haematological analysis. Thanks also to Professor P. H. Holmes and the staff of the Department of Veterinary Physiology for their assistance and advice with the radioisotope studies. I would also like to thank Dr. S. G. Deans for advice with electrophoresis and Dr. D. I. West for histology. Thanks are also expressed for facilities and advice to Dr. H. V. Smith, Department of Bacteriology, Stobhill Hospital, Glasgow for the ELISA study and to Dr. D. McEwan-Jenkinson, Electronmicroscopy Department, Moredun Research Institute, Edinburgh for electronmicroscopy. Thanks also go to Mrs. A. Bugby of the British Leather Confederation, Northampton for assistance with the tanning of skins.

Appreciation and thanks are due to Mr. R. M. Stewart for proof reading and advice during writing and to Miss K. H. Colquhoun for typing of the manuscript. Also to Mr. D. P. Arnott for statistical advice.

The author gratefully acknowledges the financial support of Bayer U.K. Ltd. to purchase calves for the radioactive experiment and the British Leather Confederation to buy animals for the leather analysis study. Appreciation is also expressed to Coopers Animal Health and Robert Young and Co. Ltd. for supplying pour-on insecticide preparations.

These studies were financed by a studentship awarded by the Department of Agriculture and Fisheries for Scotland.

CONTENTS

Page

CHAPTER ONE
LITERATURE REVIEW

CHAPTER TWO
ANALYTICAL TECHNIQUES

Page

CHAPTER SIX
HISTOLOGY OF SKIN SECTIONS AND EXAMINATION OF TANNED SKINS FROM CALVES INFESTED WITH LINOGNATHUS VITULI AND DAMALINIA BOVIS

CHAPTER SEVEN
CONCLUSIONS

SUMMARY

The literature relating to the effects of pediculosis on cattle, particularly classification and general biology, lifecycles, distribution of louse eggs on host pelage, feeding mechanisms, seasonal changes affecting host pelage, effect on host blood profile, effect on host liveweight gains, control methods, effects of host grooming and effect on host skin, have been reviewed.

Blood profiles and liveweight gains were followed in two experiments using housed calves. Blood sampling was carried out every 14 days along with the weights of the animals being noted. The blood samples were used to determine red blood cell count, white blood cell count, haemoglobin concentration, packed cell volume, *reticulocyte counts, eosinophil counts,* total *serum protein* determination and total serum albumin determination. Lice counts were also conducted on the blood sampling days. This consisted of parting the hair five times on the shoulders of each calf and counting the number of lice revealed at each hair parting.

In experiment A there were significantly greater values in the louse infested calves for haemoglobin concentration (10 weeks of age), lymphocyte count (7 weeks) and monocyte counts (30 weeks) with significantly lower values for monocyte counts (6 weeks), serum albumin determination (20 weeks) and weight (8 weeks). For experiment B significantly greater values for the infested animals were observed for leucocyte counts (7 and 8 weeks), monocyte counts (8 weeks), reticulocyte counts (7 weeks) and total serum protein (22 weeks) with significantly lower values for lymphocyte counts (24

weeks) and erythrocyte counts (12 weeks). However, all of the significant results for both experiments fell within the accepted normal range observed by Schalm, Jain and Carroll (1975) for normal animals. There was only one occasion when weight gain was significantly lower in the infested calves which occurred at 8 weeks in experiment A.

The lice population was found to be slight throughout the duration of the experiments with only a few isolated instances of moderate or severe counts being noted. Due to the infestation being slight there was no overall significant effect on the blood profile or liveweight gains in the calves infested with **L. vituli** lice.

The blood content of lice was investigated using ^{51}Cr, ^{59}Fe and ^{125}I radioactive isotopes. Each animal received 15 MBq ^{51}Cr, 15 MBq ^{125}I and 12 MBq ^{59}Fe using a jugular catheterisation technique. Blood samples were taken at regular intervals. Blood and plasma were prepared from each of the blood samples for radioactivity determination. Packed cell volume was also measured from each of the blood samples with serum albumin, protein and iron determinations also being made.

All faeces produced over a 24 hour period were weighed and random duplicate (8) 10 g samples taken for radioactivity determination. Urine produced over a 24 hour period was also collected and weighed with random, duplicate (2) samples taken for radioactivity determination.

On the last two days of the experiment 600 lice were manually removed from each infested animal, weighed and their radioactivity

determined.

Using these radioisotopes circulating red cell volumes and plasma volumes were not significantly different between infested and control calves so ensuring that the total blood volume in infested calves was not significantly different from control animals. Red cell survival was significantly greater in the infested calves. Plasma iron, plasma iron disappearance rate and plasma iron turnover rates were not significantly affected between infested and lice free animals. Serum albumin levels and intravascular (CA) pools were unaffected but the extravascular (EA) pools were significantly greater in the infested animals. However this elevation was not sufficient to cause a difference in the EA/CA ratio. There were no significant differences in the plasma ^{125}I-albumin half-lives or the fractional catabolic rate between the two groups of calves. Both ^{51}Cr and ^{59}Fe radioactivity measurements gave similar results when the blood content of the lice was determined. This was found to be approximately 0.443 ul determined by ^{51}Cr-red cells and 0.350 ul using ^{59}Fe-red cells. Using ^{125}I-albumin the albumin content of a louse was determined as being 0.984 ug.

No significant difference in liveweight gain was seen between infested and louse free animals.

Protein content of salivary glands were measured using freshly dissected salivary glands and a commercial protein assay kit. The mean concentration of protein from the salivary gland samples was found to be 106.13 ug/ml. Isoelectric focusing was carried out using a flat-bed apparatus, on freshly dissected salivary glands. The

isoelectric focusing point of the glands was determined to be at pH 3.29. Polyacrylamide gel electrophoresis was used to determine the molecular weights of substances in a homogenate of salivary glands. Electrophoresis of the gels revealed four main bands present in the salivary gland sample. The applicaton of known molecular weight standards to the gels allowed the molecular weights of the four bands to be estimated. These were found to be 80320, 58665, 42310 and 32320 daltons.

Sera from infested calves and from louse free uninfested animals were used to set up an enzyme linked immunosorbent assay (ELISA) looking at the antibody production in louse infested animals against lice salivary glands. Titrations of sera and conjugate were carried out initially to determine their optimum working dilutions. When these were known four plates were used to analyse sera from four separate sampling days with each plate being equally divided between infested and louse free sera. All four sampling days for the louse infested sera showed significantly greater antibody levels than in the control sera. The antibody levels appeared to parallel the level of louse infestation but remained at peak level even when the louse population had begun to recede.

Histological examination was made of skin sections from calves infested with **L. vituli** or **Damalinia bovis** lice as well as from lice free uninfested control animals. Both paraffin wax and plastic resin was used to embed the tissue, however, wax embedding was found to be unsatisfactory. From the resin embedded samples it was noted that approximately two-thirds of the stratum corneum was removed when **D. bovis** lice were present but no damage was noted in **L. vituli** infested

skin or in any of the uninfested control skin samples. Scanning electron microscopy was also used to study skin sections from infested and lice free calves. Holes which penetrated to the bottom of the dermal layer were observed in **L. vituli** infested skin.

When skins infested with either **L. vituli** or **D. bovis** or a mixture of the two were sent for tanning, damage was seen on the tanned, finished skin as opposed to none being observed on tanned skin from the control animals. **L. vituli** caused dark pin head sized marks which were found in the neck region and down the shoulders of the host animal. **D. bovis** lice caused white spot or white fleck marks at the base of the neck, top of the shoulders and along the backbone. Where a mixed infestation was present there were clear divisions where each type of louse could be located with **D. bovis** on the neck and along the backbone and **L. vituli** down the shoulders. Both types of damage did not take up dyes and so considerably reduced the market price of the leather.

CHAPTER ONE

LITERATURE REVIEW

1.1 INTRODUCTION

The literature relating to pediculosis on cattle is reviewed. This includes classification and general biology, life cycle, distribution of louse eggs on host, feeding mechanisms, seasonal changes affecting host pelage, effect on host blood profile, effect on host liveweight gains, control methods, effect on host grooming and effect on host skin. Some of this review may not relate directly to the results presented, but is relevant to gain a full understanding of these results.

Throughout this review the terms light, moderate and heavy are used to describe the levels of lice infestation on cattle as described by a number of authors. However, these authors do not actually define the numbers of lice these terms refer to. Titchener (1985) stated that numbers of lice could be determined by making five hair partings on the shoulders of each animal by counting the numbers revealed at each parting and formed the following criteria:

Mean count >50 lice = very severe infestation
Mean count of 20-50 lice = severe infestation
Mean count of 10-20 lice = moderate infestation
Mean count of 5-10 lice = slight infestation
Mean count of >5 lice = very slight infestation.

This definition of lice infestation is used to explain the levels observed by other authors and where they indicate the infestation was light this has been interpreted as being slight.

1.2 CLASSIFICATION AND GENERAL BIOLOGY

"A parasite ..... must not only be in intimate association with an individual of a different species but it must be metabolically dependent on it to some degree. Parasitism is seen thus as a relative phenomenon ..... At one end of this hypothetical (metabolically dependent) scale is zero dependence, that is a free-living organism; at the other end is 100 per cent dependence or total parasitism" (Smyth, 1962). Ecto-parasites are semi-independent organisms which live on the surface of their host but which possess the ability to live free from their hosts for short periods or to move from one host to another. Their host is alive and mobile. They obtain their oxygen from outside the host and, to a greater or lesser extent, they are subject to influences of the same physical environment as their non-parasitic hosts (Simpson and Beck, 1965). Ectoparasites are thus totally dependent on the host for nutritional materials, often for developmental stimuli and occasionally for control of maturation.

Ectoparasites may be divided into three groups according to their habits or the ecological niches they employ. The first group, field ectoparasites, include those that feed only for a limited period on their hosts and are free-living for most of their life cycle (e.g. ixodid ticks). Nest ecto-parasites, the second group, are most often to be found in the habitat of the host rather than on the host itself (e.g. argasid ticks). The final group comprises the host ectoparasites which are permanent residents of the integument of the host (e.g. lice).

Lice are permanent, obligatory ectoparasites which spend their entire life cycle on their host. They tend to be host specific and are usually limited to a single host species or very closely related hosts. In both the adult and nymphal stages they can seldom survive for more than a few days away from the host (Munro and Telford, 1943).

Eggs, often erroneously referred to as nits, of both biting lice and sucking lice are cemented individually to the hair or feathers of the host. These eggs are usually placed close to the skin because warmth from the host is required for egg development. Nits are the egg cases left after the lice have hatched.

Before reaching the adult stage lice go through three nymphal instars. Sex ratios vary greatly with biting lice having no males (parthenogenetic reproduction) or ratios of more than nine females to one male.

The evolution of the louse is thought to parallel that of the host. Lice are host specific, a feature which is particularly apparent in mammals where characteristics of skin, hair, surface temperature, moisture, host immune response, and grooming behaviour are important factors in regulating louse populations.

The classification of the order Phthiraptera, which contains all the lice species, has been somewhat confused in the past. Imes (1947) gave the name Anoplura to the whole order. Ferris (1951) and others suggested that lice should be classified into two orders instead of one. They classified the sucking lice in the order Anoplura and the remainder, most of which have chewing mouthparts, in

the order Mallophaga. It was considered by Ferris (1951) that the union of these two groups in a single order depends chiefly on similarities in their life histories, habits, and also the fact that they all attach their eggs to the hairs or feathers of their hosts. He adds that other and quite different insects, such as the warble-flies and the Polycteridae which are hemipteran bugs parasitic on bats, also attach their eggs to the hairs of their hosts, as indeed do bot-flies (Gastrophilidae). Although there are arguments for and against the rationale for the classification of lice in two separate orders, they are classified in this report in a single order, Phthiraptera, and the classification devised by Hopkins (1949) will be used. Hopkins gave the name Phthiraptera to the whole order and he divided it into the two sub-orders, Anoplura and Mallophaga.

All the Phthiraptera are wingless, small and have dorsoventrally flattened bodies. The antennae are short and have three to five segments. The eyes are reduced or absent. The segmentation of the thorax is indistinct. The legs are usually short, but in some species the third pair of legs is longer than the abdomen. The tarsi have one or two segments and on each tarsus there are one or two claws with which the lice cling to the hairs or feathers of their hosts. The thorax has one pair of spiracles, the mesothoracic spiracles, while the abdomen bears six pairs of spiracles at the outer margins of each of its first six segments although some species have fewer than the normal six pairs. The abdomen never bears cerci.

The Mallophaga are biting lice with chewing mandibles and are generally separated into two groups, Amblycera and Ischnocera. Thought to be the more primitive, Amblycera are adapted as body lice

found on birds and mammals. Mammal lice have one tarsal claw on the legs while bird parasites are characterised by having paired tarsal claws. In all the antennae are concealed in pouches in the head and maxillary palps are present.

Amblycera are chewing lice which feed on skin debris but by using a rasping action with their mandibles they may also feed on blood. The more important families of Amblycera on domestic animals include Menoponidae on poultry, Boopidae on dogs, Trimenoponidae on chinchillas and Gyropidae on guinea pigs.

The Ischnocera are adapted for clinging to hair or feathers rather than skin and are therefore referred to as hair or feather lice. They are generally more host specific than Amblycera, with each species often confined to a particular area on the host. They ingest only solid food, made up primarily of keratin, and need enzymes or symbionts to digest it. The important families of this suborder include Trichodectidae on mammals and Philopteridae on poultry.

The sucking lice, or Anoplura, are generally considered the most advanced lice and tend to be very host specific. They feed on the blood of their host through specialised sucking mouthparts which include three "piercers" drawn back into a pouch under the pharynx and a blood tube formed by the hypopharynx plus labium. These mouthparts are used to feed on small capillaries in the skin.

Anoplura feed exclusively on mammals and about 450 species have been described combining to make up the six families of this sub-order. The three most frequently occurring lice species in cattle

are **Linognathus vituli, Haematopinus eurysternus** and **Solenopotes capillatus** (Titchener, 1985).

The largest populations of both biting lice and sucking lice are found during the late winter in temperate regions or correspondingly the late dry season found in the tropics. These large populations reflect substantial louse reproduction during the late autumn. Usually some animals in a herd will sustain low populations during the "off" season and it will be these populations that successfully reproduce to reinfest the remainder of the herd during the autumn. This herd-infestation phenomenon can also be observed when louse infested animals are introduced into clean herds, so exposing all the animals to new infestations.

Transmission of lice from animal to animal and from herd to herd can also be achieved by louse attachment to flies (phoresy) as both biting and sucking lice have been collected from house and horn flies moving from animal to animal.

In biting lice the host immune response of triggering hair loss resulting in the shedding of biting lice and egg is generally accepted as a method of louse population regulation. Another factor which may also affect the efficiency of sucking lice reproduction and therefore regulate population numbers is that the capillaries of the host from which the lice feed may constrict so decreasing blood flow and thereby reducing the amount of blood available to the louse to feed on.

Grooming of the host by itself and by others may be considered a method of behavioural control to help decrease louse populations.

Self-dusting which is thought to help reduce louse populations occurs in poultry, horses, and other animals.

Animals which have louse populations tend to appear unthrifty in comparison with the rest of the louse-free members of the same host population. Their skin often shows signs of physical damage while the hair or plumage is matted and dull in appearance. Such infested animals are often to be found scratching as reflected in tufts of hair caught in barbed wire fences and on scratching poles. The loss of hair together with extreme itching and self-grooming can result in hairball accumulation in cattle, which at times occludes the digestive tract and causes death.

In general, louse infested animals are more difficult to manage, may show signs of anaemia depending on the severity of the louse infestation and have reduced productivity in comparison to the louse free members of the herd. If these effects persist during the winter or dry season when limited food is available to the weakened animals, they may trigger stress factors causing losses through diseases which the animal would otherwise been able to tolerate had they been louse free.

Research on ectoparasites is often difficult because of large size and mobility of the hosts and the parasites tend to be small and difficult to observe. Detailed work can be undertaken in the laboratory but expensive facilities are required to house statistically adequate numbers. However, some progress has been made by selecting host-parasite systems involving small animals. Even this has not often been done, except in research on vector-

transmitted disease and investigations on bite reactions to mosquitoes, fleas and ticks. Previous lack of knowledge of the economic effects of parasitism often prevented intensive research on these problems.

1.3 LICE LIFE CYCLES

The eggs of all cattle lice are attached to the hairs of their hosts are often incorrectly called nits. Nit is the name given to the egg case remaining attached to the hair after the louse has hatched. The exterior of the eggs of some species is sculptured in various ways.

Eggs of **L. vituli** and **S. capillatus** are similar to each other, being dark blue with soft transparent egg shells (Craufurd-Benson, 1941a). They are of an elongated oval shape, measuring, in the case of **L. vituli**, on average 0.95 mm long and 0.39 mm wide.

The eggs are laid, usually in clusters, near the skin, the proximity varying with each louse species. The precise egg-laying areas, as well as seasonal, climatic and coat changes of the host are discussed later.

Nelson *et al* (1975) in a review article on host-ectoparasite relationships stated that the general incubation period of eggs for the 2800 species of Mallophaga is 4-5 days with the attachment on the feathers or hair of the host. There are three nymphal instars each of which lasts approximately three days. In adults there are usually two sexes, although in some species it has taken many years before the male has been found and described (Andrews, 1971).

Studies on the life cycles of **H. eurysternus, L. vituli** and **Bovicola bovis** were first carried out by Lamson (1918). By removing eggs laid by mature females he was able to discern the incubation period, the life cycle egg to egg, and the number of eggs laid by a single female. For **H. eurysternus** and **L. vituli** the incubation period was 7 to 8 days and 8 to 9 days respectively with 22 to 30 days to complete the cycle egg to egg. He also noted that **H. eurysternus** females laid 35 to 50 eggs over a ten to fifteen day period. He did state, however, that because of the difficulty in keeping **B. bovis** confined in order to carry out the studies it was very difficult to determine the life cycle with any degree of accuracy.

Matthysse (1946) investigated the life history of a number of lice including **L. vituli.** He obtained data on oviposition rate, incubation period and the time necessary for the complete life cycle but was unable to ascertain the duration of the instars. He did confirm that the incubation period for 34 **L. vituli** eggs varied from 8 to 13 days, averaging 11 days. Only four lice were reared through to adult females with the complete cycle from egg to egg requiring from 23 to 27 days, averaging 25 days. It must be noted that the number of lice successfully reared was too small to give this data any real significance. A number of eggs were removed from the host soon after oviposition and incubated at a constant temperature of $90^{o}F$. The incubation period varied from 11 to 14 days, averaging 13 days.

In New Zealand Chalmers and Charleston (1980) obtained data on the life cycle of **L. vituli** with their studies being carried out in

winter on a housed calf, tethered to minimise rubbing and self-grooming. Air temperatures ranged from 3 to 20°C and relative humidity ranged from 77 to 94%. The life cycle was completed in 26 to 31 days with the following subdivisions: egg laying to hatching 10 to 12 days, first nymphal instar 6 to 7 days, second instar 3 to 5 days, third instar 5 days, moulting to egg laying 2 days. These results agree with those of Lancaster (1957).

Using similar methods to Matthysse (1946) and under optimum conditions (35°C and 75% relative humidity) Chalmers and Charleston (1980) noted the life cycle for **Damalinia bovis** was completed in 27 to 32 days; the various phases of the cycle taking the following times: egg laying to hatching 7 to 8 days, first nymphal instar 6 to 7 days, second instar 5 to 6 days, third 6 to 7 days, moulting to laying first egg 3 to 4 days. These results are similar to those obtained by Matthysse (1946).

1.4 DISTRIBUTION OF LOUSE EGGS ON HOST PELAGE

Oviposition by **D. ovis** was carried out successfully when tactile stimulus was present (Murray, 1957a). This stimulus was provided by the host fibres which could be held next to the abdomen by a gonopod and which resulted in secretion of the cement substance followed by expulsion of the egg. The required thickness of fibres to elicit such a stimulus, however, was found to be approximately 0.02 mm in diameter. If larger diameter fibres were used the number of eggs laid per louse decreased substantially (Murray, 1957a). On examining at the development of the egg within the female, Murray (1957a) established that when lice are starved, only those which contained an

egg longer than 0.48 mm subsequently lay an egg.

Murray (1957a) described lice oviposition as being in three separate stages. Stage one consisted of lice detecting any temperature gradient present in the hosts pelage and being attracted to warmth, in particular temperatures between 35 and 40^{o}C where they align themselves along the hair with their head towards the warm end of the gradient. In stage two, orientation to any particular direction was not seen when lice were exposed to constant temperature and humidity. However, orientation was apparent when they were exposed to either a temperature gradient without a humidity gradient or vice versa. In the presence of antagonistic temperature and humidity gradients, the orientation to temperature dominated. Humidity influenced the number of eggs laid but only high humidities had an adverse effect. During this stage lice were attracted to other ovipositing lice. When temperatures were suitable, oviposition proceeded to the third stage where the louse turned around, thus reversing its alignment to temperature and grasped a hair with a gonopod.

The vertical distribution of eggs was found by Murray (1957a) to be determined by the distance to which the temperature zone, 35-38^{o}C, suitable for oviposition extends from the skin. As fleece thickness increases so does the depth of the temperature zone. The typical vertical distribution of lice is usually the result of an attraction to warmth in stage 1 of the behaviour pattern at the time of oviposition. This leads the louse to the skin where a temperature zone suitable for the completion of stages 2 and 3 is located (Murray, 1957b).

1.5 SEASONAL CHANGES AFFECTING HOST PELAGE

It is well known that through its effect on gonadotrophic activity of the anterior pituitary, seasonal change in the length of day regulates the time of year at which a great many animals breed. A perhaps less well known effect of light is that of regulating, or at any rate influencing seasonal coat character (Yeates, 1955). It is commonly known that lice populations are greatest during the winter. Cyclic occurrance of lice has been attributed to crowding, diet, light intensity and temperature but the evidence is inconclusive (Lamson, 1918; Craufurd-Benson, 1941b).

Variations in susceptibility have also been reported among dairy breeds. Holsteins have been generally considered particularly subject to lice infestations (Lamson, 1918) but no breeds of cattle have been found to be immune to infestations of lice (Matthysse, 1946). Gojmerac, Dicke and Allen (1959) noted a positive correlation between lice populations and hair diameter. These same authors, however, found that although the difference in diameter of hair within the Holstein breed was highly significant there was no significant difference between breeds of cattle.

Seasonal fluctuation in populations of cattle lice has many puzzling aspects. As a rule, numbers increase during the late autumn and winter and decline during the spring and summer. In New York, Matthysse (1946) reported that changes in host diet played no major part in the spring decline. In England, Craufurd-Benson (1941b) attributed the spring decline to changes in light intensities but Matthysse thought it likely that reductions were caused by high skin

temperature of host animals in direct sunlight. Neither worker determined what factors were responsible for population increases in autumn and winter. It was noted by Lewis and Christenson (1962) that high populations of the cattle biting louse, **B. bovis**, can be maintained during the summer months if animals are restrained to reduce self-licking and rubbing. The same authors concluded that self-licking by cattle appears to be an important mechanism in reducing or restraining louse infestations. This argument was further strengthened by the decline of louse populations, during the month of April, on four yearlings kept in a covered pen where direct sunlight could not be a contributory factor.

With cattle, shedding of the coat starts anteriorly and proceeds caudally (Yeates, 1955). Reductions occurred first on the neck, then on withers, shoulders, sides and lastly on the hips. During shedding of both a heifer and a bull, the densities of **B. bovis** populations paralleled the changes in density and length of hair coats (Lewis and Christenson, 1962). This backwardly progressing reduction strongly suggests that shedding is a factor in the spring decline of populations of this louse species. Conversely Lewis and Christenson (1962) proposed on both an intra- and inter-herd basis, that longer hair coats in the autumn and winter could contribute to the increase in buildup of louse populations.

Utech, Wharton and Wooderson (1969) confirmed that self-grooming is an important mechanism by which cattle limit louse infestations and a similar controlling mechanism is said to operate with the louse **Polyplax serrata** on mice (Bell and Clifford, 1964). Self-grooming is also known to reduce the cattle tick, **Boophilus microplus** (Riek,

1962). However, it is possible that the difference in behaviour of the two parasites (ticks remaining attached to the skin while lice move up and down the hairs except when feeding or egg laying) could result in different responses to grooming. It was Lewis and Christenson (1962) who suggested that the shedding of the winter coat in spring reduces the louse populations which have built up over the winter months. Utech **et al** (1969) concluded from their observations that it was unknown whether lower planes of nutrition directly or indirectly caused reduced grooming, which certainly lead to a delay in the shedding of the winter coat, was the main reason for the decrease in louse populations on the cattle in the spring. Data produced by Lewis, Christenson and Gaines (1967) indicated that in shaded stalls, populations of the **L. vituli** build up and persist during the warmer months on hosts restrained from self-grooming. The data also support the conclusion of Lewis and Christenson (1962) that self-grooming is one mechanism by which cattle protect themselves from lice.

Chalmers and Charleston (1980a) found that in herds where observations covered the entire winter, **L. vituli** populations peaked earlier than those of **D. bovis**. Both species were most abundant on animals of up to approximately one year of age; only older cattle that were diseased or inadequately fed carried substantial burdens. They also observed that within herds, lighter cattle tended to carry more lice than heavier ones. Animals kept on a low plane of nutrition were more heavily infested than those on a higher plane. It was found that the majority of animals in a group harboured louse populations of a similar order. Generally, **D. bovis** infestations

were more common and heavier on beef herds while **L. vituli** infestations predominated on dairy breeds. Presumably, differences in breed susceptibility reflect variations in the environment provided by the host animals for the lice. The tendency for the sucking louse **L. vituli** to be more numerous on dairy breeds could possibly be because the thinner skins of these animals are easier to penetrate for feeding. The relative insusceptibility of dairy breeds to **D. bovis** is more difficult to explain, though it might well reflect differences in the nature of the coat, especially in winter.

No evidence was found by Chalmers and Charleston (1980b) that would indicate that coat colour per se influenced numbers or species of lice on animals, which agrees with the finding of Craufurd-Benson (1941b). Chalmers and Charleston (1980b) did find, however, that **L. vituli** populations, which persisted on Friesian calves during the summer, were more numerous on the white areas than on black ones. The white areas were probably preferred by the lice because they were cooler.

The hair coat provides the louse with a relatively stable and protective environment. Chalmers and Charleston (1980b) proposed that its density and composition could affect density and distribution of the louse populations. Certainly, the moulting of the winter coat and the consequent loss of eggs attached to the hairs causes a dramatic reduction in louse populations on cattle other than spring-born calves, which retain their coats through the first summer and carry heavy burdens of lice through this period possibly for this very reason. Regression analysis was carried out to investigate the correlation of louse numbers with hair diameters, hair and skin

debris weight and sample sites. The only significant correlation was between sample sites and louse numbers (Chalmers and Charleston, 1980b).

Gojmerac **et al** (1959) found no correlation between hair weights and **D. bovis** populations on dairy heifers. **D. bovis** infestations could be controlled by clipping animals early in the season; louse populations rose as hair cover increased (Allen and Dicke, 1952). Gojmerac **et al** (1959) also obtained a positive correlation between hair diameter and **D. bovis** populations but Chalmers and Charleston (1980b) were unable to confirm this. They suggested that the size or distribution of louse populations is not simply determined by coat composition and density. The distribution of lice on the body is generally similar for both **D. bovis** and **L. vituli**, suggesting that some common factor may be involved.

Self-grooming has been shown to exert considerable control on louse populations (Lewis and Christenson, 1962; Lewis **et al**, 1967). The most dense populations tend to occur in those areas of the body which are most difficult or impossible for an animal to reach and groom itself. The relatively small numbers of lice found on the heads of animals appears at first sight to contradict the above statement. However, it should be remembered that louse numbers on the lower limbs also tend to be low. It is likely that the shorter hair cover in these areas renders them less suitable for lice by providing less insulation against fluctuations in ambient temperature and humidity. The body with its more dense coat provides a more stable and more suitable environment. Chalmers and Charleston (1980b) conclude that the distribution of lice on the body is

probably largely determined by the combined effects of coat density and accessibility for grooming. They go on to point out that the reasons for the seasonal pattern in louse activity are incompletely understood. Decreasing day-length was suggested by Craufurd-Benson (1941b) to be correlated with increasing **L. vituli** populations though whether or not this relationship is causal or coincidental is not known. **D. bovis** populations peak after day-length has started to decrease and the evidence indicates that increasing skin temperatures due to solar radiation cause the decline in population (Matthysse, 1946). Chalmers and Charleston (1980b) add that temperatures clearly affect survival of adults, oviposition and nymphal development in the egg. Of these three, nymphal development appears to be most sensitive, and temperatures of only 5°C on either side of the optimum (35°C) prevent the eggs from hatching. This is probably the most important effect in terms of population decline; the hatched stages are mobile and can alter their position in the coat but, if the eggs fail to hatch, the population must inevitably decline.

The loss of winter coat in spring not only results in the loss of eggs but also reduces the insulating effect of the coat so that lice and eggs are more directly affected by fluctuations in air temperature and by sunshine.

L. vituli may be less affected by heat than **D. bovis**, both species tend to persist in shaded areas of the body on most animals in summer but on calves they may remain in more exposed areas of the body. Atmospheric humidity and rainfall may have some influence on louse populations but little is known about this.

1.6 EFFECT OF HOST GROOMING ON LICE POPULATIONS

Mutual grooming or symbiotic grooming seems to be a well established social function with appreciable survival value in many species of animals. Intraspecific mutual grooming is well known in primates. It is considered a social and hygienic function of great significance to their well being and a source of satisfaction to both parties concerned. In the lower mammals, mutual grooming appears to be a relatively basic social activity, but is one that has been difficult to evaluate and quantify.

Louse infestation in laboratory mice colonies is ordinarily not a serious matter because grooming keeps the parasite populations at barely detectable levels. Nevertheless, the pressure of increase is unrelenting and requires constant attention by the hosts to prevent excessive proliferation of the lice (Murray, 1961).

An investigation on inter sex grooming relationships in normal mice by Bell and Clifford (1964) found no appreciable louse burdens resulting from mutual grooming on pairs of each sex combination. Bell, Clifford, Moore and Raymond (1966) discerned that when the efficiency of grooming is diminished even by such a minor factor as loss of toes, this leads to a proportional increase in ectoparasites. This confirms the findings of Bell and Clifford (1964) where differences in reciprocal grooming activity between male and female mice were seen most clearly in experiments when infested, hind limb amputee mice of one sex were combined with normal uninfested mice of the opposite sex. Regardless of whether the normal mouse was male or female, an appreciable infestation was acquired by it within 1 day

after pairing and there was a simultaneous rapid decline of louse population on the amputee cohabitant. Subsequent trends of parasitism was found to vary according to the sexes of the amputee and normal mice (Bell and Clifford, 1964). When the amputee was male, the rate of decrease of lice was less than when the amputee was female. Also, parasitism of the normal cohabitant was higher and more prolonged when it was a female, indicating that maintenance of a population on the amputee did depend on transfer to the normal partner.

When amputee mice of like sex, with louse burdens developed during separation were combined, marked differences in compatability became evident. Grooming reactions between two individuals of like sex ordinarily were either reciprocal or occurred minimally because louse burdens either decreased on both animals or remained high on both in nearly every combination.

In mice subjected to amputation of hind limbs the sites of apparent heaviest infestation are the interscapular area, the nape and the occiput, which are accessible to grooming only by those limbs (Bell and Clifford, 1966). Apparently, however, these authors made no observations on self-grooming. Lewis **et al** (1967) indicated that self-grooming in young cattle was of prime importance in keeping them free of **B. bovis** and **L. vituli**, and that physiological mechanisms appeared only rarely to be important.

It was pointed out by Murray (1987) that cattle, although they are large animals, can dramatically reduce the number of lice by grooming. Cattle use the tongue, which is covered with papillae that

act like a coarse brush or curry-comb. Because cattle are large, they can carry a pelage of a density and length sufficient to protect them from cold winters. This coat is shed in the spring for a thinner summer coat. It is then that grooming becomes effective and complements the adverse microclimatic changes that also reduce the abundance of lice in the summer months. In contrast, the thick winter coat buffers the effects of climatic changes and reduces the grooming efficiency, resulting in an increase in the number of lice. The inter-relationships between grooming and microclimatic factors undoubtedly vary considerably over the geographical ranges of ungulates, causing groups of lice to be associated with temperate or tropical climates. Thus the five species of lice found on cattle (**B. bovis, H. eurysternus, H. quadripertusis, L. vituli, S. capillatus**) show differences in morphology, feeding and reproductive adaptations, with different reproductive potentials as modified by according to the climatic adaptations and grooming behaviour of their host (Murray, 1986).

The distribution of ectoparasites on the surface of their host appears to be dictated primarily by their requirement for a suitable microhabitat in which to feed and complete their life cycle. The parasites may be thwarted by grooming by the host, atmospheric conditions, host resistance and other variables. Undoubtedly, the pattern of predilection sites is a result of long association between parasite and host.

1.7 FEEDING MECHANISMS

To elucidate the mechanism of disease transmission by arthropods it is important to know firstly the means by which the vector becomes infected while feeding on a diseased host and then the route by which the infected vector transmits disease organisms to a new host. Secondly, it is of interest to note how the arthropod obtains its food from the host and the host tissues selected by the arthropod. The latter may indicate the nutritional requirements of the arthropod and whether or not antigenic material is inoculated intravascularly. Thus the development of host resistance, dermatitis and other pathologic phenomena may be understood (Nelson, **et al** 1975).

Lavoipierre (1965) considered arthropod feeding upon vertebrate skin to be of two types, solenophage or vessel feeding and telmophage or pool feeding. In this context vessels included blood vessels or lymphatics while pools included haemorrhages or lysed tissue. As Laviopierre and Beck (1967) indicated, little if anything was known about the feeding mechanism of members of the Mallophaga. This author noted that, in general, evidence for blood feeding by these lice has been restricted to the discovery of red cells and haemoglobin in the gut. This evidence can only be described as circumstantial as there is no certainty that the blood exuding from lacerations is solely due to the puncturing of the skin by the lice.

1.8 EFFECT ON HOST BLOOD PROFILE

Blood-sucking ectoparasites remove blood from their host and usually inject an oral secretion, all or part of which may enter the circulation of the host and so may cause demonstrable changes in blood and bone marrow. References to anaemia caused by ectoparasites are fairly common in the literature. The earliest is that of Finkel (1933) on the anaemia caused by **Pediculus humanus humanus**, on two human volunteers over a period of almost two years. He noted significantly reduced red blood cell and haemoglobin values along with an unexplained monocytosis.

A study was made of the blood profile of normal healthy calves from the time of birth to the age of one year (Greatorex, 1954). All the samples of blood were taken from apparently healthy calves in attested herds and great care was taken not to cause the calf any excitement in order to avoid abnormal readings due to the so called "alarm factor". He found that the packed cell volume (P.C.V.) bore a close relationship to the erythrocyte count. During the first weeks of life high values occurred in both these tests with haemoglobin content also being correspondingly high. There appeared, however, to be a tendency for both the erythrocyte count and P.C.V. to show decreasing values from about the 28th week, this decrease being more pronounced in the latter. The haemoglobin content ranged between 4.60 and 16.05 g per 100 ml of blood with the majority of calves giving readings of 9.0 to 14.5 g per 100 ml. The haemoglobin content of the calf is high at birth; this decreases during the first two weeks of life and is followed by fluctuations during the remainder of the year (Greatorex, 1954).

Holman (1956) confirmed the findings of Greatorex (1954) by noting that the red blood cell count, in healthy calves, decreased in size until about the second month and then increased. The red blood cell count, although very variable for individual calves, remained at a high level for four months and then decreased as the corpuscles increased in size.

Over a period of years it was observed by Peterson, Roberts, Becklund and Kemper (1953) that some individual cattle, heavily infested with **H. eurysternus**, showed clinical evidence of anaemia. The anaemia was manifested by pale mucous membranes, particularly the conjunctivae, a lack of colour about the muzzle and, at times, even the udder. Heavily infested animals showed a lack of stamina when being driven long distances or during severe winter weather. The results showed that extremely heavy infestations of **H. eurysternus** produced severe anaemia, at times reducing the red blood cell volume to such an extent that it was necessary to destroy the lice to prevent the host from dying. The remarkable recovery of the animals following the destruction of the lice further indicated that withdrawal of blood by lice was the sole cause of the anaemia. Following a first destruction of lice on one animal the red blood cell volume increased from 13.0% to 37.0% in forty-two days. After a second destruction it increased from 10.7 to 40.3% in thirty-nine days and on the third occasion from 9.0 to 40.0% in forty-four days, 45.0% being the mean value for lice free cattle. They also found a reduction in haemoglobin, total red cells and white cells. No consistent change in the percentage values for the various classes of white cells was observed.

Shemanchuk, Haufe and Thompson (1960) noticed that all the heavily infested animals showed clinical symptoms of anaemia, such as general unthriftiness, lack of vigour and extreme paleness of the eyelids and in the case of females, the udder. The erythrocyte count and haemoglobin content of the blood in infested animals were approximately 50 per cent lower than those of louse-free animals. Attempts to move the weakened cattle, even short distances such as 100-200 m, resulted in exhaustion and in five cases, death. It was apparent to these authors that the reduction in the oxygen-carrying capacity of the blood, directly related to the low erythrocyte and haemoglobin levels, resulted in anoxic collapse of cellular metabolism. The erythrocyte count and haemoglobin content increased in the infested animals when the lice were destroyed. Approximately 35 days were required for the blood condition to improve and for the animal to recover from the anaemic symptoms. The haemoglobin levels and erythrocyte counts in steers of louse-free group were significantly higher than those in a infested group over a period of 15 weeks. From one experiment it was concluded that blood transfusion was probably of little value in the treatment of animals suffering from anaemia associated with severe infestations unless the lice was also destroyed. Blood transfusion, which involves considerable expense and labour, provided relief for only a short period of time.

These researchers also found that six severley infested cows aborted in mid-winter at the peak of louse infestation. Four of the animals were examined independently by three veterinarians and they agreed that the anaemic condition was probably responsible for the

abortion.

Numbers of leucocytes were slightly lower in the infested animals than in the louse-free animals (Shemanchuk **et al**, 1960). Values varied greatly at intervals and between animals. Leucocyte counts could vary from day to day and even within a single day in the presence of an infection. They concluded, therefore, that the reduction in the leucocyte counts in infested animals could not be attributed to the louse infestations, except in the most heavily infested animals.

Periods of high louse numbers can be recognised by lowered red cell, haemoglobin and P.C.V. (Nelson, Shemanchuk and Haufe, 1970). Anaemia was generally more severe in the **H. eurysternus** infested group whereas no anaemia was observed in any of the uninfested animals. Usually the more louse-infested the animal, the lower were its red cell, haemoglobin and packed cell values. It was noticed, however, that similar infestations on different animals did not always produce similar degrees of anaemia.

The white cell count usually varied between 5000 and 11000 mm^3 in healthy animals. In louse-infested animals, however, as the anaemia progressed the count dropped lower and the extreme variability which had been apparent in healthy animals tended to disappear. A suggestion that heavy louse infestations may place stress on the animal was reflected in a decline in eosinophils and an increase in neutrophils. Nelson **et al** (1970) found that blood values (red, white and eosinophil counts; haemoglobin; P.C.V. and reticulocyte determination) of lice free animals and infested

animals, kept free of lice by treatment, fell mostly within the ranges indicated by Schalm (1961) as being normal.

In animals that became progressively more anaemic, the level of haemoglobin at which reticulocytosis was first observed in blood smears, though not found consistently, appeared to lie near 5 g per 100 ml of blood. Anaemic animals did not appear to be in great difficulty until they were moved at which time obvious signs of exhaustion appeared. Several animals collapsed and died while being moved very short distances (Nelson **et al**, 1970).

It was suggested to Nelson **et al** (1970) by Schalm (personal communication) that as the anaemia developed slowly, there were physiological adjustments in size, rate and output of the heart and in the respiratory efficiency, and that the animals adjusted their physical activity to the developing anaemic condition. The combination of these factors may have led to the release of erythrocytes larger than normal.

Mehrotra and Singh (1986), in India, carried out a study on the blood constituents of calves infested with **L. vituli** and found that the infested animals suffered from anaemia. They established that the lice infested calves had lower haemoglobin values than the normal calves but had both higher packed cell volumes and red cell counts.

Blood eosinophila occurs as a result of antigen-antibody reactions in tissue (Archer, 1960). The high circulating eosinophil levels in cattle infested with **H. eurysternus** suggests allergy occurred in response to louse feeding, and that this served to keep louse numbers under control (Nelson **et al**, 1970). One such mechanism

had already been suggested by Nelson and Bainborough (1963) as forming the basis for the acquired resistance of sheep to keds (**Melophagus ovinus**). Andrews (1942) noted a positive correlation between eosinophil numbers and the development of resistance of lambs to infestations of the nematode **Haemonchus contortus.**

The ability of animals to maintain heavy infestations of lice without severe effects can break down in later life. This was suggested by Nelson **et al** (1970) as one of their cows showed a gradual decline in eosinophil count from the average of 308 mm^3 in her fifth year to 244 mm^2 in her sixth year. By her ninth year the average count was 191 mm^3 during a sustained increase in louse numbers. Nelson **et al** (1970) point out that the fact that numbers of circulating eosinophils were also low in naturally louse-resistant cattle suggests that a different mechanism of resistance was operating in these animals.

In New Zealand Chalmers and Charleston (1980c) found, however, that there were no significant differences in weight gain and in haematocrit levels between louse-infested and louse-free groups in any experiment. They suggested, however, that the difference between these results and those obtained by North American workers (Collins and Dewhirst, 1965) could be due to the fact that **Haematopinus eurysternus,** one of the predominant louse species in North America, was not present in any of the experiments conducted by Chalmers and Charleston (1980c). The findings of Chalmers and Charleston (1980c) agree with those from two other New Zealand experiments described by Kettle (1974) and in Australia, Cummins and Tweddle (1977) were also unable to demonstrate a significant response

to the treatment of **L. vituli** infestations.

The differences between New Zealand and North America findings may relate to the fact that **H. eurysternus,** one of the predominant species in North America, is relatively unimportant in New Zealand and was not involved in any of the experiments. **H. eurysternus** infestations tend to increase as animals grow older as do the associated detrimental effects. In contrast to this **D. bovis** and **L. vituli** occur mainly on animals in their first year of life and are less numerous on older animals unless exceptional circumstances prevail.

From the results of Chalmers and Charleston (1980c) and those of Kettle (1974), under New Zealand conditions, there is little likelihood that treating cattle for lice will yield a financial return from increased weight gains. However, the former point out that, irrespective of financial considerations it is likely that the mere presence of lice or evidence of discomfort or poorer appearance of infested animals will be sufficient justification for many farmers to proceed with treatment.

Holman (1956) stated anaemia is more likely to occur in a calf than a cow and there is a tendency for calves to become anaemic at some period between 6 and 18 months of age; development of anaemia appeared to be associated with environment and not with age. Calves can show a slight or moderate degree of hypochromia even when the haemoglobin level in the blood is too high to record as anaemia and the term "simple hypochromia" has been suggested to describe this state.

1.9 EFFECT ON HOST LIVEWEIGHT GAINS

It has been contended that the presence of lice on a host animal retards potential weight gain and ultimately affects the overall productivity, these effects being especially important in beef animals when young. Two experiments were carried out by Kettle (1974) to determine if the presence of lice had any effect on productivity. The first experiment studied two year old steers while the second used yearling calves. In each experiment there were two groups of twenty calves, one harbouring a mixed infestation of **L. vituli** and **D. bovis** and one with lice free controls. Results for both experiments indicated that there was no significant difference in weight gain between groups in either experiment. Kettle (1974) concluded that the louse infested group may have fetched a lower price on the basis of poor coat condition but no real economic benefit from louse treatment could be found. Likewise work by Tweddle, Cummins and Graham (1977) observed that cattle with light to moderate lice infestations, and kept on two differing nutritional levels failed to demonstrate a significant liveweight advantage from lice treatment.

They found that the difference in lice numbers between low and high planes of nutrition made up of 4 kg pasture hay and 5 kg pasture hay plus 1.5 kg oaten grain per head per day respectively, confirmed previous conclusions of Utech **et al** (1969) which showed that cattle on low planes of nutrition carried heavier louse burdens. Light **L. vituli** infestations may not cause significant reductions in weight gains with the plane of nutrition being the only important factor in influencing liveweight change (Cummins and Tweddle, 1977).

In contradiction to the view of Collins and Dewhirst (1965) that untreated louse infested cattle gained less weight than treated louse-free animals, Cummins and Graham (1982) found in Australia that analysis of the body weight gains during the 131 day experimental period indicated that initial bodyweight and plane of nutrition had significant effects but that lice control treatments had no effect and there was no interaction between lice treatment and plane of nutrition. The conclusion was that animals on a high plane of nutrition were better able to control their lice populations than animals on a low plane of nutrition.

Data to suggest that the lice populations do indeed affect the liveweight gain in cattle were put forward by Collins and Dewhirst (1965). It was demonstrated that on some Arizona rangelands, cattle not given supplemental feed lose considerable weight over the winter because of poor grazing conditions and cold weather. Cattle lice were most abundant and numbers built up rapidly when the hair was long and thick. These authors found in every case that the lice observed were **H. eurysternus.** The mean P.C.V. for mature lice free hereford cattle was 38.4%, whereas the mean P.C.V. of the heavily infested group in both sexes was significantly lower than this, with 22.2% for heifers and 25.8% for bulls. The P.C.V. of the heavily infested heifers ranged from 14.5 to 31.0% and that of the bulls from 21.5 to 28.0%. The heavily infested cattle also had clinical signs of anaemia, extreme weakness and poor condition. On both sexes light and moderate infestations had no significant effect on the mean P.C.V. as compared with that of the lice free groups with normal P.C.V.. Analysis of variance revealed a highly significant

correlation between degrees of louse infestation and mean P.C.V. in both sexes. In heifers only moderate and heavy louse infestations significantly increased winter weight loss, whereas in bulls only heavy infestations significantly increased weight loss.

The finding of Collins and Dewhirst (1965) that heavy infestations of **H. eurysternus** cause anaemia in cattle is consistent with results reported by other investigators (Peterson, Roberts, Becklund and Kemper, 1953 and Shemanchuk **et al**, 1960). Effects were more pronounced in female cattle and evidence indicated that treatment was economically justified on all heavily infested cattle and moderately infested heifers but as **H. eurysternus** is so fecund lousicidal treatment of the entire herd was suggested as being worthwhile. They further noted that infestations built up rapidly during the winter when the cattle were under stress and predisposed them to other diseases.

Recent work by Bailey, Carrol and Knee (1984) indicated that **L. vituli** infestation has little effect on the productivity of beef cattle. It was found that the incidence of lice increased during winter and declined in spring. Although the infestation was light in all the experiments, insecticide effectively controlled lice in the treated groups. In eight of the ten experiments the infestation did not result in lower growth rates but this may have been due to the generally light level of infestation present in the untreated animals. The decline in lice population during spring may have been due to effects of previous exposure as described by Callinan (1980) and Cummins and Graham (1982). Plane of nutrition, as expressed through liveweight change, in addition to seasonal effects may also

have influenced lice population. The significant correlations between infestation with **L. vituli** and decreased growth rate in two separate experiments were each influenced by one individual heifer with a high louse score and a lower growth rate than the remainder of group. The low percentage of the variation expressed by regression equations indicated that moderate levels of **L. vituli** infestation did not depress growth.

Examination of heifers within the untreated group indicated that no relationship existed between increasing infestation of **H. eurysternus** and liveweight change (Bailey **et al**, 1984). Callinan (1980) found that higher levels of artificially induced infestations of **L. vituli** than were observed in the experiments of Bailey **et al** (1984) did not significantly affect P.C.V., the levels of lymphocytes, polymorphs, eosinophils or red and white cells. The results of Bailey **et al** (1984) question the necessity for routine whole herd treatment against lice.

Scharff (1962) found that only about 1% of a herd had a very heavy infestation of **H. eurysternus** and thus concluded that cattle lice were, on average, of minor economic importance in Montana and that control measures were probably of little or no economic benefit on more than 95% of the cattle in the State. Unfortunately, the justification for this conclusion is reduced somewhat by the fact that most of the population data, collected on slaughter cattle, included details not only from cattle that had never been subjected to louse control measures, but also from undetermined numbers of those that had been treated at one time or another. However, the conclusion was supported by the fact that only 1-2% of a herd were

highly susceptible to the heaviest infestations of **H. eurysternus**, which were unquestionably capable of causing death in the most susceptible animals if timely control measures had not been taken. Rapid reinfestion with life-threatening populations recurred after control measures had been taken, so that occasionally as many as three treatments per year were required. Before symptoms of anaemia become apparent, heavily infested animals were readily recognisable in a herd. Their hair, especially in the brisket area, assumed a dirty, greasy appearance; and lice and their eggs were visible from a considerable distance (Scharff, 1962).

When heifers were restrained from self grooming, natural or artificially transferred infestations of **L. vituli** built up to heavy infestations and persisted (Lewis, Christenson and Gaines, 1967). Wood (1970) reported that lice rarely affect the physical condition of the cattle. Animals with highest louse numbers were otherwise debilitated causing a reduction on self-grooming, a feature also shown by Callinan (1980) using two different planes of nutrition, found that the calves were able to control lice without self-grooming.

Work carried out by Nelson **et al** (1970) suggested that louse infested cattle had blood eosinophilia, an indication of an allergic reaction in response to louse feeding. Periods of high louse numbers were associated with lowered red and white blood cells, haemoglobin and P.C.V.. Significant eosinophilia and depressed red cell counts in calves naturally infested with **L. vituli** were also found by Cummins and Tweddle (1977). In the work carried out by Callinan (1980) none of these described changes were apparent.

Roberts (1938), Scharff (1962) and Tweddle **et al** (1977) found that **L. vituli** occurred most frequently on calves with numbers decreasing as the animals matured. It was concluded by Rich (1966) that in most instances previous exposure to the parasite enables the host to control the development of subsequent populations of **L. vituli.** Callinan (1980) substantiated these findings by noting that louse numbers could only be increased by, and always declined, after artificial infestation.

1.10 EFFECT ON HOST SKIN

The skin constitutes the largest single tissue of the body and it is the tissue which is constantly being exposed to arthropods. The skin by its anatomical and histological structure also constitutes one of the main barriers against invasion of the body by pathogenic organisms including arthropods. It is tough, resilient and has a great capacity for expansion without losing much of its protective function. The hair can also be useful occasionally by preventing many arthropods from coming into direct contact with the skin surface (Gaafar, 1972).

Histologically, several layers of epithelial cell in various stages of keratinization within the epidermis form another important part of the skin and adds to the efficiency of the skin in its role as a barrier against the action of many physical and chemical factors in the environment, including many arthropods. The epithelial cells of the epidermis have the added strength of desmosomal attachments which obstructs all but very specialised organisms from penetrating the skin layers below. Epidermal cells also have a remarkable

ability to regenerate which is extremely important in healing and covering any damaged areas underneath.

It is only those arthropods which are able to penetrate the epidermal layers and so reach the dermis, that are able to obtain blood or deposit their products in a reactive part of the host's body. However, if the epidermis layer is covered in thick layers of keratin, many arthropods are incapable of penetration. Thus the avascularity of the epidermis could possibly be termed an adaptive resistance factor. Also important in preventing arthropods and other harmful organisms from penetrating deeper layers of the skin are the sebaceous and sweat glands. The lipoidal nature of the sebum and the electrolyte contents of sweat may actually neutralize and also limit the passage of any foreign material deposited on the skin surface (Gaafar, 1972).

Lloyd (1980) stated that the surface inhabitants of the mammalian skin may be divided into two groups. In one group are the visitors which spend a limited time on the skin while, in the other group, are the residents which are capable of completing their life cycle on or within the epidermis and hair coat. It is into the latter category which lice fall.

These residents can be divided into three groups. The first of these incorporates the obligate parasites such as lice whose presence on the skin is usually associated with clinically detectable damage to the epidermis and hair coat. The rest of the residents can be separated into saprophytes, commensals or the facultative parasites, such as scavenging mites (Lloyd, 1980).

The primary effect on the skin of any insect or acarine inhabitant is to break the integrity of the surface barrier layers (McEwan Jenkinson, 1980). The extent of this damage and therefore the resulting reaction in the host skin is dependent on the nature of the mouthparts and other structures used in penetration, and the mode of nutrition. It was Lavoipierre (1965) who classified the feeding methods of arthropod ectoparasites as telmophage feeders, which includes lysed material, tissue fluid and extravascular blood and solenophage feeding in which the mouthparts penetrate directly into the dermal vasculature. Pool feeding is most generally seen in the arthropods which possess blunt, round mouthparts and which tend to feed on the strateum corneum and living epidermis. Examples are the biting lice and the burrowing and scavenging mites. The solenophage feeding arthropods are characterised by the presence of sharp, pointed mouthparts which are able to penetrate directly into the superficial dermal blood and lymph vessels. Such mouthparts are seen in Diptera and sucking lice. The damaging effects caused by both the feeding or arthropod ectoparasites and the resulting host reaction has been extensively reviewed by Nelson, Bell, Clifford and Keirans (1977). There are also considerable differences in the extent of reactions by individuals of the same host species to attack by the same parasite. These reactions tend to be determined by the presence of toxic and antigenic substances which may be released by the arthropods and by the sensitivity of the host to these substances.

Histological examination of the host-arthropod interface can reveal details of feeding mechanism of the arthropod; extent of host-tissue damage (mechanical or immunological) caused by the feeding of

the arthropod; the nature of the host response to arthropod feeding which includes the changes apparent after intermittent or continuous exposure to the host. It is difficult, however, to differentiate between changes in host response resulting from hypersensitivity and those from reaction to simple trauma or irritation. The situation is further complicated in that allergic reactivity and inflammation caused by irritation share common characteristics. Knowledge of previous exposure, duration and level of infestation must be known to prevent such confusion.

As pathological changes in various inflammatory cell differ, distinction must be made between host response at known intervals at the feeding site of a single resident arthropod, and the response seen when random biopsies are taken from an area where unknown numbers of resident arthropods have fed repeatedly over an extended period.

Nelson **et al** (1977) hold the opinion that the net result on the host of periodic feeding depends to a somewhat large extent on what occurs at the host-parasite interface. Feeding involves the mouthparts, their total time in contact with the host tissue and the biochemical nature of oral secretions introduced into the host. Sprent (1969) and Theis and Budwiser (1974) hold similar views.

Acquired resistance of mice to **Polyplax serrata** was described as being the result of previous exposure and was more apparent on reinfested areas (Bell, Clifford, Moore and Raymond, 1966). The evidence these researchers put forward was that after a certain period of time the initial area reinfested was found unsuitable for

feeding and was vacated by the lice, which moved peripherally in order to continue feeding. Thus the physiologic expression of the state of acquired resistance becomes a function of the area available for reinfestation, along with the size and duration of the initial infestation. By injecting whole louse extract into the ears of resistant mice, followed by Evans blue dye intravenously, Nelson, Clifford, Bell and Hestekin (1972) demonstrated that the mice were indeed sensitised to the lice, even though normally the ears are not fed upon by lice. But if this sensitisation were totally protective, one would not expect a second population comparable to the first to be demonstrated on the same mouse. Histopathologically, a mixed inflammatory response indicated a possible sensitisation process in the first two weeks of infestation, followed by vasoconstriction and empty blood vessels for the following two weeks with achronic irritation response in the last eight weeks. Nelson **et al** (1977) concluded that chronic irritation played a major role in the acquisition of resistance.

As cattle lice are most numerous in winter when the animals skin is dry and scaly, and least in the spring, when the coat is shed and the skin becomes oilier, it has been suggested that lice are affected by this seasonal change (Underhill, 1923; Schull, 1932 and Roberts, 1938). Further evidence is required before deciding whether the lice decrease as a result of the theoretical physiological change of the skin or a higher oil content of the skin after coat casting in winter or a combination of the two in conjunction with other factors such as areas protected from sunlight. In summer lice tend to be found on the long dense hair of the ears and other long haired parts of the

body. The coat appears to be acting as a filter of the light intensity, which supports the previous arguments that the light factor is important in regulating the seasonal activities of lice.

The typical skin reaction that follows the bite of a blood-sucking insect has been studied in some detail, notably by Theodor (1935) in sandflies; Peck, Wright and Grant (1943) in body lice; Mellanby (1946) in mosquitoes; Gordon and Crewe (1948) in mosquitoes and tsetse flies; Goldman, Johnson and Ramsey (1952) in mosquitoes, bedbugs and fleas.

Gillet (1967) states that an immediate, and more or less violent reaction on the part of a sensitised host must present a special hazard to an insect if it is to obtain its blood meal and escape unharmed. The irritation which typically heralds the onset of the reaction often starts suddenly and unless the insect can complete its meal and leave the host, or the site of biting, it is in danger of being disturbed or even killed by the host.

If the speed of feeding in a species which elicits a sudden and violent reaction in a sensitised host has an inheritable component, then one may suppose that slower feeders in a population will be selected out. Moreover, in insects that depend on a blood meal for egg-production, only those that complete the meal before the onset of irritation will lay a full complement of eggs; the others will either be killed or injured by the host before egg development begins, or they will be disturbed before completion of the meal and so lay fewer eggs.

Five stages occurring in the allergic reactivity of guinea pigs to flea bites were defined by Larrivee, Benjamini, Feingold and Shimizu (1964). These are:

(i) **induction stage** during which no abnormal macroscopic or microscopic changes occur in the skin.

(ii) **stage of delayed skin reactions only,** characterised by intense mononuclear infiltration at the vicinity of the bite site some 24 hours following the bite.

(iii) **stage of immediate and delayed reactions,** where immediate reactions appearing 20 minutes after the bite and characterised by an eosinophilic infiltration; the delayed reaction appearing within 24 hours, characterised by mononuclear inflitration.

(iv) **stage of immediate reactivity only,** the reaction appearing 20 minutes after the bite and characterised by an inflitration of eosinophils at the bite site.

(v) **stage of nonreactivity resulting from desensitisation,** where no skin reactivity is observable and no cellular abnormalities occur.

However, the work of Larrivee **et al** (1964) differs from that of Nelson **et al** (1972) in two distinct ways. In the first place Larrivee **et al** (1964) found no neutrophils among the inflammatory cells at any stage of reactivity, though eosinophils were found. This suggests either that neutrophils were not present, or that biopsies were not taken often enough in the early sensitisation

period to reveal them, or that they were not distinguishable from eosinophils.

The second difference is that Larrivee **et al** (1964) showed five stages of reactivity, whereas Nelson **et al** (1972) inferred that the two phases of cellular reactivity seen in their work represented two mechanisms. The first was primarily an immune reaction during which the resistance was initiated, possibly with some added effect of the embedded stylets and prestomal teeth of the insects. The second is primarily a nonimmune reaction to chronic irritation, which serves to maintain the state of resistance until numbers of lice are reduced to minimal levels.

1.11 CONTROL MEASURES

Whilst it is known that lice cause irritation and restlessness there are conflicting views concerning their effects on livestock production. Chalmers and Charleston (1980a) in New Zealand found no significant difference in weight gain or haematocrit levels between louse-infested and louse-free animals. In Australia, Cummins and Tweddle (1977) were also unable to find a positive response to treatment. In America, however, Snipes, 1948 and Collins and Dewhirst, 1965 observed significant weight gains after control measures had been taken.

Understandably, commercial companies are interested in the economics involved in the development and production of any new chemical. More often than not, these new chemicals afford protection against a wide range of insects and acari of agricultural and

veterinary importance.

It was demonstrated by Price, Prigge and Dicke (1949) that lice infestations appeared to be lower on clipped than unclipped animals housed in the same pen. Allen and Dicke (1952) found that clipping the hair coat of heifers infested with chewing lice reduced the louse population to a very low level within a matter of hours, although lice were observed clinging to the short stubs of hair near the skin following clipping. Nits also remained on the hair stub. Price **et al** (1949) suggested that clipping appeared to be an effective means of control for the chewing louse. They pointed out, however, that re-establishment of heavy infestations on animals clipped early in the season may occur and that these infestatons may be prolonged beyond the normal seasonal incidence. They also stated that clipping did not control the sucking louse as effectively as it controls the chewing lice but that it does reduce the population substantially.

Application of 5 to 7 ounces of a dust containing 0.5% piperonyl butoxide, 0.05% pyrethrins and 0.20% rotenone, in sulphur, were very effective in controlling **H. eurysternus** on beef cattle (Snipes, 1948). Initial results showed a 40% reduction in populations within ten days with effectiveness lasting four months. However, the level of infestation and time of year the powder was applied was not stated.

Louse powder containing permethrin was found to reduce louse numbers by 85 to 95% with the residual population located on the brisket (Madder and Surgeoner, 1979). These authors suggest that the survival of these lice was probably due to insufficient powder

reaching the area.

Where lice were detected on cattle farmers usually treated them with louse powder containing gamma HCH (BHC). However, Titchener (1985) observed small populations of **L. vituli**, usually in the brisket area, present on cattle treated with this louse powder.

Although insecticidal powders have been used for many years, their labour intensive method of application, requiring the powder to be rubbed into the host hair coat, now makes them less favourable to farmers as new, easy to apply preparations are commercially available.

Systemic insecticides were first used in the treatment of cattle lice in 1947 when 2-pivalyl-1-1,3-indandione was administered orally to cattle infested with **H. eurysternus** (Bushland, Radeleff and Drummond, 1963). Unfortunately, the extrapolation of toxicological data from laboratory experiments using rats and mice to field conditions with cattle proved faulty and the animals died.

Dermal systemic insecticides were developed to combat cattle grubs, and were applied directly to the sites of infestation (Bushland **et al**, 1963). Initially, pour-on insecticides were generally recommended for use against cattle lice using a schedule involving two treatments 14 to 20 days apart but the trend has been toward developing a satisfactory one-treatment formulation. The second application was required because most insecticides neither affect louse eggs nor do they have sufficient residual activity to destroy newly emerged nymphs which may hatch after initial treatment, thus creating a source of reinfestation. A control measure requiring

only one treatment would increase convenience and decrease cost (Madder and Surgeoner, 1979).

Shemanchuk, Haufe and Thompson (1963) found that spraying cattle with insecticide was an effective method of louse eradication. Once the problem of how much pressure should be used to produce an optimum spraying mist was overcome they noted isolated colonies of lice present on all animals two weeks after spraying with two quarts of 0.5 per cent malathion, while no lice were observed to survive on animals treated with amounts greater than one gallon of the formulation. The authors conclude that successful treatment requires adequate coverage of the animals body. From the data provided by Craufurd-Benson (1941) the most suitable interval between spray treatments for insecticides with residual effect, was sixteen days. Two spray treatments at sixteen day intervals adequately encompassed the incubation period, the development of the three instars and the pre-oviposition period. The timing of spray treatments should be such that complete treatment is carried out before unfavourable weather sets in.

Two treatments were found superior to one by Buchanan and Coles (1971) when using an insecticide based on chlorpyrifos. When bound to cattle hair, or more likely to the organic detritus in the coat, it could have useful residual activity against lice. They also found that this insecticide is an extremely effective ovicide for the control of **L. vituli** and **D. bovis**. It was found that the emergence from the eggs was suppressed to some extent with diazinon and death generally occurred in the first few days after treatment. As with malathion, it appeared that older eggs were more susceptible. The

chlorpyrifos containing insecticide, on the other hand, appeared to have true ovicidal activity. Partial emergences were not recorded and the pattern of emergence showed that hatching of eggs of all ages was suppressed. Treated eggs soon became lighter in colour, flatter and desiccated while the embryos of these eggs failed to become defined or lost definition in a short time.

After pour-on systemic insecticide treatment in late November, December, January and early February, infestations of **L. vituli** on calves were below their pre-treatment densities in the following March (Rich 1966). He suggested that pour-ons provide an extremely practical method, requiring a minimum of application equipment for protection of calves against heavy infestations during late winter.

Nickel, Hyland, Gjurekovic and Brondke (1970) also found that two treatments with the pour-on fenthion was much more satisfactory than one. However, Kettle (1972) found that three insecticide formulations that had been used in an experiment were effective in controlling **L. vituli** but they did not give complete eradication even although each calf had received approximately twice the recommended amount of insecticide on a volume for weight basis.

Pour-on insecticides were suggested by Kettle and Pearce (1974) to have application in agriculture where small numbers of calves are to be treated or where lack of facilities makes spraying or shower treatment impossible. Their ease of application, and therefore a tendency to cause little disturbance to the calves is probably the greatest point in their favour.

It was reported by Kettle and Lukies (1979) that two separate pour-ons containing chlorpyrifos and temephos respectively gave a high degree of control but two others made up of phosmet and methidathion respectively were less effective. They pointed out difficulties in using pour-ons in order to control lice at that particular time. These negative effects included the expense of pour-ons as compared to sprays when large numbers of animals were to be treated; also no insecticide to that date in New Zealand had killed all the lice on the animals.

Until recently the control of infestation of stationary ectoparasites was essentially based on the use of organo phosphorous compounds of low toxicity which were applied to the cattle by the spray or dip method. The main problem with all these preparations, however, has always been the time-consuming and awkward application and the long withdrawal periods for meat and edible tissues. With the introduction of the pyrethroids into ectoparasite control there are a number of advantages to be gained from their usage, the most important being outstanding knock-down effect against all insects, combined with relatively long periods of effectiveness. In addition, the pyrethroids also possess the advantages of low mammalian toxicity and failure to accumulate in the environment. As there is no risk of accumulation in milk and edible tissue, their use entails no withholding periods for products after application (Liebisch, 1986).

Insecticidal ear tags have recently become a valuable new tool for the control of livestock pests. Ear tag effectiveness has been well documented by researchers across the United States of America. Insecticidal ear tags are commonly referred to as controlled release,

sustained release or slow release devices. Two key parameters critical to the effectiveness of such slow release devices for the delivery of pesticides to cattle are the rate of release of pesticide and the change in that rate over time on the animal. Miller, Oehler and Kunz (1983) noted that the release of pyrethroids from insecticidal ear tags follows Ficks Laws of diffusion. They also found that doubling the concentration in decamethrin tags more than doubled the release rate which was more pronounced the early stages of release. Both fenvalerate and permethrin ear tags lost their effectiveness when greater than 50 per cent of the pesticide was still present in the tag. Despite the great increase in efficiency of pesticide use afforded by the tags in comparison with convential spray treatments, ear tag formulations are still capable of considerable improvement (Millar **et al**, 1983). There have been resistance problems with ear tags for other ectoparasites in the United States of America, suggesting perhaps potential problems in lice control.

Different pour-on synthetic pyrethroids have been recently introduced to control flies on cattle at pasture. A study by Titchener (1985) showed that these too are highly effective against lice and, at the appropriate dose rate, they can be used on any age of animal at any time. Titchener (1985) also concluded that these new preparations, in the development of lice control, showed to be more efficacious than existing compounds. All are equally suitable for use on calves, although the author points out that if there are respiratory problems, spraying is best avoided. He goes on to suggest that larger animals found to be louse infested are best

treated with pour-on preparations, treatment being carried out effortlessly when the animals are feeding.

CHAPTER TWO

ANALYTICAL TECHNIQUES

2.1 INTRODUCTION

The analytical techniques used in this project will be described in the order in which they appear in the text. These methods will be described in full.

2.2 BLOOD ANALYSIS TECHNIQUES

2.2.1 Red Blood Cell Count

Blood samples in heparinised tubes were mixed. Using a Coulter Counter diluter (Coulter Electronics Ltd., Dunstable, England) each of the blood samples was diluted to a 1:500 ratio with Isoton (Coulter Electronics Ltd., Dunstable, England). 1 ml of this solution was further diluted to give a final blood to Isoton ratio of 1:50,000. Each of these final dilutions was read on a Coulter Counter (Model TA II) multichannel particle counter to give the number of red cells x 10^{12}/l of whole blood.

2.2.2 White Blood Cell Count

Blood samples in heparinised tubes were mixed. Using a Coulter Counter diluter each of the samples was diluted to a 1:500 ratio with Isoton. The diluent was mixed and then six drops of Zaponin (Coulter Counter Electronics Ltd.) was added to lyse and red blood cells. The solutions were read on a Coulter Counter Model TA II Multichannel particle counter to give the number of white blood cells x 10^{9}/l whole blood.

2.2.3 Haemoglobin

Heparinised blood samples were mixed and then haemoglobin concentration determined by the cyanmethaemoglobin method as described by the International Committee for Standardisation in Haematology (1967). The diluted samples were read on the absorbance mode of a spectrophotometer (Pye Unicam PU 8610 uv/vis kinetics spectrophotometer, Philips Scientific, Cambridge, England). Each of the observed absorbances was adjusted using a haemoglobin conversion table to give the haemoglobin content in g per 100 ml of whole blood.

2.2.4 Packed Cell Volume

Samples of blood in heparinised tubes were mixed. From each sample a capillary tube was three-quarters filled and then sealed with Cristalseal (Hawksley and Son Ltd., London, England). The capillary tubes were centrifuged at 1200 rpm for 6 minutes in a micro-haematocrit centrifuge (Hawksley and Son Ltd., London, England). After this each of the tubes was placed in a haematocrit reader (Harksley and Son Ltd., London, England) and the packed cell volume read.

2.2.5 Reticulocyte Counts

Heparinised blood samples were mixed and then three drops of blood from each tube were placed into individual plastic vials using a separate pastette for each sample. Into each vial was added 3 drops of methylene blue vital stain. The vials were incubated in an oven at 37^{o}C for 20 minutes. A smear on a labelled, glass slide was made from each sample. To carry out the reticulocyte count on each

slide one hundred red cells were counted and the number of reticulocytes contained in the same area noted to give a percentage value.

2.2.6 Eosinophil Counts

Heparinised blood samples were mixed and then a smear from each tube was made on a labelled glass slide. The smears were stained using Wrights giemsa (Sigma, Poole, England), 1 ml per slide. After one minute 2 ml of deionised water was added to each slide. Five minutes later the slides were washed with deionised water and allowed to air dry.

On each slide a random field of one hundred cells was observed and the number of eosinophils noted as a percentage.

2.2.7 Serum Total Protein Determination

2.2.7.1 SERUM PREPARATION

Blood samples were placed in heparin-free tubes and a wooden applicator stick inserted to help retract the clot and so extrude the serum. After being left overnight at room temperature the stick with clot attached was removed and discarded. The tubes were centrifuged at 1800 g (3200 rpm in a Super Minor Centrifuge, Measuring Scientific Equipment, London, England) for 10 minutes, during which time any remaining erythrocytes formed a sediment at the base of the tube. The serum was then aspirated off using a separate disposable pastette for each sample and placed in individual labelled plastic vials. Serum remaining after protein and albumin tests was frozen at -20^{o}C for future use.

2.2.7.2 TEST PROCEDURE

2.5 ml biuret reagent (Sigma, Poole, England) was put into labelled test tubes, 50 ul Autoset (Wellcome Foundation Ltd., Crewe, England) was added to two tubes to act as standards. For the two control tubes, 50 ul Seronorm 66 (Nyegaard and Co., Oslo, Norway) was added to the biuret reagent. 50 ul of each sample was placed in separate reagent only tubes. All tubes were mixed for 15-30 seconds. After leaving to stand at room temperature for 20 minutes the tubes were read on a spectrophotometer (Pye Unicam PU 8610, Philips Scientific, Cambridge, England) at 540 m to give the amount of protein in grammes per litre of whole blood.

2.2.8 Serum Albumin Determination

2.2.8.1 SERUM PREPARATION

As described in 2.2.7.1.

2.2.8.2 TEST PROCEDURE

2.5 ml Bromocresol green reagent (Sigma, Poole, England) was placed in disposable, labelled plastic test tubes. 10 ul Autoset (Wellcome Foundation Ltd., Crewe, England) was also placed in two of the tubes to act as standards and 10 ul Seronorm 66 (Nyegaard and Co., Oslo, Norway) in another two designated as controls. Into the remaining reagent only tubes 10 ul of serum was added, each sample being placed in a separate tube. All the tubes were mixed for 15-30 seconds and then allowed to stand at room temperature for 20 minutes. The tubes were then read on a spectrophotometer (Pye Unicam PU 8610, Philips Scientific, Cambridge, England) at 630 m to give the amount

of albumen in grammes per litre of whole blood.

2.3 ERYTHROKINETIC AND PLASMA PROTEIN STUDIES

2.3.1 Packed Cell Volume

From each of the heparinised blood samples packed cell volumes (P.C.V.) were determined. The microhaematocrit capillary tubes were sealed with Cristaseal (Hawksley and Son Ltd., London, England), centrifuged for 6 minutes at 1200 rpm in a microhaematocrit centrifuge (Hawksley and Son Ltd., London, England). The packed cell volume was read on a microhaematocrit reader (Hawksley and Son Ltd., London, England).

2.3.2 Whole Blood and Plasma Radioisotope Determination

One millilitre of heparinised blood was pipetted out from the sample into radioisotope counting vials and made up to the standard volume (10 ml) with dilute sodium hydroxide. The plasma was separated from each of the raw blood samples by centrifugation (Super Minor, 3200 rpm, Measuring Scientific Equipment, London, England). 1 ml of this plasma was pipetted into counting vials, then made up to the standard volume with dilute sodium hydroxide.

2.3.3 Serum Albumin Determination

2.3.3.1 SERUM PREPARATION

As described in 2.2.8.1.

2.3.3.2 TEST PROCEDURE

2.3.3.2.1 Reagents

(i) Stock Bromocresol Green Dye Solution

0.432 g of the sodium salt of bromocresol green was dissolved (or 0.432 g of bromocresol green in 20 ml of 0.1 N NaOH) and diluted to 1 litre with distilled water.

(ii) Buffer-Indicator, 0.05 M phosphate, pH 7.0, containing 4.5×10^{-5}M bromocresol green

2.654 g of KH_2PO_4 and 4.330 g anhydroyuws Na_2HPO_4 was dissolved in 500 ml of distilled water in a 1 litre volumetric flask. 75 ml of the stock bromocresol green dye (point (i) above) was added and diluted to 1 litre with distilled water.

(iii) Albumin standard

A solution of approximately 3%, using bovine serum albumin (Sigma, Poole, England) and distilled water was prepared.

2.3.3.2.2 Procedure

(i) 15 ml of the buffer-indicator solution was placed in the required number of tubes for duplicate tests, blanks and standard estimations.

(ii) 100 ul of 'test' solution was added to the tubes containing buffer-indicator solution.

(iii) 100 ul of the albumin standard solution was added to two tubes containing 15 ml buffer-indicator solution.

(iv) A 'reagent blank' was prepared by adding 100 ul distilled water to 15 ml buffer-indicator solution.

(v) A reference (or zero) blank was prepared by adding 5 ml of water to 10 ml of the buffer-indicator solution.

(vi) All the tubes were mixed gently.

(vii) After setting the spectrophotometer (SP 600, Philips Scientific, Cambridge, England) at zero with the zero blank, the absorbance of each tube was read at 615 mu.

2.3.3.2.3 Calculation

$$C_T = T \times \frac{Cs}{s}$$

Where:

CT = concentration of test sample

Cs = concentration of standard solution

T = difference in absorbance between test and protein-free sample

s = difference in absorbance between standard and protein-free sample

2.3.4 Serum Total Protein Determination

2.3.4.1 SERUM PREPARATION

As described in 2.3.3.1.

2.3.4.2 TEST PROCEDURE

2.3.4.2.1 Reagent

(i) Biruet reagent

90 g of sodium potassium tartrate was dissolved in 400 ml of 0.2 N NaOH. 10 g of cupric sulphate was added. Following complete solution of the cupric sulphate, 10 g of potassium iodide were dissolved in the solution. The solution was then diluted to 2 litres with 0.2 N NaOH.

2.3.4.2.2 Procedure

(i) 5.0 ml of distilled water was pipetted into the required number of tubes.

(ii) 0.2 ml of the test serum (in duplicate) was added to the 5.0 ml of distilled water.

(iii) 5.0 ml of Biruet reagent was added to each tube, mixed well and allowed to stand at room temperature for 30 minutes.

(iv) 'Blanks' were prepared as above using 0.2 ml of distilled water in place of serum.

(v) After 30 minutes the test and standard samples were read against the 'blank' on a SP 600 spectrophotometer at wavelength 540 mu (Philips Scientific, Cambridge, England).

2.3.4.2.3 Calculation

$$CT = OD \text{ of } T \times \frac{Cs}{OD \text{ of } Std}$$

Where:

CT = concentration of test sample

OD of T = optical density of test sample

Cs = concentration of standard solution

OD of Std = optical density of standard solution

2.3.5 Serum Iron Determination

2.3.5.1 SERUM PREPARATION

As described in 2.2.8.1.

2.3.5.2 TEST PROCEDURE

2.3.5.2.1 Reagent

An Iron BP Test Kit was used for the serum iron determination which was supplied by Roche Diagnostics, Switzerland. The reagent was made up using the instructions enclosed in the kit.

2.3.5.2.2 Procedure

(i) 1.5 ml of reagent was pipetted into the required number of tubes.

(ii) 0.5 ml of test serum (in duplicate) was added to the 1.5 ml reagent.

(iii) 'Blanks' were prepared as above using 0.5 ml distilled water in place of serum.

(iv) All test samples were read against the 'blank' on a SP 600 spectrophotometer at wavelength 546 nm (Philips Scientific,

Cambridge, England).

2.3.5.2.3 Calculation

$$A(T) - A(RB) = A(T)$$

Where:

A(T) = absorbance of test samples

A(RB) = absorbance of 'blank' samples

A(T) = correct absorbance of test samples

To convert to millilitres each of the A(T) figures are multiplied by 188:

$$A(T) \times 188 = \text{millilitres (M)}$$

To convert to mg/100 ml:

$$M \times \frac{5584}{10_6} = \text{mg/100 ml}$$

2.4 PROTEIN ESTIMATION OF SALIVARY GLANDS

2.4.1 Salivary Gland Preparation

Salivary glands were removed from recently obtained **Linognathus vituli.** Fifty glands were placed in separate plastic microcentrifuge tubes with lids (Sigma, Poole, England) and frozen at -20°C until required.

2.4.2 Test Procedure

2.4.2.1 PREPARATION OF STANDARD SOLUTIONS

2 mg/ml bovine serum albumin (BSA) was used to prepare the protein standards as follows:

Diluting 100 ul of 2 mg/ml BSA and 900 ul distilled water gives a 200 ug/ml dilution.

900 ul distilled water + 100 ul BSA (2 mg/ml) = 200 ug/ml (i)

740 ul distilled water + 60 ul of (i) = 150 ug/ml (ii)

400 ul distilled water + 400 ul of (ii) = 100 ug/ml (iii)

400 ul distilled water + 400 ul of (iii) = 50 ug/ml (iv)

400 ul distilled water + 400 ul of (iv) = 25 ug/ml

2.4.2.2 PREPARATION OF WORKING REAGENT

Using Pierce BSA Protein Reagent supplied by Pierce, Cambridge, England, working reagent was prepared by mixing 50 parts of Reagent A with 1 part Reagent B. This working reagent was stable for one week at room temperature.

2.4.2.3 PROCEDURE

(i) 2 ml of working reagent was pipetted into the required number of tubes.

(ii) 100 ul of distilled water was added to each defrosted test salivary gland sample and added (in duplicate) to the 2 ml working reagent.

(iii) 100 ul of each standard (in duplicate) was added to the 2 ml working reagent.

(iv) 'Blanks' were prepared as above using 100 ul distilled water in place of the salivary glands.

(v) All solutions were mixed immediately using a multi-vortex mixer and then incubated in a 60°C water bath for 30 minutes.

(vi) All the tubes were cooled to room temperature and the absorbance of each read at 562 nm on a uv spectrophotometer (Pye Unicam PU 8610 uv/vis kinetics spectrophotometer, Philips Scientific, Cambridge, England).

2.4.2.4 CALCULATION

The blank absorbance readings were subtracted from the standard and sample readings. A standard curve was plotted of blank corrected absorbance at 562 nm against protein concentration of the standards. From the graph produced the protein concentration of the samples was determined.

2.5 ISOELECTRIC FOCUSING OF SALIVARY GLANDS

2.5.1 Salivary Gland Preparation

500 **L. vituli** salivary glands were placed in 0.5 ml of Ringers Solution which consists of:

sodium chloride	2.250 g/l
potassium chloride	0.105 g/l
calcium chloride	0.120 g/l

sodium bicarbonate 0.050 g/l

pH 7.0 0.050 g/l

0.2 ml disaggregating buffer was added and the samples placed in a 100°C water bath for 3 minutes.

(Disaggregating buffer: Tris 1.21 g/l
EDTA 0.29 g/l
containing 1% S.D.S. and 5% mercaptoethanol).

2.5.2 Focusing Procedure

2.5.2.1 APPARATUS

A flat bed apparatus FBE-300 (Pharmacia Ltd., Central Milton Keynes, England) was attached to the cold water supply and mains power. Several drops of n-decane (Serva, Heidelberg, West Germany) were added down one side of the cooling plate on the flat bed apparatus. A Precote Gel (Serva, Heidelberg, West Germany) was aligned along the n-decane solution, the protective cover from the back of the gel being peeled off whilst wearing disposable gloves and taking care not to touch the gel. The gel wicks were saturated with the appropriate fluid (anode or cathode; Serva, Heidelberg, West Germany) and placed on the gel:

(i) corresponding to where the electrodes were to be placed;
(ii) parallel to each other;
(iii) with a 5 mm space from each wick end to the edge of the gel.

The applicator strip was placed parallel to the wicks and in the centre of the gel.

2.5.2.2 APPLICATION OF SAMPLES ONTO GEL

The samples were placed in the slots of the applicator strip using a microsyringe. Molecular weight standards (Sigma, Poole, England) were also injected into separate spaces in the applicator strip.

2.5.2.3 POWER

The electrodes were centred onto the wicks and the power applied to a maximum voltage of 1500 volts and 3800 volthours using an electrophoresis constant power supply, ECPS 3000/150, and volthour integrator, VH-1 (Pharmacia Ltd., Central Milton Keynes, England).

2.5.2.4 STORAGE OF GEL

Once focusing was complete the gel was removed, placed on absorbant paper and left to dry at room temperature. When dry the gel was stored in a protective transparent cover.

2.6 POLYACRYLAMIDE GEL ELECTROPHORESIS

2.6.1 Apparatus

2.6.1.1 GEL HOLDER AND ELECTROPHORESIS TANK

The cooled vertical slab gel apparatus used was the Protean Cell (Bio-Rad Laboratories, New York, U.S.A.).

Up to two slab gels can be electrophoresed simultaneously. Each slab is formed between two glass plates 16.0 x 18.0 x 0.3 cm, which are held apart by P.V.C. spacers, 0.15 cm thick, placed down each of the two vertical sides of the sandwich. The spacers are held in position by one-piece plastic clamps. The bottom of the sandwich is sealed using polycarbonate cams which press the base against a silicone rubber gasket in a casting stand. After gel pouring, polymerisaton, sample-well formation and sample loading, as described later in the text, the slab gel sandwich is released from the casting stand and locked in position against another silicone rubber gasket in the upper buffer reservoir using the same cam system. The upper and lower reservoirs are filled with the correct electrophoresis buffer and that in the lower reservoir is cooled by collant passing through a glass tube heat exchanger, placed between two slab gels, and stirred by a magnetic stir bar.

2.6.2 Additional Items of Equipment Required for Electrophoresis

Additional items of equipment which are required apart from standard laboratory glassware and magnetic stirrers are given below.

(i) Microlitre syringes

These are used for loading samples onto the gel. The control needed for careful sample loading the narrow sample wells in slab gels was best coped with by using a 50 ul or 100 ul microsyringe.

(ii) Power pack

This was capable of supplying about 500 V and 100 mA.

2.6.3 Preparation of Solutions for Polyacrylamide Gels

2.6.3.1 STOCK SOLUTIONS

2.6.3.1.1 Acrylamide-bisacrylamide (30:0.8)

30 g of acrylamide (Sigma, Poole, England) and 0.8 g bisacrylamide (Sigma, Poole, England) were dissolved in 80 ml distilled water. 2 g amberlite (BDH Limited, Poole, England) was added with the solution being stirred for 30 minutes. After filtering the solution was made up to 100 ml with distilled water and stored at 4°C until required, storage time being 1-2 months.

2.6.3.1.2 Temed (N,N,N', N'tetramethylenediamine)

This was used as supplied (Sigma, Poole, England). It was stable in undiluted solution at 4°C in a dark bottle.

2.6.3.1.3 Ammonium persulphate (1.5%, w/v)

0.15 g of ammonium persulphate (Sigma, Poole, England) was dissolved in 10 ml distilled water. The solution is unstable and was made fresh just before use.

2.6.3.1.4 Stacking Gel Buffer: pH 6.8

7.57 g Tris (Sigma, Poole, England) was placed in 20 ml of distilled water and titrated to pH 6.8 with 0.1 M hydrochloric acid. 2 g S.D.S. (sodium dodecyl sulphate; Sigma, Poole, England) was then added and enough distilled water to make the solution up to 500 ml. This buffer was stored for several weeks at room temperature.

2.6.3.1.5 Resolving Gel Buffer: pH 8.8

22.7 g Tris was placed in 20 ml of distilled water and titrated to pH 8.8 with 0.1 M hydrochloric acid. 2 g of S.D.S. was then added and enough distilled water to make the solution up to 500 ml. This buffer was stored for several weeks at room temperature.

2.6.3.1.6 Electrophoresis Buffers

(i) Lower tank buffer

30 g Tris, 144 g glycine (Sigma, Poole, England) and 5 g S.D.S. were added to 4.5 l of distilled water, stirred until dissolved and then made up to 5.0 l with more distilled water. This solution was stored at 4^{o}C but was used within 48 hours.

(ii) Upper tank buffer

4 ml mercaptoethanol (Sigma, Poole, England) was added to 2 l of lower tank buffer. Mercaptoethanol is extremely toxic, therefore gloves and a fume cupboard were used.

2.6.4 Preparation of Slab Gels

Disposable gloves were worn during slab gel preparation to prevent contamination of clean glass plates with skin proteins.

2.6.4.1 DISCONTINUOUS BUFFER SYSTEM

The use of a stacking gel polymerised on top of the resolving gel, as required by the discontinuous buffer system, meant that sample wells were formed in the stacking gel.

2.6.4.1.1 Glass Plates

It is most important to ensure that the slab gel plates are perfectly clean to obtain good cell adhesion to the glass. The sides of the plates which were to be in contact with the gel were swabbed with an acetone-soaked tissue held in a gloved hand. After a final rinse with ethanol the plates were allowed to air dry.

2.6.4.1.2 Assembly of Gel Plates in Casting Stand

The glass plates were held the correct distance apart by thin Teflon spacers (1.5 mm wide) which must be of uniform thickness, both with respect to each other and along their length to ensure good contact with the plates and a gel of uniform thickness. The spacers were placed down the sides of one of the plates and the other plate then placed on top of this using gloved hands. The plate assembly was clamped together at each side using one-piece plastic clamps. The assembly was then stood vertically in a casting stand where the base of the sandwich was pressed against a silicone rubber gasket. The base of the plate assembly was sealed with a 1% water agar (agar number 3; Sigma, Poole, England) solution which was boiled and then immediately placed at the bottom of the sandwich by a pasteur pipette and allowed to set.

2.6.4.1.3 Resolving Gel Mixture: 12.6%

20.5 ml acrylamide, 12.5 ml resolving gel buffer and 50 ul TEMED were placed in a measuring cylinder and the mixture made up to 50 ml with distilled water. This solution was then degassed for 1 minute using a water pump. 0.5 ml ammonium persulphate was added and the

gel cast immediately.

2.6.4.1.4 Pouring Resolving Gel

The resolving gel mixture was poured into the space between the two glass plates and a plastic bridge inserted to allow sufficient space at the top for a stacking gel to be polymerised later and sample wells formed. Polymerisation took 30 minutes.

2.6.4.1.5 Stacking Gel Mixture: 4%

6.5 ml acrylamide, 12.5 ml stacking gel buffer and 50 ul TEMED were placed in a measuring cylinder and made up to 50 ml with distilled water. The mixture was then degassed for 1 minute using a water pump. 0.5 ml ammonium persulphate was added and the gel cast immediately.

2.6.4.1.6 Pouring Stacking Gel

After the stacking gel was poured on top of the resolving gel a comb was inserted immediately into the stacking gel mixture, being careful to avoid trapping any air bubbles beneath it. The assembly was left undisturbed whilst the stacking gel polymerised which took 45 minutes.

2.6.4.1.7 Sample Wells

After polymerisation the comb was carefully removed to expose the sample wells which were rinsed with upper tank electrophoresis buffer and then filled with this buffer. Any divisions between wells that were displaced during comb removal were straightened with a syringe needle. Once the stacking gel had polymerised the slab gel

was used immediately.

2.6.5 Sample Preparation

2.6.5.1 SALIVARY GLANDS

Salivary glands from live lice were removed and stored in disagregating buffer (0.2 ml) in a plastic microcentrifuge tube with lid (Sigma, Poole, England). 100 glands were placed in each tube and frozen until required. Prior to electrophoresis the required number of glands were pooled together and heated in a boiling water bath for 3 minutes. This ensured denaturation of the proteins. After heating the sample was allowed to cool at room temperature.

2.6.6 Molecular Weight Standards

Whenever S.D.S.-PAGE (polyacrylamide gel electrophoresis) is used, it is wise to include a mixture of polypeptides of known molecular weight. Whilst this is essential for determining the molecular weight of sample polypeptides, it is also worthwhile if only undertaking a quantitive assessment of the protein composition of samples since it provides a measure of reproducibility between different gel runs.

Each of the standard proteins (Sigma, Poole, England) were dissolved in disagregating buffer at a concentration of 1 mg/ml, heated at 100^{o}C for 3 minutes and stored frozen in small aliquots. When a sample was to be analysed, an aliquot of molecular weight markers was thawed out, warmed to dissolve any precipitated S.D.S. and run on parallel lanes of the slab gel.

2.6.7 Sample Loading and Electrophoresis

2.6.7.1 APPARATUS

After polymerisation of the stacking gel, the slab gel sandwich was released from the casting stand and locked into position against a silicone rubber gasket in the upper buffer reservoir.

2.6.7.2 ADDITION OF BUFFERS

Reservoir buffer was added to the lower reservoir of the electrophoresis apparatus. Upper buffer was placed into each sample well using a pasteur pipette. This served to fill the space above the gel with reservoir buffer, hence removing any air bubbles present.

2.6.7.3 SAMPLE LOADING

The samples were carefully loaded onto the gel surface using a microsyringe. The tip of the sample applicator was held 1-2 mm above the gel surface to minimise sample mixing with the reservoir buffer during loading. The dense sample solution flowed onto the gel surface and formed a sharply defined layer.

2.6.7.4 APPLICATION OF POWER

The electrophoresis apparatus was connected to the power pack with the anode connected to the lower reservoir and the cathode connected to the upper one.

2.6.8 Recovery of Gels

Slab gels were easily recovered by removing the side spacers and gently levering the glass plates apart. Once the gel had been recovered it was essential to mark which end was which and also indicate the right and left sides of the gel to ensure the lanes were read correctly.

Both of these requirements were met by making a small notch into the lower left side of the gel. Alternatively one could inject a small amount of India ink at this point.

2.6.9 Staining of Gels

2.6.9.1 COOMASSIE BLUE STAIN

2.6.9.1.1 Staining

Coomassie blue R250 (0.1%; Sigma, Poole, England) was dissolved in water, methanol, glacial acetic acid (5:5:2 by volume) and filtered through a Whatman No 1 filter paper (Gallenkamp, Loughborough, England) to remove any insoluble material before use. The slab gel was placed in a plastic tray containing the stain solution and left overnight.

2.6.9.1.2 Destaining

After staining was complete, the excess stain had to be removed to allow the protein bands to be clearly seen.

The destaining solution was made up of 25% methanol, 10% glacial acetic acid and 65% distilled water. The gel was placed in this solution for 36 hours during which period the destaining solution was changed four times. Gels should not be stored in this solution since the protein bands are also eventually destained.

2.6.9.2 SILVER STAIN

2.6.9.2.1 Reagents Using Silver Stain Kit for Polyacrylamide Gels AG-5 (Sigma, Poole, England)

(i) Fixing solution (30% ethanol, 10% glacial acetic acid solution)

270 ml of ethanol was mixed with 90 ml glacial acetic acid and then made up to 900 ml with distilled water.

(ii) Silver equilibrium solution

1.5 ml of silver concentrate was diluted to 300 ml with deionized water.

(iii) Development solution

30 ml of developer 1 concentrate was diluted to 300 ml with deionized water and then 0.17 ml of developer 2 concentrate was added. This solution was stable for up to 2 hours.

(iv) Stop solution (10% acetic acid solution)

3 ml of glacial acetic acid were added to 297 ml of deionized water.

(v) Reducer solution

2.0 ml reducer A concentrate, 4.0 ml reducer B concentrate and 0.7 ml reducer C concentrate were mixed together and then diluted to 300 ml with deionized water. This solution was stable for 1 day or until it turned green or blue.

2.6.9.2.2 Procedure

Disposable gloves were always worn as figerprints on gels are detectable.

(i) Fixing

After removal from the electrophoresis chamber the gel was immediately placed in fixing solution. The gel was left in 300 ml of fixing solution for 1 hour, the solution being completely changed three times within that period. After fixing the gel was rinsed three times with 300 ml deionized water, each change taking place after 10 minutes.

(ii) Silver equilibrium

The gel was placed in 300 ml silver equilibrium solution and allowed to equilibrate with gentle agitation for 30 minutes. The gel was then rinsed for 10-20 seconds in 300 ml deionized water.

(iii) Development of gel

150 ml of developer solution was placed over the gel. After 5-8 minutes this solution was discarded and replaced with the remainder of the developer solution. The gel was watched carefully to achieve the darkest bands while minimizing the

yellowing or darkening of the gel. To stop the development the developer solution was replaced with stop solution for 5 minutes and then rinsed three times with 300 ml deionized water, each change was at 10 minute intervals.

(iv) Reducer rinse

The gel was placed in 300 ml of reducer solution for 10-20 seconds. This solution was poured off and the gel immediately rinsed under cold running tap water for 1 minute. Afterwards the gel was rinsed three times with 300 ml deionized water, each rinse after 10 minutes.

(v) Recycle option

If increased sensitivity is desired, the gel can be recycled through the staining procedure starting with (ii) to (iv).

(vi) Storage of stained gel

The gel can be stored in deionized water indefinitely or dry after equilibration in 2% aqueous glycerol solution. Gel bands did not fade for up to three months.

2.6.10 Determination of Molecular Weights for Sample

The relative mobility, Rf, refers to the mobility of the protein band of interest measured with reference to a tracking dye where:

$$Rf = \frac{\text{distance migrated by protein}}{\text{distance migrated by dye}}$$

Using standard marker polypeptides or known molecular weight a plot of $\log_{10}$ polypeptide molecular weight versus relative mobility

(Rf) reveals a straight line relationship. For each gel, therefore, a set of marker polypeptides of known molecular weight were electrophoresed and the distance migrated by each used construct a standard curve from which the molecular weight of the sample polypeptides could be calculated based on their mobility under the same electrophoretic conditions.

2.7 ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)

2.7.1 Pre-ELISA Analysis

As a preliminary step before the ELISA assay was carried out a test using fresh salivary glands on microscope slides was completed. This was done to establish whether or not salivary glands would adhere to the solid phase and the possible concentrations of sera and conjugate necessary for the analysis. The test was carried out on microscope slides using fresh salivary glands in order to maintain the limited number of glands already prepared and in the frozen state.

The test was carried out as follows:

(i) Five freshly dissected salivary glands were placed on a slide and allowed to air dry. Five slides of this nature were prepared.

(ii) The slides were placed in 100 ml methanol containing 75 ul concentrated hydrochloric acid and 500 ul hydrogen peroxide.

(iii) The slides were washed with 3 five minute changes of wash buffer (see 2.7.3.4).

(iv) Four slides were flooded with a 1:10 dilution of louse infested calf sera. The remaining slide was flooded with 1:10 dilution of louse free calf sera. The slides were left at room temperature for one hour.

(v) Three, five minute changes in wash buffer.

(vi) Conjugate was added to each slide. The four louse infested sera slides had conjugate added in 1:50, 1:100, 1:200 and 1:500 dilutions with that added to the louse free sera slide being 1:200. All the slides were left for one hour at room temperature.

(vii) Three, five minute changes with wash buffer.

(viii) All the slides were flooded with substrate. The substrate was made up by adding 15 mg 3,3'diaminobenzidine to 50 ml phosphate buffered saline. Immediately before use 16 ul hydrogen peroxide was added to the solution. The slides were left for 20 minutes at room temperature.

(ix) Three, five minute changes with wash buffer.

(x) The slides were immersed in:

30% methanol	5 minutes
50% methanol	5 minutes
70% methanol	5 minutes
100% methanol	5 minutes
100% methanol	5 minutes

The slides were preserved by the addition of D.P.X. mounting medium (Sigma, Poole, England) and a coverslip.

2.7.2 Preparation of Samples

One hundred and fifty salivary glands were removed from **L. vituli** lice and placed in 2 ul of distilled water in separate, plastic microcentrifuge tubes with lids (Sigma, Poole, England) and stored at -20^{o}C until required.

2.7.3 Stock Solutions

2.7.3.1 COATING BUFFER (Carbonate-Bicarbonate pH 9.6)

1.50 g of sodium carbonate (Sigma, Poole, England), together with 2.93 g sodium hydrogen carbonate (Sigma, Poole, England) and 0.20 g sodium azide (Sigma, Poole, England) were mixed in distilled water to a final volume of 1 litre. This solution was stored at 4^{o}C and was made fresh every two weeks.

2.7.3.2 DILUTING BUFFER

Phosphate buffered saline (PBS) pH 7.2 was made initially by mixing 8.50 g sodium chloride (Sigma, Poole, England), 0.32 g sodium di-hydrogen phosphate and 1.10 g di-sodium hydrogen phosphate in distilled water to a final volume of 1 litre. To make the diluting buffer 100 ml of PBS had 50 ul Tween 20 (Sigma, Poole, England) and 10 g Marvel (skimmed milk powder product used in coffee, manufactured by Cadbury-Tetley, Manchester, England) added, mixed on a magnetic stirrer and then filtered before use. This solution was made fresh daily.

2.7.3.3 SUBSTRATE

For one ELISA plate 5 ml distilled water, 2.5 ml 1 M citric acid, 2.5 ml disodium hydrogen phosphate, 100 ul OPD* and 5 ul hydrogen peroxide were mixed together. This solution was made fresh just before use.

*WARNING OPD - orthophenylenediamine
This was made up as follows:

OPD (brown powder or crystals) was obtained as a free base. Care was required as OPD is a mutagenic compound. Contact and inhalation was avoided whilst making up the solution. 0.40 g OPD was dissolved in a 1 litre solution of distilled water containing 5.11 g 1 M citric acid and 9.15 g di-sodium hydrogen phosphate. The solution was colourless or had a very slight tinge of brown. It was stored in dark bottles at $4^{o}C$ for one week and discarded if the solution turned brown before one week had elapsed.

2.7.3.4 WASH BUFFER

5.35 g di-sodium hydrogen phosphate (andhydrous) (Sigma, Poole, England) was mixed together with 1.95 g sodium di-hydrogen phosphate (Sigma, Poole, England), 42.50 g sodium chloride (Sigma, Poole, England) and 2.5 ml Tween 20 (Sigma, Poole, England) in distilled water to a final volume of 5 litres.

2.7.4 Preparation of Sera

The sera which was used was that obtained from the blood profile experiment (Chapter 3), the preparation of which is described in

Chapter 2, section 2.2.8.1. Before being applied to the ELISA plate the sera was diluted to the required concentration with diluting buffer.

2.7.5 Preparation of Conjugate

The conjugate used was anti-bovine IgG (whole molecule) peroxidase supplied by Sigma, Poole, England, and before being applied to the ELISA plate was diluted to the required concentration with diluting buffer.

2.7.6 ELISA Procedure

2.7.6.1 APPLICATION OF ANTIGEN TO ELISA PLATE

For each plate one microcentrifuge tube containing 150 salivary glands was mixed with 5 ml coating buffer in a trough for multichannel pipettes. Using a multi-tip micropipette 50 ul of this solution was added to each well of the ELISA plate (flat bottomed microelisa plates, immulon cobalt sterilized M129B, supplied by Dynatech Laboratories Ltd., Billinghurst, England). The plate was then left overnight at 4^{o}C or put in an incubator at 37^{o}C for two hours.

2.7.6.2 WASHING

After the appropriate incubation period the plate was washed. The principle was to discard the contents of the wells by "flicking" them into a sink (or suitable container-bowl, etc.), then adding wash buffer which was then flicked away. This washing was repeated four times and then the plates were turned onto absorbent paper to remove

the majority of the residual wash buffer by gently tapping the plates against the paper, well openings down.

2.7.6.3 APPLICATION OF SERA

In order to discern the appropriate working dilution of the sera it had to be titrated. This was done by means of a chessboard titration. The sera was diluted to a 1:5 concentration, 1 ml being prepared. Using a single channel micropipette 100 ul of diluted sera was added to each well of row A (column 2 to 12, see Figure 2.1).

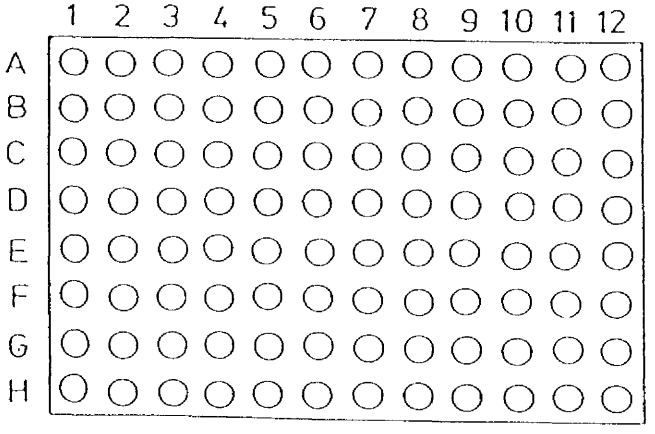


Figure 2.1

50 ul of diluting buffer only was added to all the other wells on the plate. Using a multi-tip micropipette placed in row A the contents of the row were mixed by pipetting 6 times. 50 ul were transferred to row B (2 to 12), the mixing process repeated. This transfer process was repeated to row H (2 to 12). After the last mixing in column 12 50 ul was removed and discarded. Column 1 contained only buffer and acted as a blank reference for the plate reader. A lid was placed on the plate which was then placed at 37^{o}C for 30 minutes. After the incubation period was complete the plate was washed as described in 2.7.6.2.

When the optimum dilution of the sera had been discerned all the wells had 50 ul of this concentration of sera added to them and then the plate was placed on a shaker (Titertek, Flow Laboratories, Richmansworth, England) for 30 minutes at room temperature and then washed before conjugate could be added. Before the sera was added the plate was "blocked" for 30 minutes by the application of diluting buffer only to all of the wells and then shaken for 30 minutes, the plate being washed afterwards.

2.7.6.4 APPLICATION OF CONJUGATE

Initially the conjugate optimum working dilution had to be discerned. This was done by means of completion of the chessboard titration initiated by the sera. The conjugate (anti-bovie IgG (whole molecule supplied by Sigma, Poole, England)) was diluted to 1:200, 1 ml being prepared. Using a multi-tip micropipette 100 ul of conjugate wad added to column 2 (A to H). All the other wells had 50 ul of diluting buffer added to them. Using a multi-tip micropipette placed in the wells of column 2 the contents were mixed by pipetting eight times. 50 ul was then transferred from column 2 to column 3 (A to H). Column 3 was then mixed. The transfer process continued to column 12, where after mixing 50 ul was removed and discarded. A lid was placed on the plate which was then put in an incubator at 37^{o}C for 30 minutes. Washing was repeated when incubation was complete.

When the optimum working dilution of the conjugate had been discerned 50 ul of this concentraton was placed in all of the wells and the plate shaken at room temperature and then washed.

2.7.6.5 ADDITION OF SUBSTRATE

When washing was completed after addition of conjugate the substrate was added. Using a multi-tip micropipette 50 ul of substrate was added to each of the wells. The plate was then shaken at room temperature for 20 minutes or until a bright yellow colour appeared in some of the wells, after which time the plate was read.

2.7.6.6 READING OF ELISA PLATE

An ELISA plate reader (Titertek Multiskan) supplied by Flow Laboratories, Rickmansworth, England was used to read the plates.

Initially the plates were read at 450 nm. After this 50 ul 2 M sulphuric acid was added to each well and the plate re-read at 492 nm. The protocol for initial reading at 450 nm was that of being an indicator. Should the reaction have required more time the plate could have been put back on the shaker for another period of time, read again at 450 nm and then sulphuric acid added since this stops the reaction after which the plate could be read at 492 nm.

2.8 HISTOPATHOLOGICAL TECHNIQUES

2.8.1 Wax Embedding

Skin (1 x 1 x 0.5 cm) was obtained from Ayrshire calves infested with **L. vituli** and a separate group of non-infested lice-free animals to act as controls.

Ayrshire calves infested with **Damalinia bovis** were also sampled as were lice-free animals of the same herd which were regarded as

controls.

2.8.1.1 EMBEDDING MATERIAL

2.8.1.1.1 Fixation of Material

All skin samples were fixed using neutral buffered formalin.

2.8.1.1.2 Dehydration

Using a Histokinette Tissue Processing Machine (British American Optical Co. Ltd., Slough, England) the skin sections, now in the form of 2 x 2 mm blocks with the hair coat cut off, were placed in the following:

distilled water	2 hours
50% alcohol	1 hour
70% alcohol	2 hours
90% alcohol	1 hour
90% alcohol	2 hours
100% alcohol	1 hour
100% alcohol	2 hours
100% alcohol	3 hours
chloroform	1 hour and 30 minutes
chloroform	2 hours and 30 minutes
wax	3 hours
was	3 hours

2.8.1.1.3 Embedding

Additional accessories needed for embedding:

(i) Vaccuum embedding bath

On immediate removal from the histokinette the sections were placed in a vaccuum embedding bath (Charles Hearson and Co. Ltd., London, England) to ensure any air present in the sections was removed prior to embedding.

(ii) Plastic moulds and liquid wax

Once removed from the vaccuum embedding bath each section was placed, with the correct orientation, in a plastic mould and then covered in liquid wax. The mould was then rapidly cooled in cold water to set the wax as quickly as possible. Each mould was labelled.

2.8.1.2 SECTIONING MATERIAL

2.8.1.2.1 Preparation of blocks

The wax blocks containing the sections were removed from the moulds and, using a hot palate knife, were shaped to fit onto the cutting chuck. The blocks were fixed onto labelled chucks by liquid wax.

2.8.1.2.2 Cutting sections

Sections from 5 to 10 um were cut on a Riechart-Jung Model 1130/biocut microtome (British American Optical Co. Ltd., Slough, England) and floated out on a water bath at 45 to 50^{o}C. They were collected onto clean, dry glass slides and placed in an oven (60^{o}C) to dry.

2.8.1.3 STAINING

2.8.1.3.1 Stains Used

Slides were stained using Erlichs Haematoxylin and Eosin.

(i) Composition of Erlichs Haematoxylin

Haematoxylin	2 g
100% alcohol	100 ml
Glycerin	100 ml
Distilled water	100 ml
Glacial acetic acid	10 ml

Potash alum ($K_2SO_4Al_2(SO_4)_3.24H_2O$) in excess (10-14 g)

The haematoxylin was dissolved in the alcohol before adding the other ingredients. The stain was allowed to ripen naturally for six weeks in a large flask, loosely stoppered with cotton wool. The glycerin acted as a stabilizer and retarded evaporation, and as the haematoxylin became oxidized, the colour of the solution changed from purplish to deep red, while the pungent odour of the acetic acid was replaced by a pleasant vinous aroma.

(ii) Eosin

Used as supplied by Sigma, Poole, England.

2.8.1.3.2 Staining Procedure

(i) Wax removal

The sections were placed in the following:

xylene	5 minutes
100% alcohol	3 minutes
100% alcohol	3 minutes
90% alcohol	3 minutes
70% alcohol	3 minutes

(ii) Staining time

The sections were then placed in Erlichs Haematoxylin for 15 minutes, washed in distilled water and then placed in Eosin for 45 seconds, again washed in distilled water afterwards.

(iii) Clearing

After staining, the slides were immersed in:

100% alcohol	1 minute
100% alcohol	1 minute
xylene	5 minutes

2.8.1.3.3 Mounting

After being allowed to air dry, a cover slip was placed on each slide being secured with D.P.X. mounting medium (Sigma, Poole, England).

2.8.2 Resin Embedding

Skin was obtained from young Ayrshire calves infested with **L. vituli**. A separate group of non-infested lice-free animals were used as controls.

Skin was also obtained from Ayrshire calves infested with **D. bovis** and non-infested lice-free animals of the same herd which acted

as controls.

2.8.2.1 EMBEDDING MATERIAL

2.8.2.1.1 Fixation of Material

All skin used was fixed in neutral buffered formalin.

2.8.2.1.2 Dehydration

Using a Histokinette Tissue Processing Machine (British American Optical Co. Ltd., Slough, England) the skin sections, now in the form of 2 x 2 mm blocks with the hair coat cut off, were placed in the following:

distilled water	2 hours
70% alcohol	2 hours
90% alcohol	1 hour
90% alcohol	2 hours
95% alcohol	3 hours

2.8.2.1.3 Embedding Kit and Accessories

A LKB Historesin Embedding Kit 2218-500 (Agar Aids Ltd., Stansted, England) was used to resin embed the skin samples.

The composition of the materials in the kit were explained on the instruction leaflet.

2.8.2.1.4 Embedding Instructions

(i) Infiltration

The solution was prepared according to the kit instructions.

Intermediate infiltration in a 1:1 solution (95% ethanol:inflitration solution) took place over a 24 hour period with two changes of solution. This was followed by 6 hours in inflitration solution.

(ii) Embedding

This solution was made up in accordance with the instructions in the kit. The lower half of gelatin capsules (Windsor Laboratories, Ltd., Slough, England) were filled with embedding solution. The specimens were immersed and orientated with the upper half of the capsule then being placed in position. The specimens were allowed to polymerize for 40-120 minutes at room temperature. The time to complete polymerization depended upon

(i) room temperature;

(ii) amount of embedding medium;

(iii) the relative amounts of infiltration solution and hardner.

2.8.2.2 STORAGE

2.8.2.2.1 Historesin Kit

All the components were stored at room temperature. The bottles were kept tightly closed as the plastic monomers were hydroscopic. All components had a shelf life of at least 1 year at room temperature.

2.8.2.2.2 Infiltration Solution

The infiltration solution was stored at room temperature (20°C). It can also be stored in a refrigerator, but this may cause benzoylperoxide to precipitate. In this case the solution must be stirred before use.

2.8.2.2.3 Specimen Blocks

The sectioning properties of Historesin were optimal if the sectioning was carried out directly after embedding. If the blocks dried out, the natural ribboning tendency disappeared. In this case ribboning could be achieved by moisturising the horizontal edges of the block. Since glycomethacrylate is hydroscopic the Historesin specimen blocks were stored in constant humidity to avoid sectioning problems especially with older blocks.

2.8.2.3 SECTIONING MATERIAL

2 um sections were cut with a glass knife using a Riechart-Jung Model 1130/Biocut Microtome (British American Optical Co. Ltd., Slough, England). Sections were floated on a water bath at 45 to 50°C and collected onto clean glass slides which were then allowed to dry at room temperature.

2.8.2.4 STAINING MATERIAL

Slides were immersed in Wrights stain (Sigma, Poole, England) for 45 minutes, removed, washed in distilled water and left to dry at room temperature.

2.8.2.5 MOUNTING

Sections on the slides were preserved by the addition of D.P.X. mounting medium (Sigma, Poole, England) and a coverslip.

2.9 ELECTRON MICROSCOPY TECHNIQUE - THIOCARBOHYDRAZIDE METHOD

2.9.1 Buffer Solutions Required

(i) Solution A

Solution A consists of:

$NaH_2PO_4.2H_2O$	15.6 g
Distilled water	500 ml

This solution was stored at room temperature.

(ii) Solution B

Solution B consists of:

di-sodium hydrogen phosphate	
Na_2HPO_4	14.2 g
Distilled water	500 ml

This solution was stored at room temperature.

(iii) Solution C

10 ml Solution A and 40 ml Solution B were mixed together with 50 ml distilled water. This solution was filtered through a 0.45 u millipore membrane. The solution was stored at $4^{o}C$.

(iv) Solution D

100 ml of Solution C was mixed with 50 g D-glucose (Sigma,

Poole, England). It was filtered through a 0.45 u millipore membrane. The solution was stored at 4°C.

(v) Solution E

96 ml of Solution C and 4 ml Solution D were mixed together and then filtered through a 0.45 u millipore membrane. This solution was stored at 4°C.

2.9.2 Preparation of Material

2.9.2.1 SKIN SAMPLES

Skin from Ayrshire calves infested with **L. vituli** as well as samples from a separate group of lice free animals was trimmed into 5 x 5 mm blocks with as much of the hair coat removed as possible. The same procedure was carried out on skin from **D. bovis** infested calves and lice free animals from the same herd.

2.9.2.2 FIXATION

(i) The samples were placed in Solution E (approximately 20 ml) for 15 minutes. This was repeated for 3 changes.

(ii) Three, 15 minute changes of distilled water.

(iii) The skin was placed in 1% osmium tetroxide in 0.1 M phosphate for 2 hours.

(iv) Three, 15 minute changes of distilled water.

(v) The skin was immersed in a freshly prepared, filtered, saturated solution (approximately 1%) of thiocarbohydrazide (TCH) for 30 minutes.

(vi) Three, 15 minute changes of distilled water.

(vii) The skin was placed in distilled water overnight.

(viii) Three, 15 minute changes of distilled water.

(ix) The samples were placed in fresh TCH for 30 minutes.

(x) Three, 15 minute changes of distilled water.

(xi) Samples were immersed in 1% aqueous osmium tetroxide for 1 hour.

(xii) Three, 15 minute changes of distilled water.

2.9.2.3 DEHYDRATION

The tissue was dehydrated in the following series of graded alcohols:

(i) 15 minutes in 30% acetone

(ii) 15 minutes in 50% acetone

(iii) 15 minutes in 80% acetone

(iv) Three, 15 minute changes in 100% acetone

2.9.3 Critical Point Dryer

2.9.3.1 EXCHANGE OF TRANSFER LIQUID

All samples were placed in a Critical Point Dryer, model 11 120A supplied by Blazers Unicon Ltd., Principality of Liechtenstein. The samples were placed in covered brass baskets (code number 17037, Blazers Unicon Ltd.) and kept immersed in 100% acetone at all times from the completion of dehydration to placement in the 100% acetone

filled pressure container in the critical dryer. The transitional 100% acetone fluid was completely exchanged with liquid carbon dioxide at a pressure of 50 atmospheres.

2.9.3.2 DRYING

The pressure container was automatically slowly heated to 40°C as rapid heating may cause strong turbulence which could damage the tissue. The pressure was increased to 105 atmospheres and once this and the temperature of 40°C were achieved simultaneously the change in aggregate state of the drying liquid took place and only interference streaks were still visible in the pressure container whilst the actual drying of the specimens took place. After 5 minutes the pressure was switched off and once it reached zero atmospheres the heat was also switched off to avoid recondensation taking place.

2.9.4 Mounting Specimens

Once removed from the critical point dryer the specimens were placed on 0.6 cm diameter aluminium chucks using quick drying silver paint (Agar Scientific Ltd., Stansted, England).

2.9.5 Sputter Coating Specimens

The specimens were coated to a thickness of 10 nanometers with a gold/palladium mixture using an E5100 Series II Cool Sputter Coater (Polaron Equipment Ltd., Watford, England).

2.9.6 Electronmicroscopy

Specimens were examined using a JEOL T-300 scanning electron microscope supplied by Japanese Electron Optics Ltd., Tokyo, Japan.

CHAPTER THREE

THE EFFECT OF LINOGNATHUS VITULI INFESTATION ON THE HAEMATOLOGY AND WEIGHT OF YOUNG FRIESIAN CALVES

3.1 INTRODUCTION

The three species of sucking lice found in south-west Scotland are **Linognathus vituli, Haematopinus eurysternus** and **Solenopotes capillatus.** A survey in south-west Scotland (Titchener, 1983) found that of 100 farms visited 56 had calves infested with **L. vituli,** 11 with **H. eurysternus** and 7 with **S. capillatus,** which is similar to findings in New Zealand (Chalmers and Charleston, 1980a).

It has been reported in the literature that cattle lice, in particular **H. eurysternus,** cause anaemia, reduce weight gain and in extreme cases cause the death of the host when present in large numbers (Matthysse, 1946; Kemper, Cobett, Roberts and Peterson, 1948; Collins and Dewhirst, 1965; Nelson **et al,** 1970). However, it should be noted that **H. eurysternus** is a much larger louse than **L. vituli** and consequently has the capacity to remove more blood from the host.

With cattle infested with **L. vituli** there appears to be little or no effect on the hosts blood profile or weight (Chalmers and Charleston, 1980; Cummins and Tweddle, 1977; Tweddle **et al,** 1977; Oormazdi and Baker, 1980). None of these studies had severe infestations of this louse. Mehrotra and Singh (1986) stated that anaemia was present in calves infested with **L. vituli** which were studied in four differing seasonal climates.

The economic effect caused by cattle lice has been recognised for many years. Lice free cattle gained an average of 73 lbs more per head over a 61 day period than untreated **H. eurysternus** infested cattle under identical conditions (Snipes, 1948). According to Smith and Richards (1955) losses caused by lice alone to all classes of

livestock and poultry in the United States of America were estimated to be approximately $52 million annually. Matthysse (1946) considers that the financial loss due to lice cannot be estimated, since it is not known how much loss of milk pediculosis causes, or how much extra feed is required to maintain and fatten a lousy steer or heifer. Not rejecting the claim that lice are of economic importance, Scharf (1962) considered that louse control measures were probably justified on only 5 per cent of cattle in the State of Montana, United States of America.

The purpose of the present chapter was to determine, by means of two almost simultaneously occurring experiments, the blood profile and weight gains of young Friesian calves infested with the blood sucking louse, **L. vituli**.

3.2 MATERIALS AND METHODS

The data presented was obtained from two experiments undertaken at the Brickrow Farm Unit, The West of Scotland College, Auchincruive, Ayr, Scotland, in 1986/87. These investigated the effect, on calves, of populations of the long-nosed sucking louse, **L. vituli**, with regard to blood parameters and liveweight gains of the host.

3.2.1 Animals and Treatments

All the calves, being Friesian bull calves, were kept in cattle sheds for the duration of the experiments. The control and test groups were kept separate at all times. The control groups were treated twice with Swift (Robert Young and Co. Ltd., Glasgow,

Scotland) to prevent any louse infestation. Identical rations were given throughout the experimental period so no discrepency in results could be related to nutrition.

Experiment A started blood sampling calves aged 6 weeks and continued until they reached 30 weeks of age. Experiment B began three weeks later and calves were initially blood sampled at 3 weeks, ending at 24 weeks of age.

3.2.2 Blood Sampling and Analysis

Every 14 days all calves were weighed, with the weights being noted, and blood samples taken. Two 10 ml samples, one heparinised, were taken by jugular-venipuncture at each bleeding, care being taken not to cause the calf any excitement in order to avoid abnormal readings due to the so-called "alarm factor".

The blood samples for each calf were used to determine:

(i) Red blood cell count (Chapter 2, section 2.2.1).

(ii) White blood cell count (Chapter 2, section 2.2.2).

(iii) Haemoglobin concentration (Chapter 2, section 2.2.3).

(iv) Packed cell volume (Chapter 2, section 2.2.4).

(v) Reticulocyte counts (Chapter 2, section 2.2.5).

(vi) Eosinophil counts (Chapter 2, section 2.2.6).

(vii) Total protein determination (Chapter 2, section 2.2.7).

(ix) Total albumin determination (Chapter 2, section 2.2.8).

3.2.3 Lice Counts

Each time blood samples were taken a louse count was carried out on each animal to estimate the level of infestation. This consisted of parting the hair five times on the shoulders of each calf and counting the number of lice revealed at each hair parting (see Plate 1). Lice free calves were thoroughly examined to ensure they remained lice free throughout the experimental period.

For the purpose of data analysis and discussion, the following criteria will be applied to the level of infestation:

Mean count >50 lice = very severe infestation
Mean count of 20-50 lice = severe infestation
Mean count of 10-20 lice = moderate infestation
Mean count of 5-10 lice = slight infestation
Mean count <5 lice = very slight infestation

(After Titchener, 1985).

3.2.4 Statistical Analysis

One way analysis of variance was used to test for significant differences between mean values for all parameters studied for each calf on all sampling days.

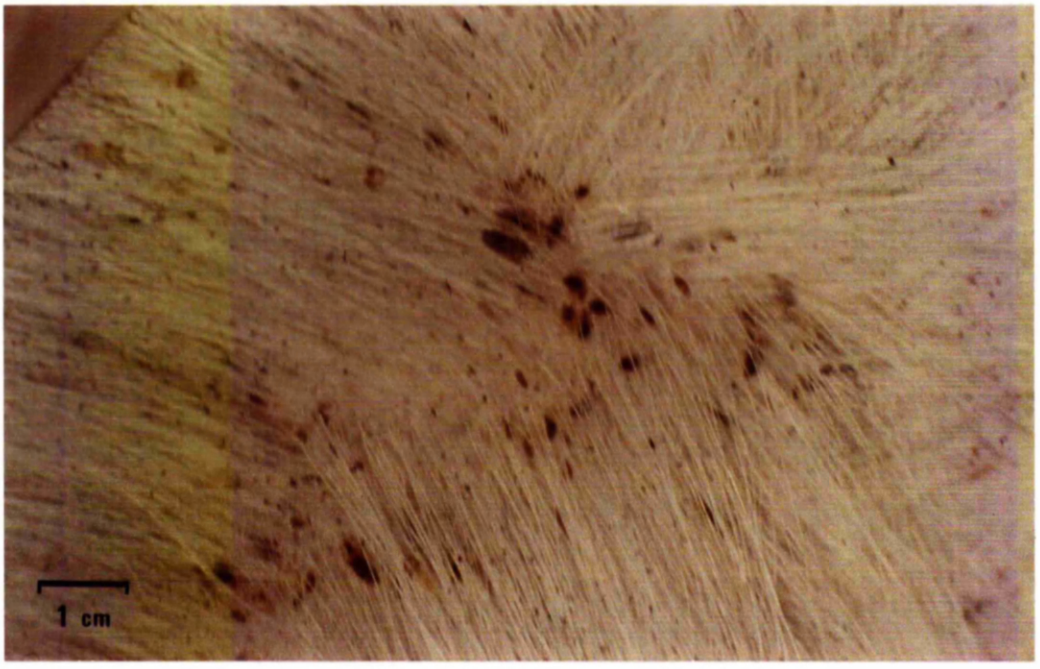


Plate 1 Hair parting on the shoulders of a calf revealing louse population.

3.3 RESULTS AND DESCRIPTION OF TRENDS IN THE DATA

The results will be presented along with a description of the data. This will be derived directly from, and will contain no interpretation of, the results.

3.3.1 Packed Cell Volume

3.3.1.1 GENERAL TRENDS

The mean percentage packed cell volumes are shown in Figure 3.1 and Appendix 1.

For experiment A the infested animals mean values ranged from 30.23 to 35.46% with the lice free or controls having a range of 31.75 to 34.25%. The test animals appear to parallel the values of the control animals on each sampling date with the greatest difference occurring at 18 weeks when the infested calves had the greater mean value.

In experiment B the louse infested animals had 33.44% as the lowest mean value with 34.56% the highest, whilst the controls ranged from 33.71 to 34.43%. The values of the two groups were similar at each sampling throughout the duration of the experiment, the largest difference occurring at 18 weeks of age when the infested calves had the greater mean value.

3.3.1.2 STATISTICAL EVALUATION

One way analysis of variance indicated that there were no significant differences in packed cell volume percentages between infested and louse free calves throughout the duration of both

Mean Packed Cell Volume

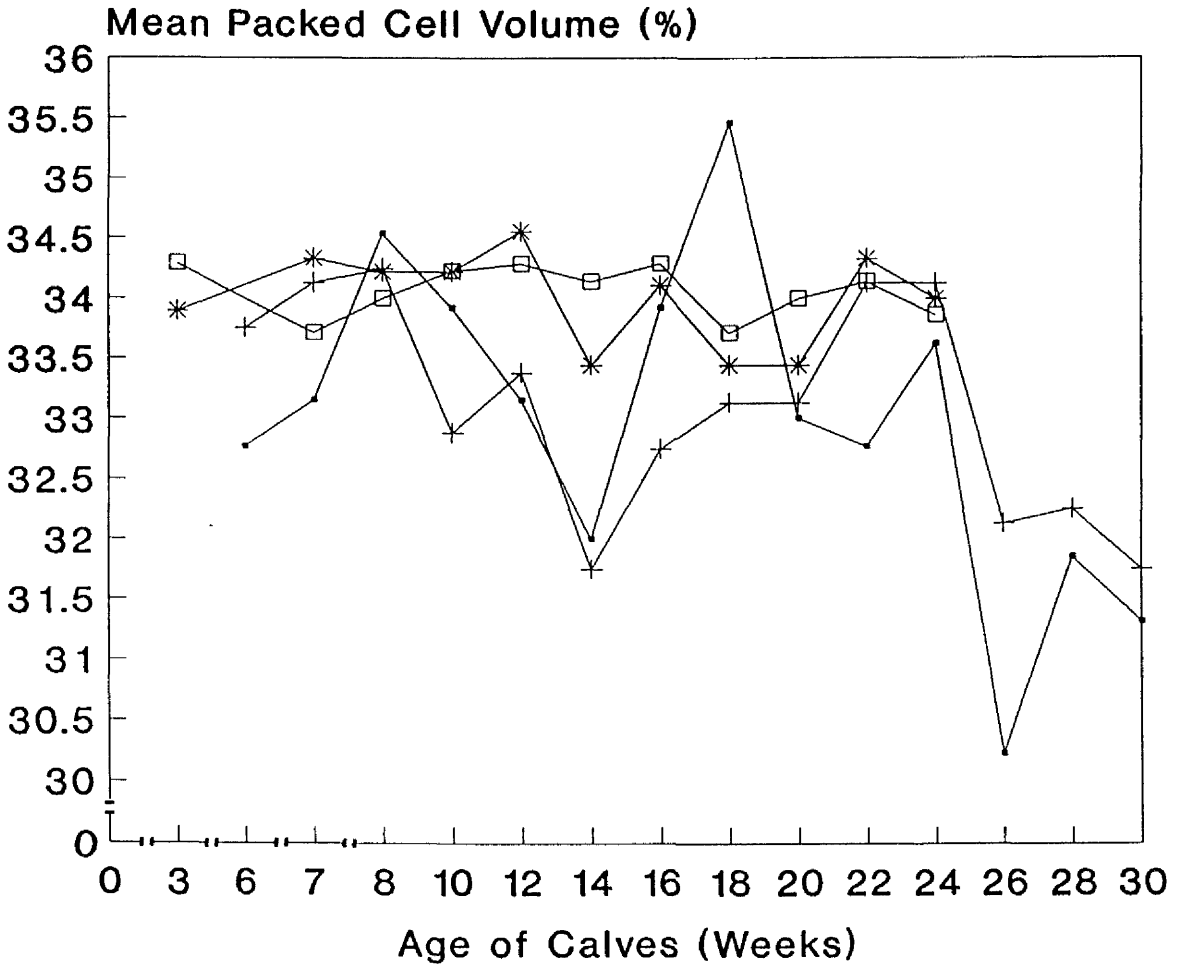


Figure 3-1

experiments.

3.3.2 Haemoglobin Concentration

3.3.2.1 GENERAL TRENDS

The mean haemoglobin concentrations are shown in Figure 3.2 and Appendix 2.

Values varied between 10.08 and 12.02 g/dl for experiment A infested calves and 10.46 to 11.26 g/dl for the lice free animals. The test values followed the same pattern throughout the study as that of the controls, the largest difference occurring at 18 weeks when the infested calves showed the greater value. Both groups of animals showed a sharp decrease from 22 to 26 weeks although this increased slightly at the end of the experimental period.

For experiment B infested calves the range was 10.90 to 11.97 g/dl and 10.89 to 12.23 g/dl for the lice free controls. Again, both groups of calves showed similar values on each sampling day throughout the experiment. At 14 weeks the largest discrepancy was seen with the infested group being the lower of the two values.

3.3.2.2 STATISTICAL EVALUATION

Analysis of the data revealed no significant differences in experiment A until the calves were 10 weeks old when the haemoglobin concentration was significantly higher in the infested than control animals ($P<0.05$). This was the only sampling day at which there was a significant difference in experiment A. Applying the same statistical technique to experiment B data found no significant

Mean Haemoglobin Concentration

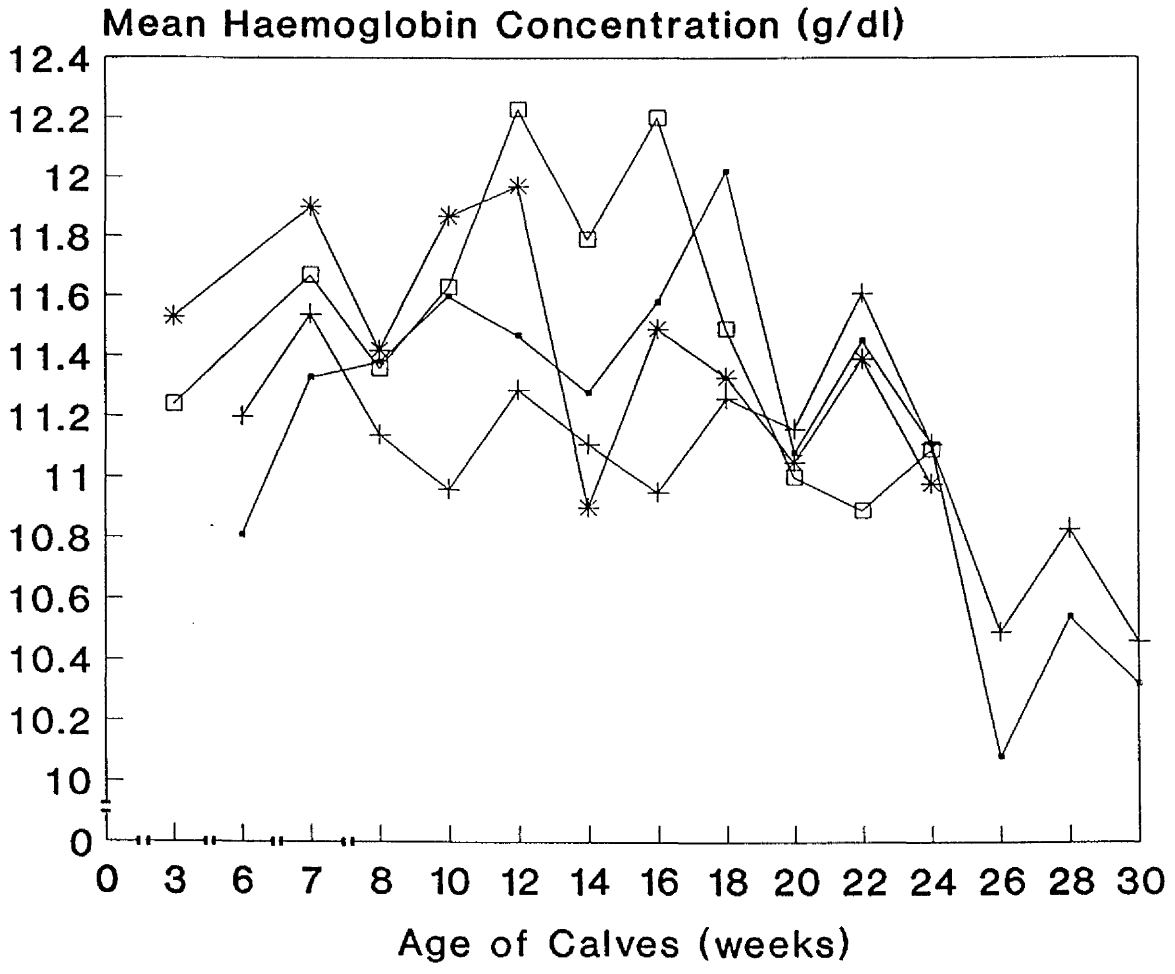


Figure 3·2

differences between infested and lice free animals at any time during the experimental period.

3.3.3 Total Leucocyte Counts

3.3.3.1 GENERAL TRENDS

The mean leucocyte counts are shown in Figure 3.3 and Appendix 3.

Values for the test animals in experiment A varied from 8.72 to 10.33 x 10^9/l and from 8.50 to 11.90 x 10^9/l for the controls. Both infested and louse free calves showed slight fluctuations from the start of the experiment to 18 weeks of age. After this the test animals decreased sharply, to their lowest level, at 20 weeks and then increased consistently on the following sampling days, peaking at 28 weeks old with a very slight decrease on the final sampling day. The louse free calves began a consistent steep rise after 18 to 22 weeks followed by a similar value at 24 weeks and then another steep rise to 28 weeks which was the highest value observed for the control animals. It was also the second largest difference between the two groups although the infested value for this date was the largest noted throughout the experiment. From 28 weeks to the final sampling at 30 weeks there was a sharp decline to a final value very slightly below that of the infested calves.

In experiment B the test animals ranged from 7.07 to 8.59 x 10^9/l and 8.01 to 9.44 x 10^9/l for the louse free calves. Except for 22 weeks, when the values for infested and control calves were similar, the infested group had lower values for total leucocyte

Mean Total Leucocyte Counts

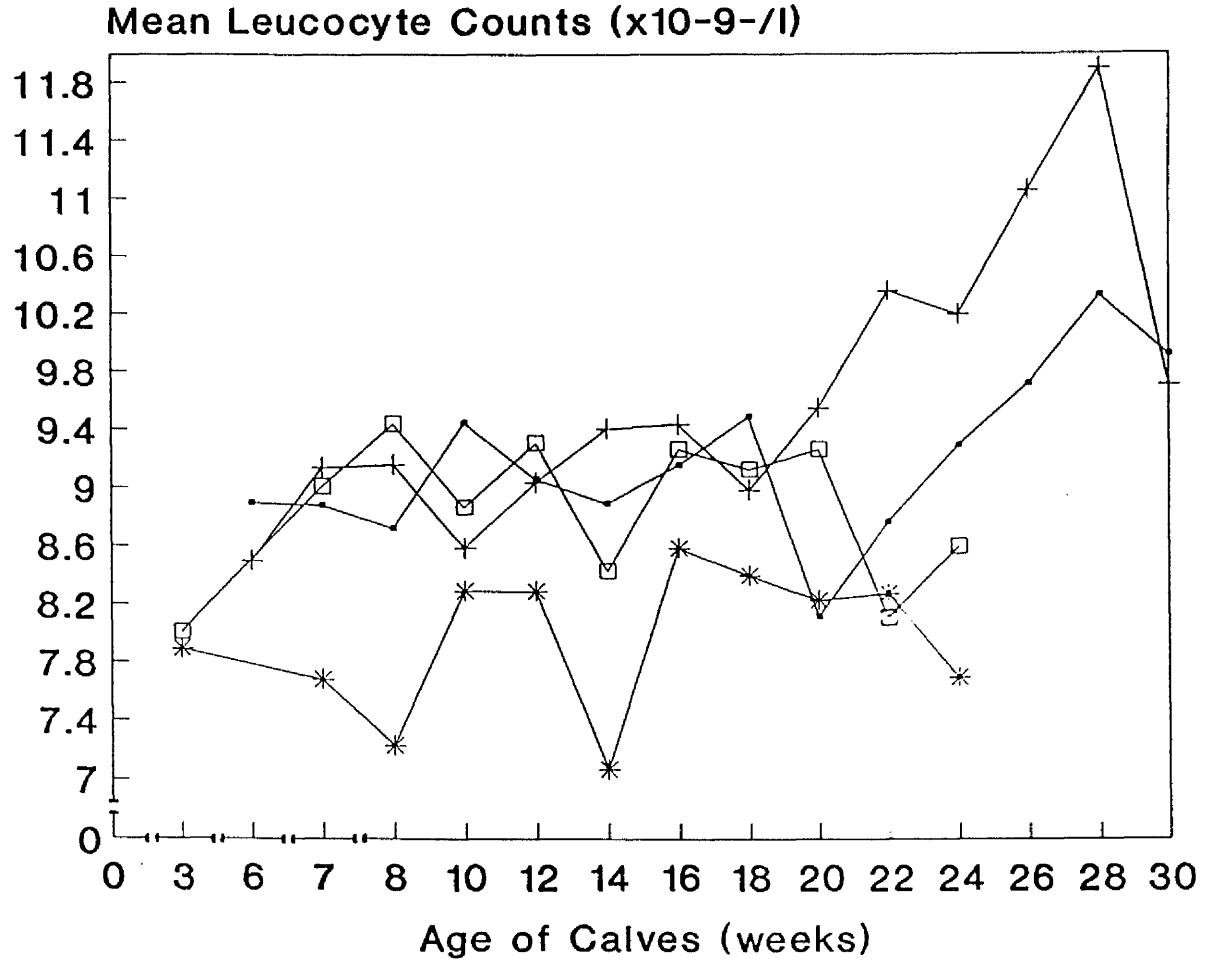


Figure 3.3

Infested A
Control A
Infested B
Control B

counts than the controls. Although starting off with a similar value to the lice free calves the infested animals decreased until 8 weeks, rose to slightly above the starting value, plateaued and then a steep fall was seen at 14 weeks to the lowest value recorded. This was followed by an equally sharp rise at 16 weeks and then a gradual decline for the remainder of the experiment.

The control calves in experiment B started at approximately the same value as the test animals. This was followed by a sharp rise at 8 weeks, some fluctuations and then the lowest value observed at 14 weeks. A sharp rise occurred to similar levels observed prior to the decline which was followed by a sharp decrease at 22 weeks, recovering slightly at the end of the experiment.

3.3.3.2 STATISTICAL EVALUATION

Analysis of the data showed no significant difference in louse infested and louse free calves in experiment A. In experiment B no significant differences were revealed until sampled at 7 and 8 weeks of age. Both sampling days showed that the total leucocyte count for the test animals was significantly lower than the controls ($P<0.05$). For the remainder of the experiment no significant differences were observed.

3.3.4 Lymphocyte Counts

3.3.4.1 GENERAL TRENDS

The mean lymphocyte counts are shown in Figure 3.4 and Appendix 4.

Mean Lymphocyte Counts

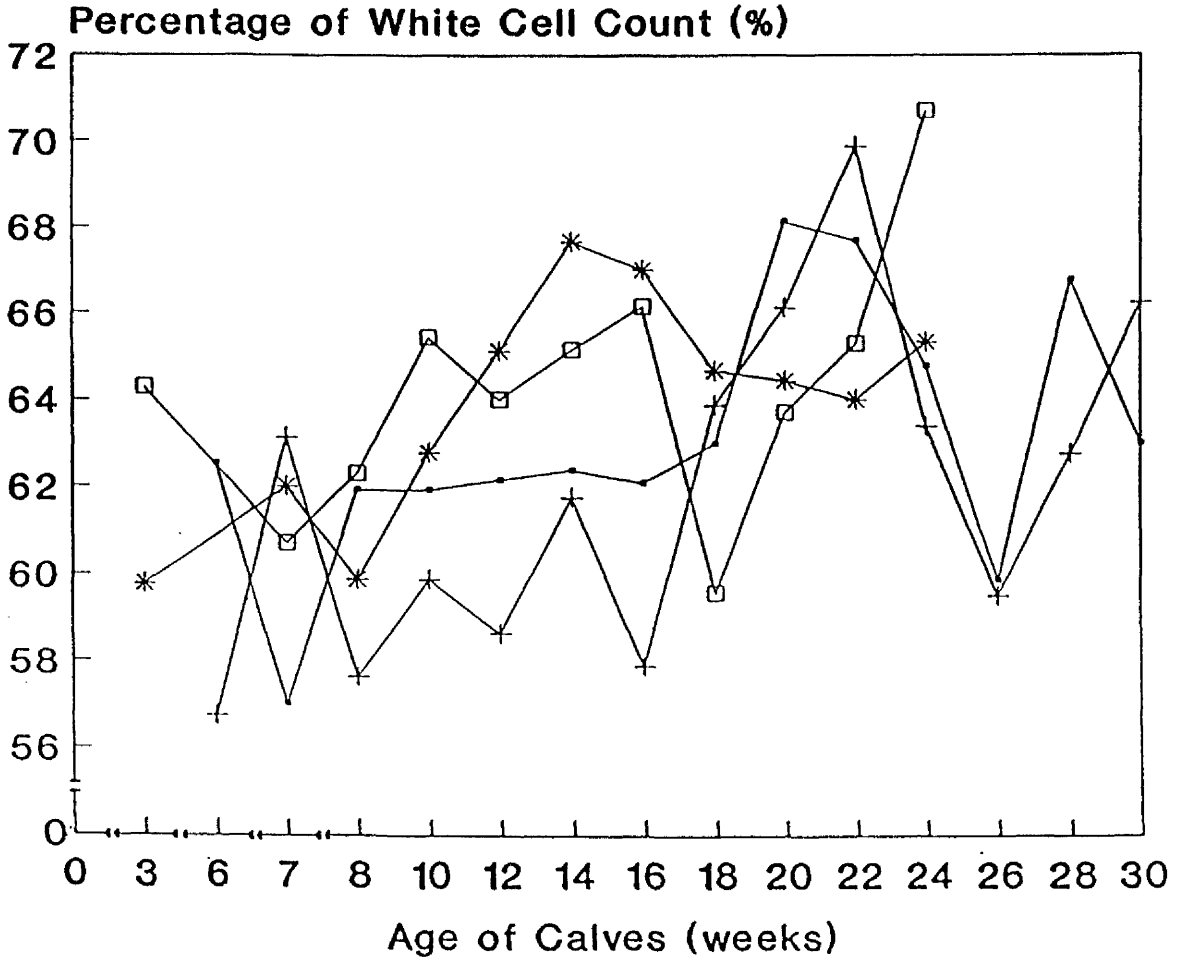


Figure 3-4

Infested A
Control A
Infested B
Control B

For experiment A the louse infested calves mean values ranges from 57.00 to 68.15% of the total leucocyte count and the louse free animals 56.75 to 69.88%.

In the infested group there was a slight decrease from the first sampling day to the second followed by a similar increase. The values remained steady until 16 weeks followed by a rise to a peak value at 20 weeks after which there was a steady decline to the end of the experiment. The control calves followed a similar pattern although the rise from 16 weeks reached a peak at 22 weeks and then declined.

The infested calves in experiment B increased steadily, with the exception of 8 weeks, from the start of the experiment to 14 weeks after which was a decline with a slight recovery on the final sampling day. After an initial decline the controls increased to 10 weeks, remained fairly constant until 16 weeks and then dropped sharply at 18 weeks only to increase steadily to the end of the experiment where they produced the highest value observed during the experimental period.

3.3.4.2 STATISTICAL EVALUATION

There were no statisticallay significant differences in experiment A until seven weeks of age when there was a statistically significant higher percentage of lymphocytes present in the louse infested calves than the controls ($P<0.05$). On no other sampling day in experiment A were there any other significant differences.

Applying the same statistical analysis to experiment B showed no significant differences until the final sampling day when the calves were 24 weeks old. On this day there was a significantly lower percentage of lymphocytes in the louse infested calves than the lice free controls ($P<0.05$).

3.3.5 Neutrophil Counts

3.3.5.1 GENERAL TRENDS

The mean neutrophil counts are shown in Figure 3.5 and Appendix 5.

For experiment A the values ranged from 21.31 to 33.15% of the total leucocytes present in the louse infested animals and 22.75 to 33.13% for the controls. Both the test and control calves had similar values throughout the experiment. There were slight fluctuations at the beginning of the experiment followed by a relatively constant period and then a decline to similar low levels for both groups at 22 weeks. Over the next four weeks, in the control figures, there was a constant increase and then a decline to the final value with the test calves showing a steeper decline and then a rise to the final value.

In experiment B the variation was 23.56 to 30.89% and 18.57 to 31.29% for test and control groups respectively. Again infested and lice free calves showed a similar pattern of values throughout the experiment. After initial fluctuations both decreased until 14 weeks for test animals and 16 weeks for controls. This was followed by an increase for both, larger in the case of the controls, and then a

Mean Neutrophil Counts

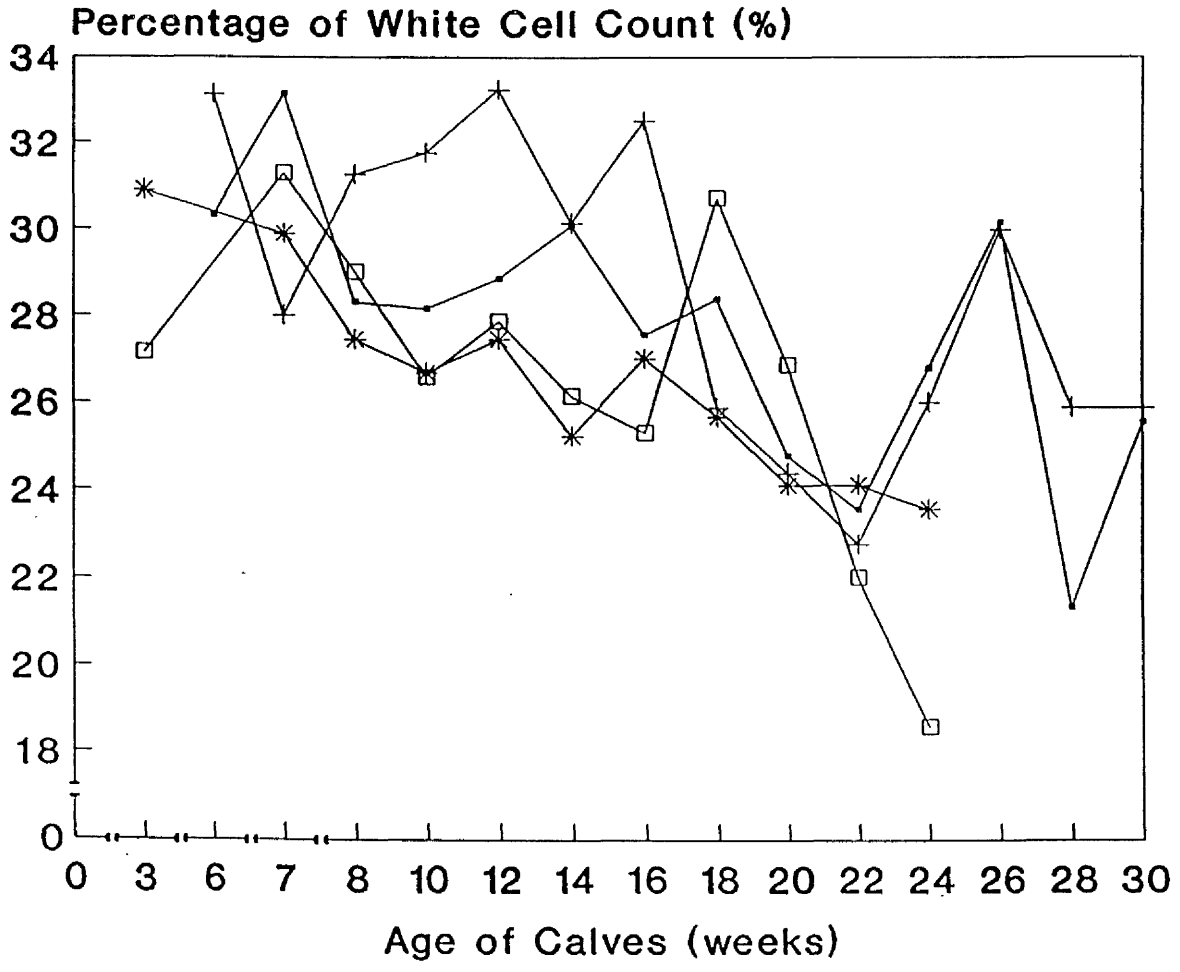


Figure 3·5

Infested A
Control A
Infested B
Control B

gradual decrease to the final value, the decline being sharper for the control calves which had a final value below that of the infested calves. The difference between these final values being the second largest of the experiment.

3.3.5.2 STATISTICAL EVALUATION

Analysis of the data indicated that for both experiments there were no statistically significant differences in neutrophil counts between infested and louse free calves.

3.3.6 Monocyte Counts

3.3.6.1 GENERAL TRENDS

The mean percentage monocyte counts are shown in Figure 3.6 and Appendix 6.

The infested calves in experiment A ranged from 6.92 to 12.46% and 7.13 to 11.38% for the louse free animals of the total leucocytes period. Although starting off slightly lower, the values for the test animals followed the same pattern produced by the control animals, the exception being a rise occurring from 24 to 28 weeks with a slight decline at 30 weeks. In contrast the lice free animals increased at 24 weeks, remained steady and then declined more sharply than the infested animals at 30 weeks giving the biggest difference in values on this sampling day.

For experiment B the values were 5.67 to 12.11% and 7.57 to 12.14% respectively for infested and lice free animals. The values for the louse infested animals remained similar until 7 weeks after

Mean Monocyte Counts

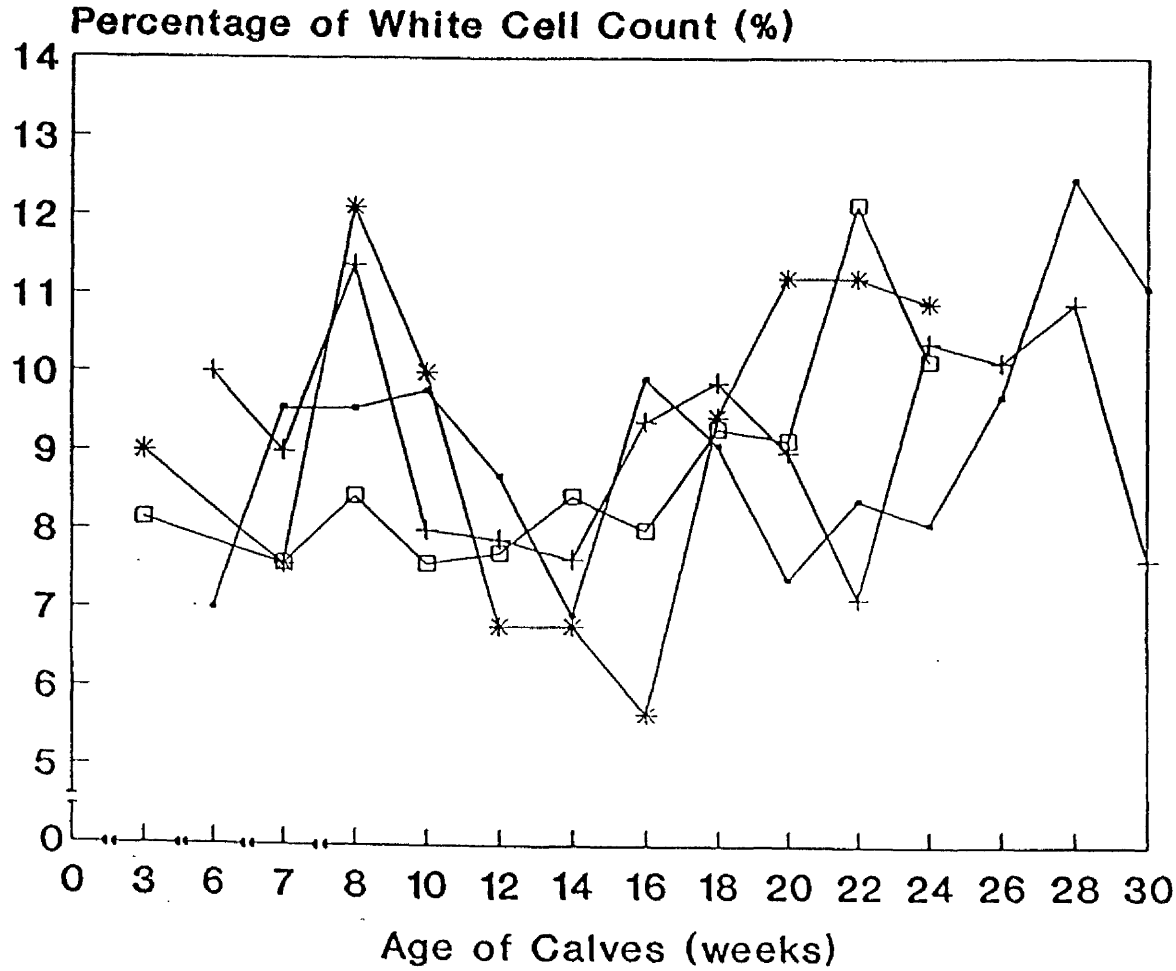


Figure 3.6

Infested A
Infested B
Control A
Control B

which there was a sharp rise to the peak value observed at 8 weeks. A decline occurred ending at 16 weeks, followed by an increase and then a plateau on the last three sampling days of the experiment which gave values slightly under the peak value. The louse free calves remained at a constant value from the start of the experiment to 16 weeks which was followed by a slight rise at 20 weeks and a sharper increase at 22 weeks with decline at 24 weeeks to a value only exceeded by the peak value.

3.3.6.2 STATISTICAL EVALUATION

Analysis of the data indicated that there were two statistically significant differences in experiment A. The first occurred on the first sampling day when the calves were 6 weeks old and showed a significantly lower percentage of monocytes present in the infested calves than the controls ($P<0.05$). The other statistically significant sampling day was the last one with the calves aged 30 weeks where there was a significantly higher percentage of monocytes present in the infested calves than the controls ($P<0.05$). Applying the same statistical techniques to experiment B data revealed no statistically significant differences until the calves were sampled at 8 weeks of age. On this date there was a significantly higher percentage of monocytes present in infested animals than was found in the control calves ($P<0.05$). This was the only time in experiment B that a significant difference was observed.

3.3.7 Eosinophil Counts

3.3.7.1 GENERAL TRENDS

Mean eosinophil counts are shown in Figure 3.7 and Appendix 7.

The infested animals in experiment A ranged from 0.077% to 0.231% with 0.111% to 0.375% for the controls of the total leycocytes present. Throughout the experiment the values for the infested animals were similar on each sampling day. The values for the control animals were slightly higher throughout the duration of the experiment with the largest difference occurring at 14 weeks where the lice free animals had the greater value.

In experiment B the louse infested calves ranged from 0.111% to 0.222% and 0.143% to 0.429% for the louse free animals. The louse infested animals started with the lowest value seen during the experiment. This remained consistent until week 12 when there was a slight rise which continued until week 18 when the value returned to the initial level and remained there until the end of the experiment when there was another rise of similar proportions to that seen at 14 weeks. The louse free animals started off with a value similar to that of the infested group which increased to a peak at 8 weeks, decreased at 10 weeks and made a recovery at 6 weeks to a value double that of the initial one. This declined slightly at 8 weeks recovered for weeks 20 to 22 and then fell again on the last sampling day.

Mean Eosinophil Counts

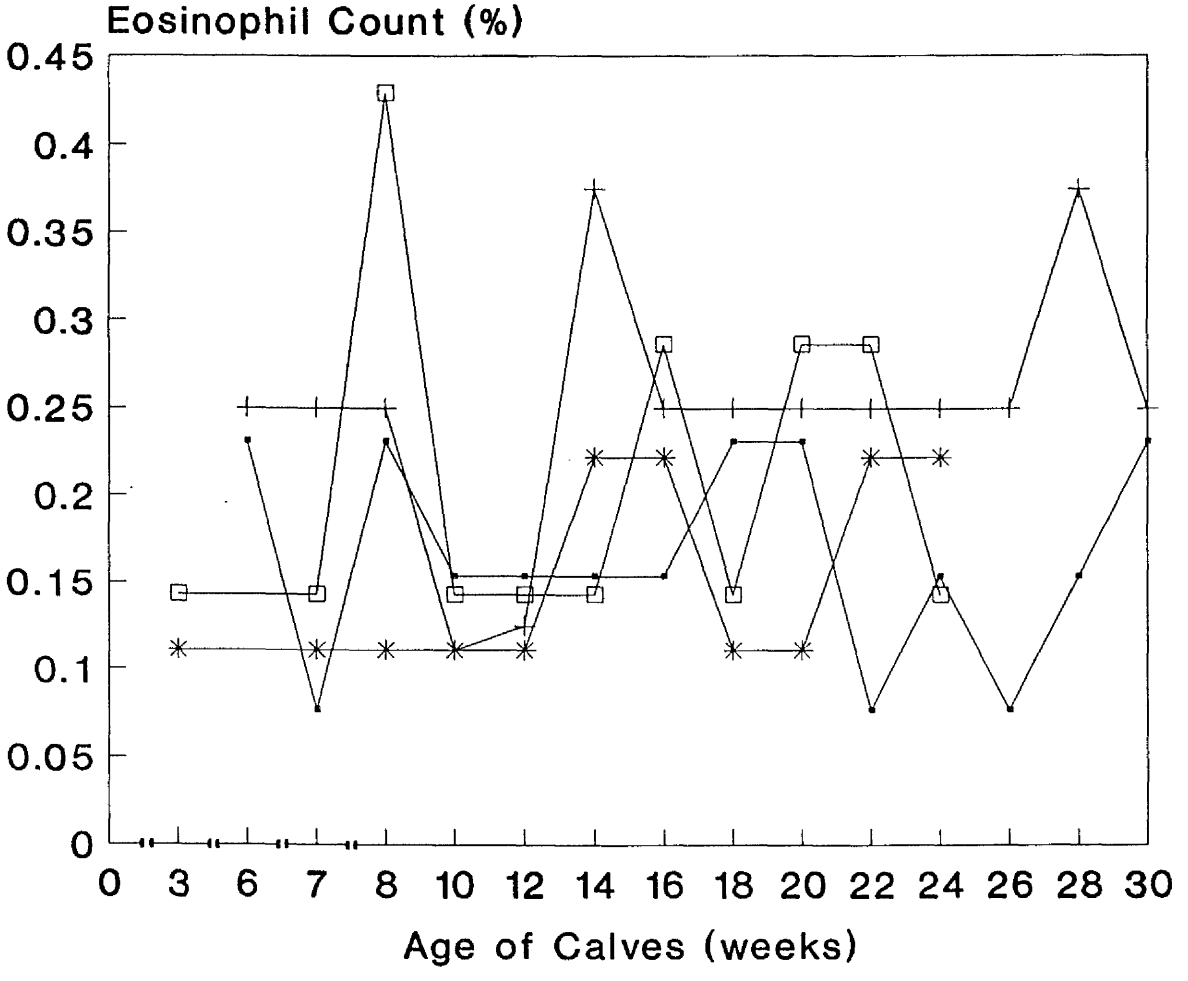


Figure 3-7

Infested A
Control A
Infested B
Control B

3.3.7.2 STATISTICAL EVALUATION

Analysis of the data showed no significant differences between louse infested and louse free animals on any sampling day throughout the duration of both experiments.

3.3.8 Erythrocyte Counts

3.3.8.1 GENERAL TRENDS

Mean erythrocyte counts are shown in Figure 3.8 and Appendix 8.

The test animals in experiment A ranged from 8.13 to 9.90 x 10^{12}/l with 8.19 to 9.89 x 10^{12}/l for the controls. Throughout the duration of the experiment there were a number of peak and trough values for both infested and non-infested calves occurring at fairly constant intervals. For the infested calves the first two peaks were at 8 to 10 weeks and then 16 to 18 weeks, both peaks having similar values. After the second peak there was a decline to the starting value, a slight increase and then a steep decrease from 24 to 26 weeks with a recovery to slightly above the initial value at 28 weeks followed by a sharp decline to the lowest value recorded for the experiment which was observed at 30 weeks.

The lice free calves had a higher initial value and increased to a peak at 8 weeks. This was followed by a decline to a slightly greater figure than the infested calves initial value and then an increase to 18 weeks where the value was similar to the controls initial figure. For the remainder of the experimental period there were peaks and troughs, the peaks being slightly less than the initial value with the troughs being similar values to those of the

Mean Erythrocyte Counts

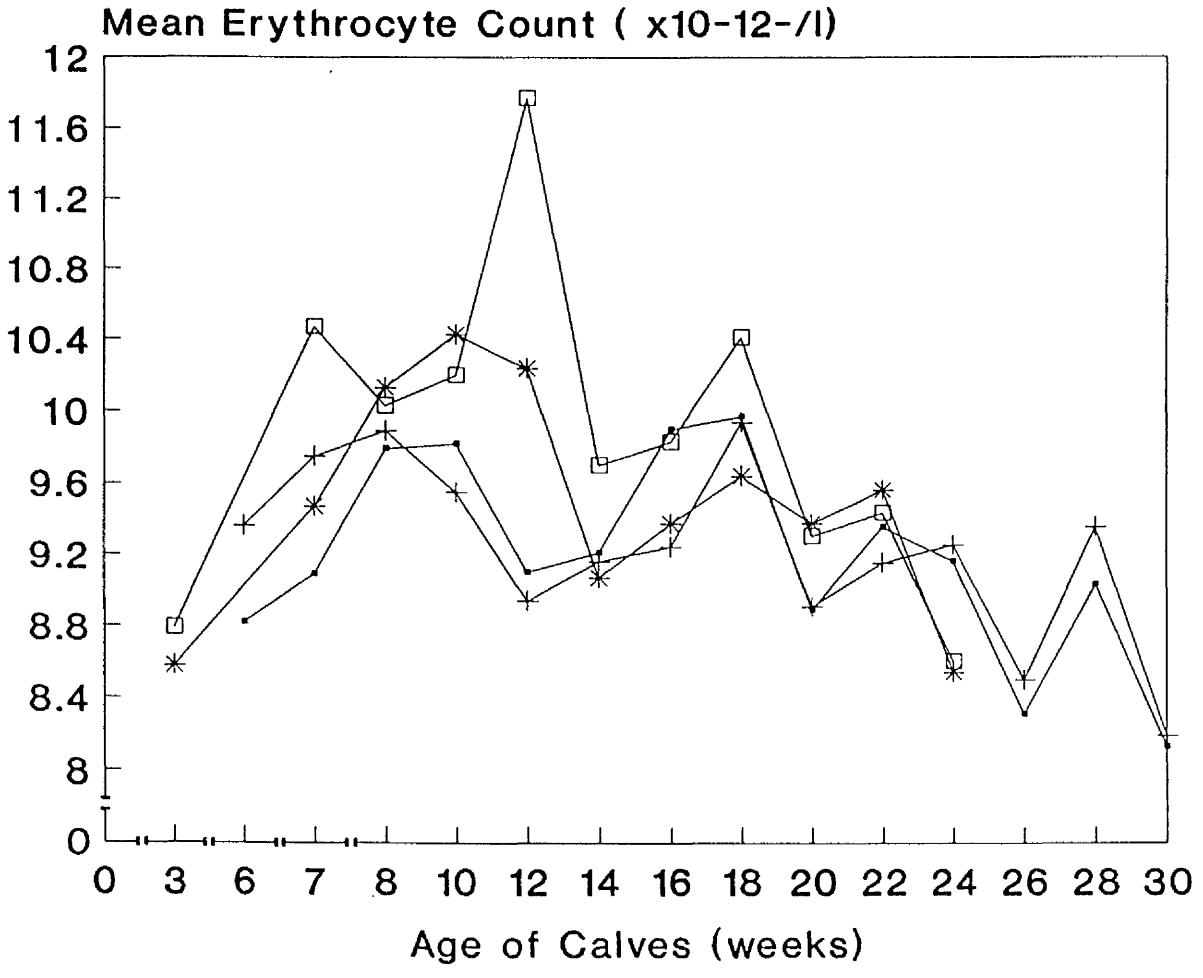


Figure 3·8

Infested A
Control A
Infested B
Control B

test animals at 26 and 30 weeks.

In experiment B the louse infested calves ranged from 8.54 to 10.43 x 10^{12}/l and 8.60 to 11.77 x 10^{12}/l for the louse free animals. The infested calves increased steadily from the initial value to a peak at 10 weeks which was followed by a decline to a value at 14 weeks slightly greater than the initial value. After this there was an increase to a plateau at 18 to 22 weeks and then a sharp decline to the final value which was similar to the initial one.

The louse free animals had initial and final values similar to those of the infested calves. Between these values were peaks of similar value to the infested calves at 7 and 18 weeks. At 12 weeks there was another peak which was approximately twice the value of the other two peaks.

3.3.8.2 STATISTICAL EVALUATION

Analysis of the data showed no statistically significant differences for experiment A in erythrocyte counts from louse infested and louse free animals. Applying the same statistical technique to experiment B indicated no significant difference until the calves were sampled at 12 weeks of age when the infested calves were significantly lower than the controls ($P<0.05$). This was the only time during the experiment that the erythrocyte count from infested and louse free animals was significantly affected.

3.3.9 Reticulocyte Counts

3.3.9.1 GENERAL TRENDS

The mean reticulocyte counts are shown in Figure 3.9 and Appendix 9.

Experiment A revealed the infested animals having a range from 0.08 to 0.38% and the controls 0.00 to 0.63% of the total erythrocytes present. The infested group started off with a few reticulocytes being present, increasing and then decreasing to a level which, although there were a few fluctuations around in the rest of the experimental period appeared to be the constant value for this group. The control group fluctuated for the first five sampling days and then appeared to reach a level which, although slight deviations were apparent, was similar to that of the test group.

In experiment B the infested calves ranged from 0.22 to 0.56% and 0.00 to 0.43% for the lice free groups. After initial fluctuations at the start of the experiment, with values which were higher than those of the test group in experiment A, the levels fell to those observed in experiment A infested group although fluctuations were again observed. The control group also had initial high increases but settled to a value comparable to experiment A lice free group.

3.3.9.2 STATISTICAL EVALUATION

Analysis of the data revealed no statistical significant differences between louse infested and louse free calves in experiment A. In experiment B there were no statistically

Mean Reticulocyte Counts

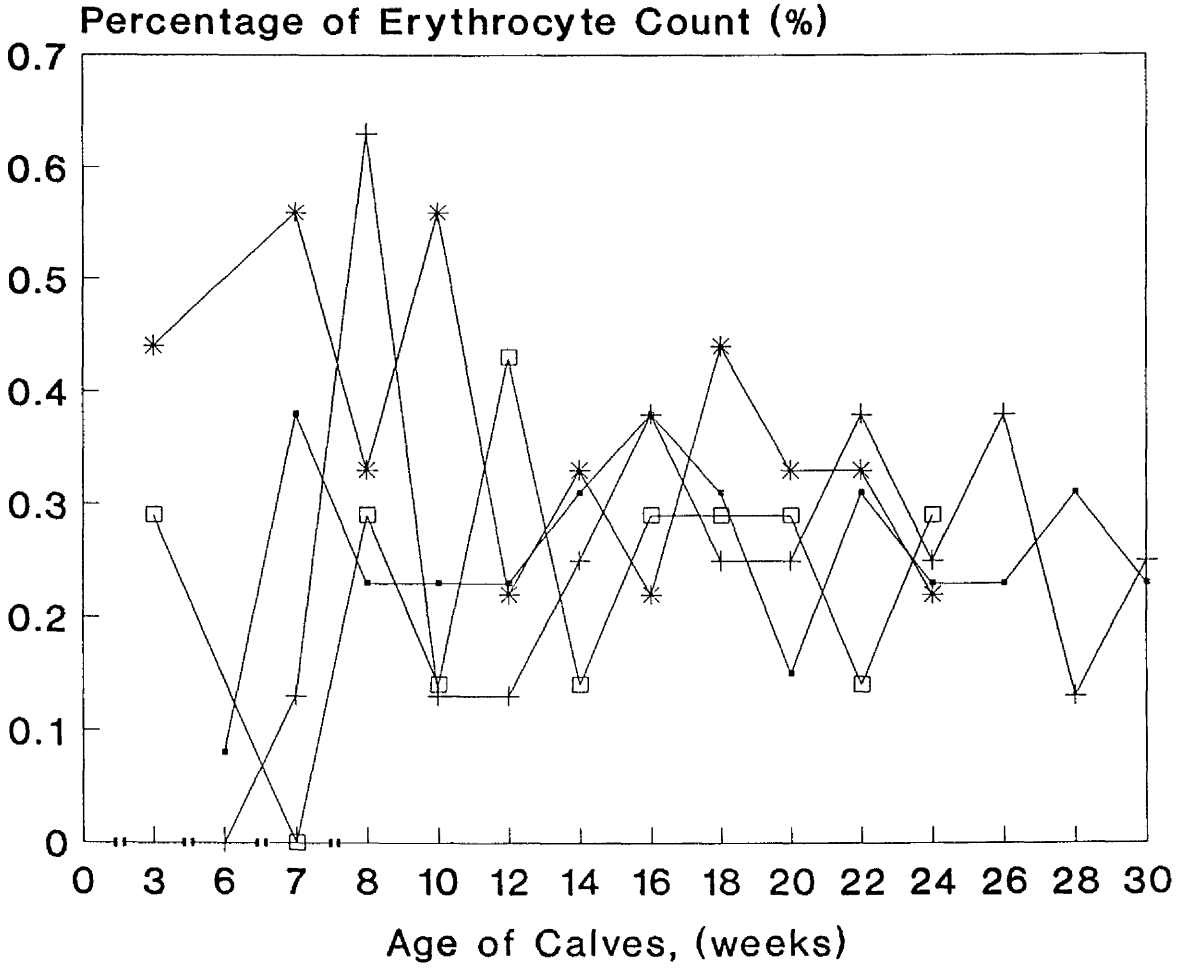


Figure 3·9

Infested A
Control A
Infested B
Control B

significant differences except the samples from calves at 7 weeks of age when there was a significantly higher mean reticulocyte count for the louse infested calves ($P<0.05$).

3.3.10 Total Protein Determination

3.3.10.1 GENERAL TRENDS

The mean total protein concentrations are shown in Figure 3.10 and Appendix 10.

For infested calves in experiment A the range was 64.23 to 65.41 g/l and 64.15 to 65.45 g/l for the louse free animals. After an initial decline in the values for the infested calves there was a steady increase to a peak at 14 weeks followed by a decline at 20 weeks and then a further increase peaking at 24 weeks. After this there was a decrease at 26 weeks with a slight recovery at the end of the experiment. The lice free calves started off with a value less than that of the infested animals on the same date but increased to this value over the next two sampling days. After a decline there was a sharp rise, a slight decline and then a rise from 16 to a peak at 20 weeks. After this there was a decline to the end of the experiment which included a plateau from 22-26 weeks and a final value approximately half that of the initial values for both infested and control groups.

In section B the infested animals ranged from 64.39 to 65.33 g/l and from 64.40 to 65.31 g/l for the lice free group. After remaining constant for 3 to 7 weeks there was a sharp decline in the infested group, seen at 10 weeks, and then a gradual recovery to a peak at 20

Mean Total Protein Determination

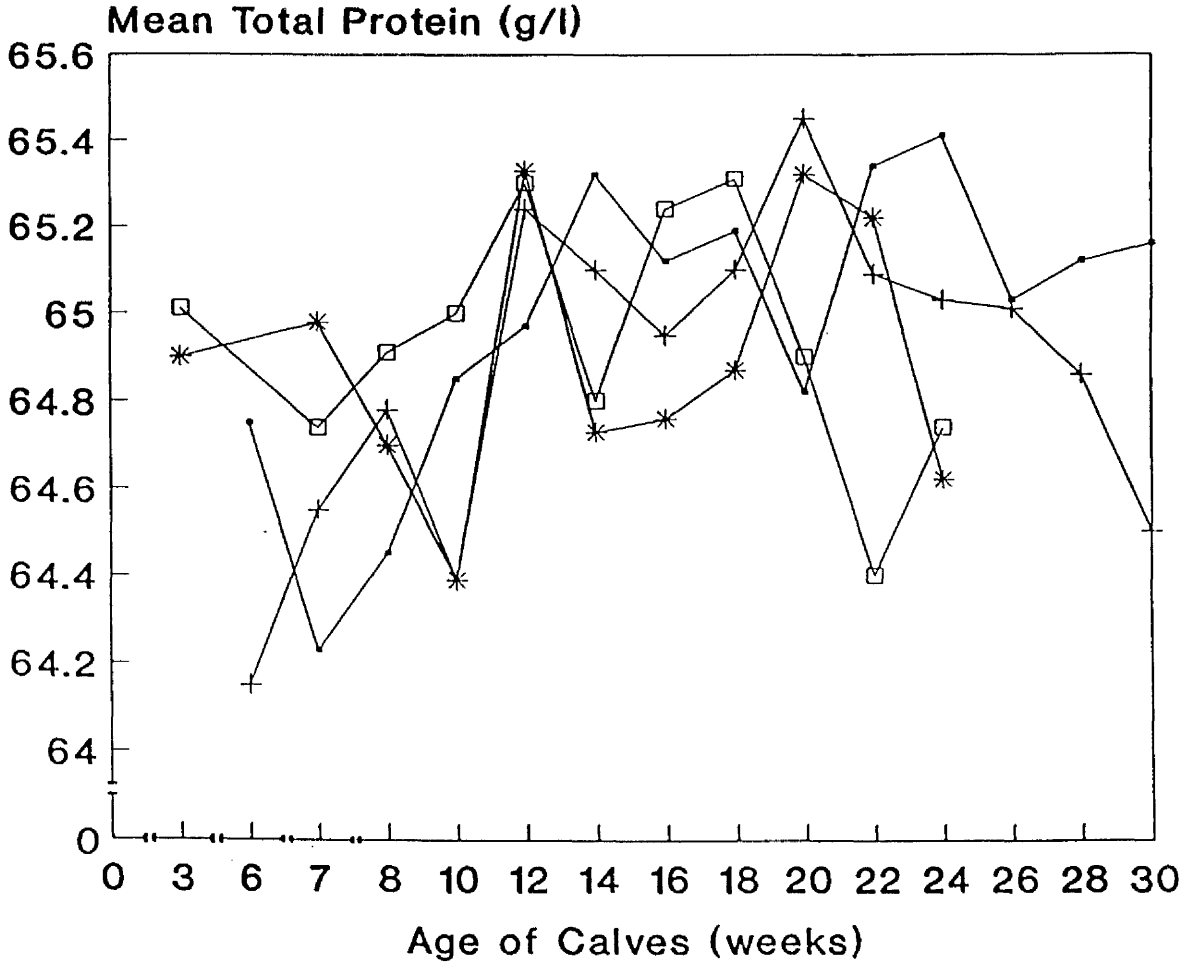


Figure 3·10

Infested A
Control A
Infested B
Control B

weeks followed by a sharp decline from 22 to 24 weeks to the final value. The lice free group started off at a similar value to that of the infested animals, had a slight decrease at 7 weeks and a steady increase to a peak at 12 weeks. After a sharp drop at 14 weeks there was another peak lasting from 16 to 18 weeks followed by a steady decline ending at 22 weeks with a recovery to a final value similar to that of the infested group.

3.3.10.2 STATISTICAL EVALUATION

There were no statistically significant differences revealed between louse infested and louse free calves in experiment A. The same was true of experiment B until the calves were sampled at 22 weeks of age when the total protein determination in the louse infested calves was significantly higher than the controls ($P<0.05$). This was the only sampling day indicating a significant difference.

3.3.11 Total Albumin Determination

3.3.11.1 GENERAL TRENDS

The mean total albumin determination is shown in Figure 3.11 and Appendix 11.

The range for the infested calves in experiment A was 34.23 to 35.05 g/l and 34.23 to 35.60 g/l for the lice free group. The infested group had their lowest mean value on the first sampling day. After this there was an increase to 12 weeks, although there was a slight drop at 10 weeks. From 12 weeks there was a gradual decline to 20 weeks, a sharp rise at 22 weeks followed by a plateau for the remainder of the experiment.

Mean Total Albumin Determination

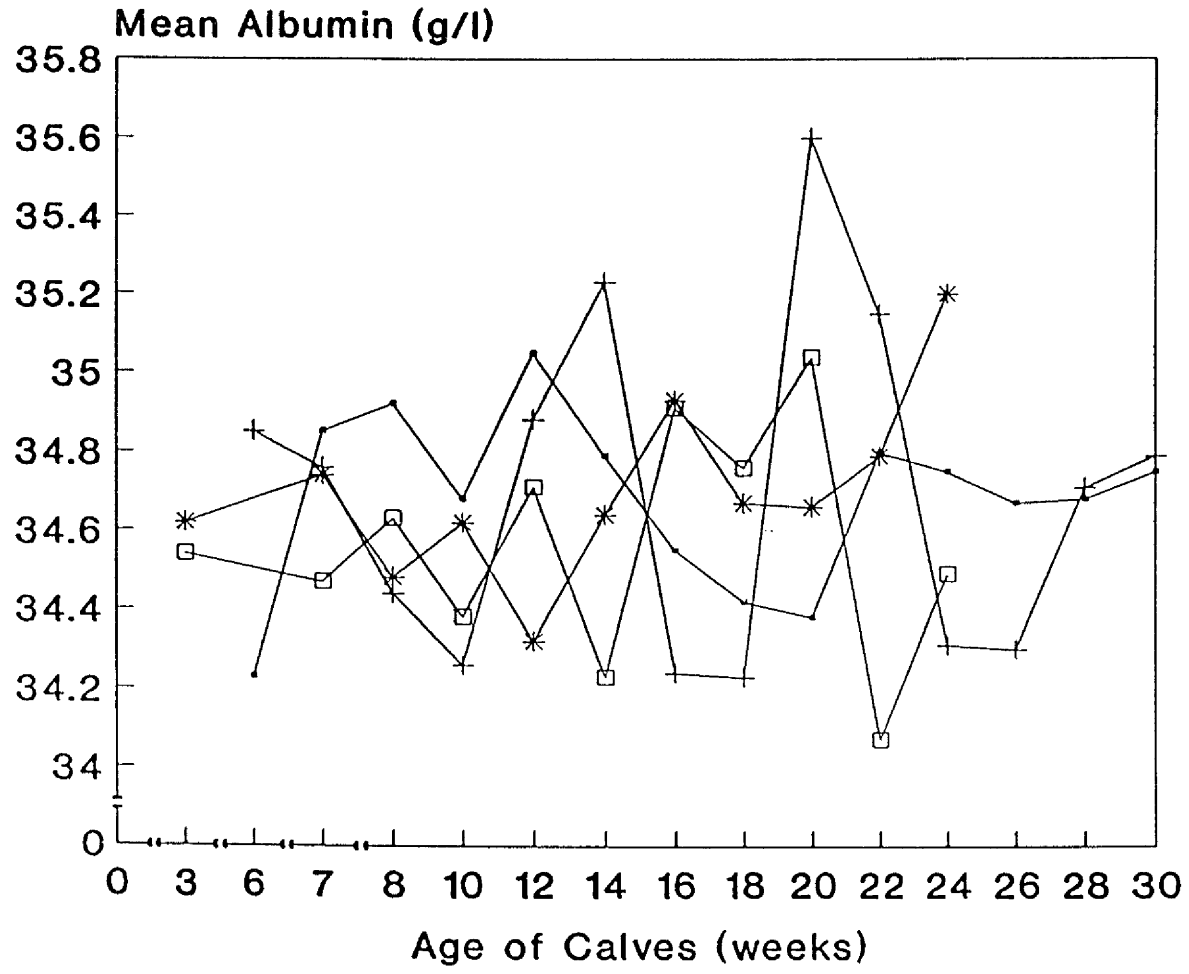


Figure 3·11

Infested A
Control A
Infested B
Control B

There was an initial decline in the lice free group occurring over 6 to 10 weeks of age followed by a peak at 14 weeks. This preceded a sharp decline to the lowest value, seen at 16 weeks, which remained until 18 weeks. A rise then occurred to a peak value at 20 weeks followed by a decline to a trough, at 24 to 26 weeks, similar to that observed at 16 to 18 weeks with a recovery from 26 weeks to the end of the experiment with a value similar to the initial one.

For experiment B from 3 to 12 weeks there was a step-wise decline to the lowest value observed, seen at 12 weeks. After this there was a constant increase to 16 weeks, a trough at 18 to 20 weeks, of similar value to the initial one, followed by an increase from 20 to 24 weeks to the highest value recorded.

The lice free group started at a similar level to the infested, had some fluctuations with a trough at 14 weeks followed by a peak which lasted from 16 to 20 weeks. After this there was a sharp decline to the lowest value, observed at 22 weeks, with a recovery to a final value similar to the initial one.

3.3.11.2 STATISTICAL EVALUATION

Analysis of the data revealed a statistically significant difference in experiment A at 20 weeks with the louse infested calves having a significantly lower total albumin concentration than the louse free group ($P<0.05$). On no other sampling day were there any statistically significant differences between infested and louse free calves in both experiments.

3.3.12 Weight

3.3.12.1 GENERAL TRENDS

The mean weight values are shown in Figure 3.12 and Appendix 12.

The initial mean weight, at 1 week old in experiment A, for the infested calves was 46.42 kg with a final weight at 30 weeks of 212.23 kg. For the lice free group the initial weight was 44.38 kg rising to 208.13 kg. All the calves in both the test and control groups increased in weight at a constant rate throughout the experimental period. The lice free group was always slightly lighter at each sampling.

In experiment B the starting weight at 1 week old for infested calves was 45.39 kg with a final weight of 160.33 kg at 24 weeks. The louse free calves were 50.00 kg at the beginning of the experiment and had a final weight of 179.71 kg. Like experiment A the infested and lice free groups in experiment B increased steadily throughout the experiment but unlike experiment A the control group in experiment B were always slightly heavier than the infested group on each sampling day.

3.3.12.2 STATISTICAL EVALUATION

Analysis of the data in experiment A revealed no statistically significant differences except when the calves were weighed at 8 weeks of age. At this time the louse infested calves were significantly heavier than the louse free animals of the same age ($P<0.05$). On no other sampling day in experiment A or at any time in experiment B were there any other significant differences with regard

Mean Liveweights

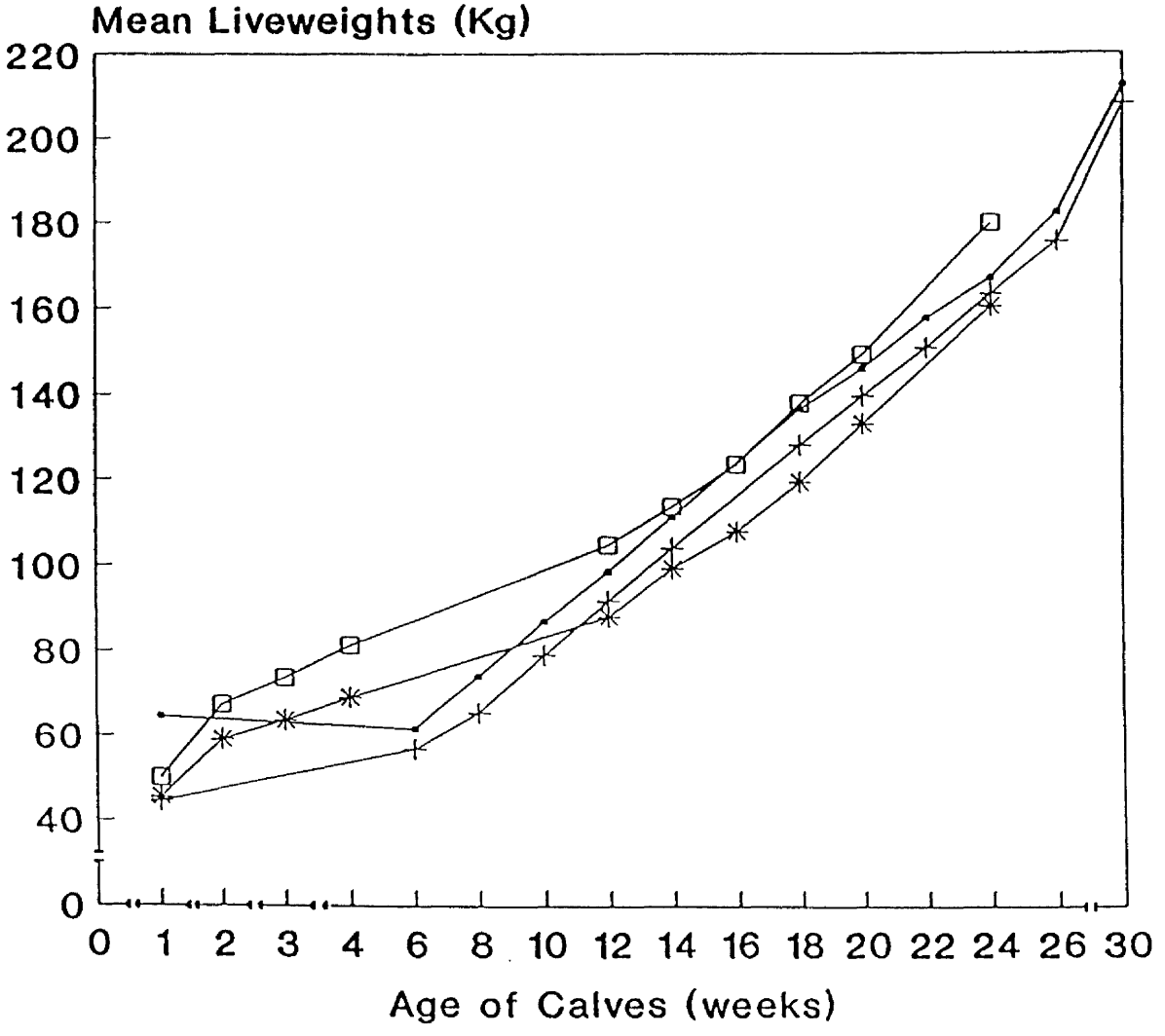


Figure 3·12

to weight.

3.3.13 Lice Counts

3.3.13.1 GENERAL TRENDS

Lice counts are shown in Figures 3.13 and 3.14 and Appendix 13.

In experiment A there was a slow, steady build up of the louse population, although at 10 weeks there was a slight drop, with a stable level of infestation occurring over a six week period (16 to 22 weeks) during which time instances of greater infestation were seen. After this the population began to recede. From the counts made the level of infestation most prevalent during the experimental period was determined as being slight, that is 5-10 lice present at each hair parting made.

For the infested calves in experiment B there was a gradual increase of the population, apart from a slight drop at 12 weeks, with a constant level occurring from 16 to 24 weeks of age which was determined as being slight. During this period there were, like experiment A, instances of counts being moderate and severe although the majority tended to be in the slight category. The counts showed that slight infestation was the degree of lice infestation most common throughout the experimental period.

The lice free calves from both experiments were examined in the same way as their infested counterparts and at no time during the experimental period was any lice found.

Mean Lice Counts
Experiment A

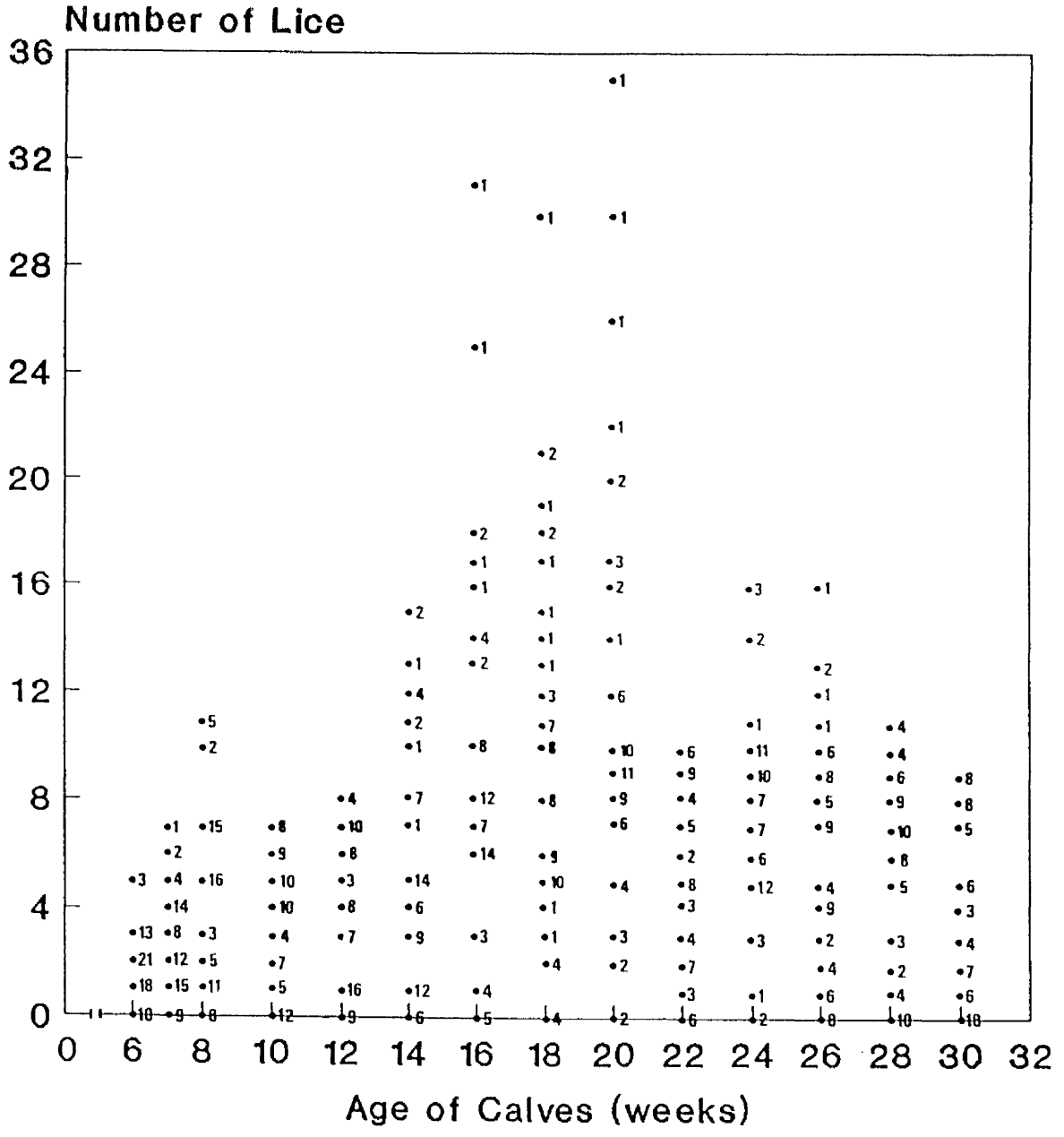


Figure 3·13

Mean Lice Counts
Experiment B

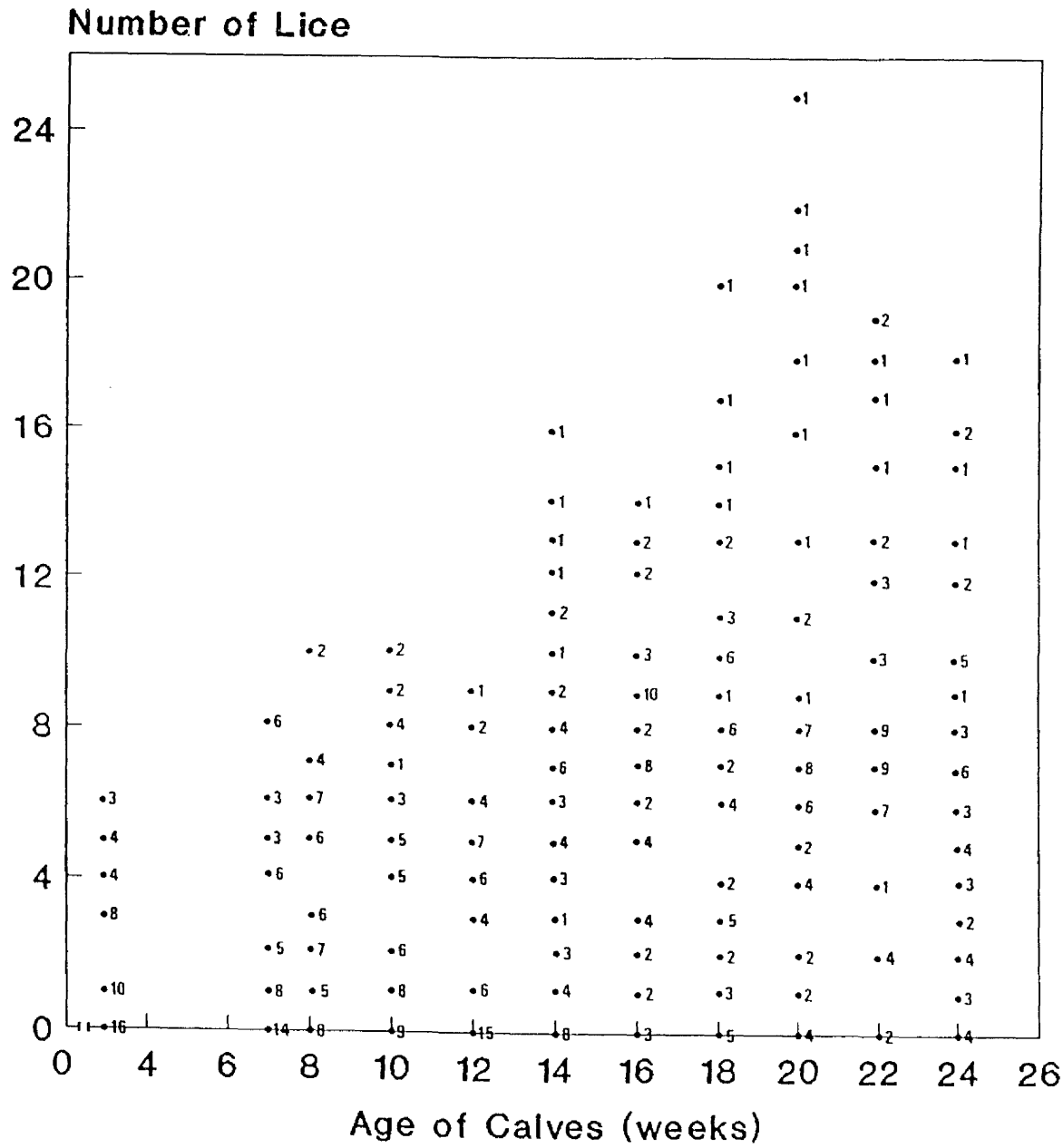


Figure 3·14

Table 3.1

Summary of Statistically Significant Results for Experiments A and B (P<0.05)

Perameter Studied	Infested Animals Exp. A	Infested Animals Exp. B
Haemoglobin concentration	10 wks - greater	ns
Leucocyte count	ns	7 wks - greater 8 wks - greater
Lymphocyte count	7 wks - greater	24 wks - lower
Monocyte count	6 wks - lower 30 wks - greater	8 wks - greater
Erythrocyte count	ns	12 wks - lower
Reticulocyte count	ns	7 wks - greater
Total protein determination	ns	22 wks - greater
Total albumin determination	20 wks - lower	ns
Weight	8 wks - greater	ns

wks - age of calves in weeks
ns - not significant

3.4 DISCUSSION

L. vituli did not significantly cause loss of weight or anaemia in young cattle during the investigation. Only a slight, naturally occurring infestation was maintained on the infested calves. These results are in agreement with the findings of Scharff (1962) in the United States of America and Kettle (1974) in New Zealand.

All calves demonstrated a constant, steady increase in liveweight throughout the experimental period which appeared to be independant from the level of infestation present on the infested calves. There were no significant differences between lice infested and lice free calves except at 8 weeks in experiment A where the infested animals were found to be significantly heavier. This difference may be the result of one of two explanations or a combinaton of them. The significantly heavier infested animals could have arisen from the gut-fill effect where some of the calves in the infested group had eaten large quantities of feed immediately prior to being weighed whereas the lice free animals had eaten less or nothing. Alternatively, the result could have been that as described by Davis and Williams (1986) who, when studying the hog louse, **Haematopinus suis**, on pigs found that infested pigs had increased growth rates and better feed efficiencies. They interpretated this as the observed decreased activity of the pigs, being caused by the lice, may reduce muscle use and thereby make more metabolic energy available for body growth.

The liveweight results in the present study are in agreement with those of Kettle (1974), in New Zealand, who also found no significant difference between slightly **L. vituli** infested calves and louse free ones. In Australia, Tweedle **et al** (1977), were also in agreement in finding no disimilarity in liveweight gains between louse free and infested animals the cattle involved harbouring **L. vituli** as their most prevalent species and only seven percent of infestations being heavy.

Cummins and Graham (1982), also in Australia, found that during the autumn-winter period untreated, louse infested heifers lost more bodyweight than treated, louse free heifers. However, over the remainder of the experiment the untreated heifers tended to gain more weight than the treated heifers so making the overall body weight changes at the end of the experimental period not significantly different. The degree of **L. vituli** infestation was considered to be light.

In eight out of ten experiments carried out by Bailey **et al** (1984) **H. eurysternus** louse infestation did not result in lower growth rates. It was pointed out that this may have been due to the generally light level of infestation. There was no relationship between an increasing infestation of **H. eurysternus** and live weight change.

In comparison to this Collins and Dewhirst (1965) found that moderate and heavy **H. eurysternus** infestations significantly increased winter weight loss in heifers, whereas only heavy infestations significantly increased weight loss in bulls. Weight

loss over the experimental period was not significantly altered when light to moderate infestations were found on bulls and light infestations on heifers. These authors suggested that the additional stress from heavy louse burdens on these cattle greatly increased the winter weight loss with heifers being more affected than bulls. Another American worker, Snipes (1948), also reported weight loss in untreated **H. eurysternus** infested cattle when compared to treated, lice free animals.

Freer and Gahan (1968) found no lice on a spray treated group of cattle and a slightly increased population of **H. eurysternus** on the untreated group after an experimental period of one hundred days. They noted significant liveweight gains during this period in the cattle which had been sprayed. They suggested that treatment to rid the cattle of any infestation should not be delayed until obvious infestation is apparent but should be part of the general herd management routine as production losses may occur even with the infestations so light that there are no obvious symptoms.

A significant negative relationship between louse numbers and daily liveweight gain in yearling steers was described by Ely and Harvey (1969). They concluded that it was either lice being the cause of the reduced daily gains or the cause of the reduced daily gains permitted greater louse infestation. They did not, however, have lice free animals to clarify this point.

Nickel **et al** (1970) provide results which show significant liveweight gains in cattle treated to rid them of their lice infestation and in fact suggested that although they found a very

high efficiency after only one treatment, two applications were more reliable particularly as the number of viable eggs largely influenced the success of the first treatment.

In considering whether eradicating lice in beef herds was justifiable from an economic point of view, Freer and Gahan (1968) stated that many farmers did not recognise that even small populations of body lice, not sufficient to show obvious signs of infestation, can still have an adverse affect on production. Their experiment showed production losses occurred even in non obvious light infestations of **H. eurysternus** and that advanced infestation caused severe economic loss. Hyland and Nickel (1968) have also shown economic gains from treatment but these probably depended on nutritional status and possibly the time of year when treatment took place in relation to the likely increase in numbers of the untreated louse population. Collins and Dewhirst (1965), Rich (1966) and Cummins and Tweddle (1977) indicated that natural louse populations rarely attain levels that cause economic losses.

Examination of the blood parameters measured during the experiment indicated no important deviations from the range observed by Schalm, Jain and Carroll (1975) for normal animals. Although there were some significant results these values were still encompassed by the normal value ranges.

There has been little work done on the blood picture of cattle infested with ectoparasites, although Greatorex (1954), Holman (1955, 1956) and many others (see Schalm, 1975) determined the blood profile of normal healthy animals. There are conflicting views as to whether

or not the presence of lice cause the host to become anaemic. Peterson **et al** (1953) were the first to publish the findings that extremely heavy infestations of **H. eurysternus** produced a severe anaemia, at times reducing the red blood cell volume to such an extent that it was necessary to destroy the lice to prevent the host from dying. They also noted a reduction in haemoglobin and white blood cell levels although there was no consistent change in the percentage values for the various classes of white cells. They suggested that the remarkable recovery of the animals following eradication of the lice indicated that withdrawal of blood by the lice was the sole cause the anaemia.

With cattle all heavily infested from natural sources with **H. eurysternus** clinical symptoms of anaemia such as general unthriftiness, lack of vigor were evident in studies carried out by Shemanchuk **et al** (1960). The erythrocyte count and the haemoglobin content of the blood of infested animals was found to be fifty per cent lower than those of louse free animals. Both these perameters increased in the infested animals when the lice were destroyed with an average of 35 days being required for the animals to recover from the symptoms of anaemia. These workers concluded that the anaemia could be attributed directly to the lice, which evidently removed more blood than the animals could replace by haematopoiesis. Nelson **et al** (1970) also recorded anaemia among cattle infested with **H. eurysternus.** They stated that periods of high louse numbers could be recognised by lowered red cell numbers, haemoglobin and packed cell volume levels (P.C.V.). They did point out, however, that similar infestation on different animals did not always produce similar

degrees of anaemia.

In America, Collins and Dewhirst (1965) noted that Hereford cattle maintained on range without supplemental feed when heavily infested with **H. eurysternus**, were anaemic with female cattle being more adversly affected. Similarly, Nickel (1971), in Australia, observed anaemia in cattle due to heavy **H. eurysternus** infestations. He stated that the control of this louse, especially on hosts with a high level of infestation, prevented the development of anaemia.

Contrastingly, there are reports in the literature of lice having no effect on the blood profile of cattle. In New Zealand, Chalmers and Charleston (1980c) found no significant differences in haematocrit levels between cattle with a mixed infestation of **Damalinia bovis** and **L. vituli** and lice free groups in any experiment. These findings afirm work carried out in Australia by Cummins and Tweddle (1977) and Cummins and Graham (1982) who observed no important deviations in **L. vituli** infested cattle from the normal range of haematological values. However, although no conclusive evidence for the development of anaemia in infested animals was obtained there was a statistically significant eosinophilia and total red blood cell count was depressed (7.2×10^6 vs 6.7×10^6 cells/mm^3, $p<0.05$). Cummins and Graham (1982) noted that although all haematological values fell within the normal range, on one sampling day infested calves had significantly lower P.C.V. and haemoglobin levels than lice free calves.

Similarly, Tweddle **et al** (1977) stated that blood perameters for all individuals and groups remained within normal clinical limits but

significant differences between **L. vituli** and louse free groups were observed in some cases although no mention of which perameters affected was made. In Ireland, Oormadzi and Baker (1980) also noted that moderate levels of infestation with **L. vituli** and **Bovicola bovis** had no significant effect on haematocrit levels in calves for nine months. They stated that although these values fell within the normal ranges (Schalm **et al**, 1975) the eosinophil counts decreased as the level of infestation decreased. Statistically, these differences were significant, particularly during the cold months when the degree of infestation reached the highest level.

In India, Mehrotra and Singh (1986), while studying some of the blood perameters of **L. vituli** infested calves, observed that the haemoglobin levels of infested animals were lower all year than was recorded for normal, uninfested animals. They found higher values for P.C.V., except in the monsoon season, for infested calves with the red blood cell counts higher all year for the same animals. Although these workers concluded that the infested animals suffered from anaemia. The six calves used were only sampled four times in the 10 month experimental period.

This present study shows that a slight infestation has no significant effect on the blood perameters or weight gains of calves infested with **L. vituli.** Although some haematological observations were found to be significantly different from the louse free animals they still came within the normal accepted range as described by Schalm **et al** (1975). Perhaps well nourished young cattle, as was present in this study, produce sufficient energy and heat to raise the skin temperature enough to cause adverse conditions for lice

breeding. As a result, the population levels of these animals would not reach the very high levels seen on undernourished animals.

Further work could be carried out to assess the effect of moderate and severe infestations of **L. vituli** on the blood profile and weight of cattle. Studies involving cattle on different planes of nutrition could also be done to determine any effects on lice populations.

CHAPTER FOUR

ERYTHROKINETIC AND PLASMA PROTEIN TURNOVER STUDIES IN AYRSHIRE CALVES INFESTED WITH LINOGNATHUS VITULI

4.1 INTRODUCTION

There have been a number of studies to determine whether or not an infestation of blood-sucking lice has an effect on the blood profile of cattle. Some workers suggest that infestations of **Haematopinus eurysternus** do cause the host to become anaemic (Collins and Dewhirst, 1965 and Nelson **et al**, 1970). However, it should be noted that **H. eurysternus** is a much larger louse than **Linognathus vituli** and therefore may remove more blood from the host. Mehrotra and Singh (1986), studying cattle infested with **L. vituli**, found an anaemic response which they related to the lice population, whilst Cummins and Tweddle (1975), working with eight month old calves with a similar infestation level, did not find this to be the case. It would seem that the level of infestation and host condition play vital roles in deciding whether the host reaches an anaemic state.

Radioisotope techniques have been widely used to study the effects of gastro-intestinal parasitism (Holmes, Dargie, Maclean and Mulligan, 1968; Holmes, Abbott and Parkins, 1986; Holmes and Maclean, 1971 and Dargie, 1975). In such infections there frequently is a pronounced loss of plasma albumin and red blood cells. These techniques have been little used in the study of ectoparasite infestations. This study reports the use of these techniques to study the pathogenesis of an ectoparasite, in particular **L. vituli**.

In order to gain a proper understanding of the changes in host metabolism that occur from parasitic infection, one must be aware of the underlying changes in metabolic pools and rates of synthesis and catabolism of these pools. This is possible by employing isotopic

labelling techniques.

An experiment was carried out to determine the effects, if any, of the blood-sucking lice on the blood profile and general body condition of young Ayrshire calves. The amount of blood being lost due to the feeding of the lice on their hosts was also evaluated. This present study describes the pathophysiological changes in Ayrshire calves with a single moderate infestation of **L. vituli.**

4.2 MATERIALS AND METHODS

The results presented were obtained from an experiment carried out at the Department of Veterinary Physiology, University of Glasgow, Veterinary School, Bearsden Road, Glasgow, Scotland. This examined the pathophysiological effects of a blood sucking louse, **L. vituli,** on young Ayrshire calves.

4.2.1 Calf Management Pre-Experiment

Before being introduced into experimental conditions it was necessary to wean 6, ten week old, Ayrshire bull calves from milk and a variety of concentrates onto water and Ruminant A diet, which is a complete ration (supplied by the Edinburgh School of Agriculture). This was carried out to ensure that the diet of all the calves remained the same throughout the duration of the experiment. After weaning, the calves had fourteen days of Ruminant A and water only before being introduced into the experimental conditions.

The calves were housed on two separate farms in groups of three, thereby making contact between the infested and the louse free group

impossible. The louse free group was also treated with 10 ml 'Spot-On' (Coopers Animal Health Ltd., Crewe, Cheshire, England), a pour-on pesticide, at three and eight weeks to prevent louse infestation. The calves were frequently and carefully examined for lice before commencement of the radioisotope studies.

4.2.2 Experimental Conditions

4.2.2.1 CALF MANAGEMENT

The calves were introduced into the metabolism stalls provided by the Department of Veterinary Physiology, University of Glasgow. Harnesses were fitted onto each calf which held faecal collection bags in place (see Plates 2 and 3).

Twice daily the animals were given 5 litres of water and 1 kg of feed, more being given if necessary, with all the residues measured and noted. The stalls were cleaned daily and the calves weighed weekly.

Daily, throughout the duration of the experiment, the calves were orally given 10 ml of 0.75% (w/v) potassium iodide solution in order to saturate their thyroid glands with iodine and so ensure the rapid excretion of ^{125}I released by catabolism of ^{125}I-albumin. Three days after introduction into the metabolism stalls the radioisotope studies began and continued for twelve days.

4.2.2.2 RADIOISOTOPE LABELLING

The techniques used for labelling red cells with ^{51}Cr, transferrin with ^{59}Fe and albumin with ^{125}I were those described by

Plate 2 Calf harnessed in metabolism stall for radioisotope studies.

Plate 3 Harness and faecal collection bag arrangement in place as used in radioisotope studies.

Holmes **et al** (1968), Holmes and Maclean (1971) and Dargie (1975).

4.2.2.3 ADMINISTRATION OF RADIOISOTOPES

Each animal received 15 MBq ^{125}I, 15 MBq ^{51}Cr and 12 MBq ^{59}Fe supplied by Amersham P.L.C., Amersham, England. Using a jugular catheterisation technique the three isotopes were injected intravenously, the injection syringes being weighed before and after administration in order to determine the total amount of isotope injected.

4.2.2.4 BLOOD STUDIES

Fifteen minutes after administration of the radioisotope a heparinised blood sample (5 ml) was collected from the jugular vein not used to introduce the radioisotopes. This was repeated at 30, 60, 90, 120, 150 and 180 minutes post radioisotope injection. For the next four days blood collections were made twice daily (10 a.m. and 4 p.m.), thereafter once daily (10 a.m.) for the remainder of the experiment. One millilitre of blood and plasma was made up to the standard volume (10 ml) with dilute sodium hydroxide for radioactivity determination (Chapter 2, section 2.2.2). From each of the blood samples packed cell volumes (P.C.V.) was determined (see Chapter 2, section 2.2.1). Serum albumin, protein and iron determinations were carried out as described in Chapter 2, sections 2.2.3, 2.2.4 and 2.2.5 respectively.

4.2.2.5 FAECAL STUDIES

Faecal collection bags were changed three times a day. All faeces produced over a 24 hour period were weighed, ensuring each

calf's amount was kept separate. This amount included any 'spill' onto the floor of the stall although this was not sampled from.

The faeces were mixed thoroughly and random duplicate (8) approximately 10 g samples were collected into radioisotope counting vials, the exact weight of the faeces being determined on tared scales to two decimal places.

4.2.2.6 URINE STUDIES

Urine produced over a 24 hour period was weighed and from each duplicate (2) approximately 1 ml aliquotes were collected into counting vials. The exact weight of each urine sample was determined using tared scales to two decimal places. These samples were then made up to the standard volume (10 ml) with dilute sodium hydroxide for radioactivity determinaton.

4.2.2.7 LICE STUDIES

On the last two days of the experiment 600 lice were manually removed from each infested animal, weighed and their radioactivity determined. By comparison with the blood radioactivity, the blood content of the lice could be determined.

4.2.3 Radioactivity Content of Samples

The amount of radioactivity present in the blood, plasma, faecal, urine and lice samples, along with the radioisotope standards was determined using a gamma scintillation counter (Packard Instruments).

4.2.4 Calculation Methods

4.2.4.1 CORRECTION OF RAW COUNTS

The ^{59}Fe counts were the only ones which could be used directly from the raw counts. The ^{51}Cr counts were influenced by the ^{51}Cr counts were influenced by the ^{59}Fe and the ^{125}I counts were influenced by both the other isotopes, making it necessary to correct the ^{51}Cr and ^{125}I raw counts before carrying out any calculations.

4.2.4.1.1 Correction of ^{51}Cr Counts

Cross over of ^{59}Fe into ^{51}Cr=

$$\frac{\text{mean Fe in Cr channel for standards}}{\text{mean Fe in Fe channel for standards}} = \text{factor (F)}$$

To correct the raw ^{51}Cr counts, each of the sample iron channel counts were multiplied by F and the resultant figure subtracted from the corresponding counts in the chromium channel. This was carried out for blood, faecal and urine counts.

4.2.4.1.2 Correction of ^{125}I Counts

(i) Plasma counts

Cross over of ^{59}Fe into ^{125}I =

$$\frac{\text{mean Fe in I channel for standards}}{\text{mean Fe in Fe channel for standards}} = \text{factor (F)}$$

To correct the raw plasma ^{125}I counts each of the sample iron channel counts was multiplied by F and the resultant figure subtracted from the corresponding counts in the iodine

channel.

(ii) Faecal and urine counts

As both ^{51}Cr and ^{59}Fe isotopes affected the ^{125}I faecal and urine counts the ^{51}Cr counts had first to be corrected as described in section 4.2.4.1.1.

These corrected ^{51}Cr counts were then used to assess their cross-over effect into the iodine channel by:-

Cross over of ^{51}Cr into ^{125}I =

$$\frac{\text{mean Cr in I channel for standards}}{\text{mean Cr in Cr channel for standards}} = \text{factor (F)}$$

The corrected ^{51}Cr counts were then multiplied by the factor (F) and these figures subtracted from each of the ^{125}I faecal and urine counts.

Each of these sample results were then used when the ^{59}Fe cross-over calculation was made as described in 4.2.4.1.2. The resultant sample figures were the corrected ^{125}I values for each animals faecal and urine samples.

4.2.4.2 MEASUREMENT OF ALBUMIN POOLS AND CATABOLIC RATE USING ^{125}I-LABELLED ALBUMIN

(i) Total injected radioactivity

See Appendix 13.

(ii) The plasma radioactivity as counts/min/ml of each daily plasma sample was expressed as a percentage of the 15 minute post-injection sample. These were plotted against time in days as

abcissa on seminlogarithmic paper. The best straight line was fitted using a computer and the half-life (t1/2) for each animal calculated.

(iii) The total radioactivity excreted each day in urine and faeces was calculated. Example:-

Total urine activity (day 5) =

Mean activity of aliquots collected on day 5 (cts/min/ml) x total weight (g) of urine.

(iv) The plasma volume (Vp), intravascular (CA), extravascular (EA) and total body (TA) pools of albumin were calculated for each animal - see Appendix 14.

(v) The fractional catabolic rate (K) was determined by graphic analysis of the plasma radioiodine disappearance curve, and by analysis of the excreted radioactivity as outlined in Appendix 14.

(vi) The faecal clearance of plasma for each 24 hour period was evaluated by dividing the total counts per minute of the faecal collection by the counts/min/ml plasma at the beginning of that period.

$$\frac{\text{faecal cts/min}}{\text{plasma cts/min/ml}} = \text{faecal clearance of plasma}$$

4.2.4.3 MEASUREMENT OF RED CELL AND BLOOD VOLUMES, RED CELL SURVIVAL AND GASTROINTESTINAL BLOOD AND IRON LOSS USING ^{51}Cr-LABELLED ERYTHROCYTES

(i) From the counts/min/ml of each blood sample and its haematocrit the counts/min/ml red blood cells was determined.

$$\frac{\text{blood cts/min/ml}}{\text{haematocrit}} = \text{RBC cts/min/ml}$$

(ii) The total radioactivity injected into each animal was calculated, see Appendix 13.

The cts/min/ml red blood cells of the samples taken 15, 30, 45 and 60 minutes after injection were plotted against time on semilogarithmic paper. The best straight line was fitted using a computer and the equilibrium radioactivity determined by extrapolation to zero time (t_0). The red cell volume was calculated by:-

$$V_{RC} = \frac{TIR}{t_0}$$

$$V_{RC\ ml/kg} = \frac{V_{RC}}{BW}$$ where BW = body weight.

(iii) Blood volume was calculated by:-

$$V_{B(RC)\ ml} = \frac{V_{RC} \times 100}{PCV}$$ where PCV = packed cell volume.

$$V_{B(RC)\ ml/kg} = \frac{V_{B(RC)}}{BW}$$ where BW = body weight.

It should be noted that the packed cell volume overestimates the mean body haematocrit. Therefore, determination of the blood volume using the red cell - haematocrit method underestimates the true blood volume, whilst the method using plasma volume overestimates the true blood volume.

$$V_{B(P)\ ml} = \frac{V_P \times 100}{100 - PCV}$$

where V_P = plasma volume (see Appendix 13).
PCV = packed cell volume.

(iv) The counts/min/ml red cells of each daily blood sample were expressed as a percentage of the activity of the 15 minute sample and were plotted against time in days as abcissa on semilogarithmic paper. The best straight line was plotted by computer and the half-life (t1/2) calculated.

(v) For each animal the total radioactivity excreted each day in faeces and in urine was determined.

For each 24 hour period a faecal blood and red cell clearance was calculated by dividing the total counts/min/ml blood and red cells for the blood sample taken at the beginning of that period. Clearance figures for urine was calculated similarly.

4.2.4.4 MEASUREMENT OF RED CELL SYNTHESIS, RED CELL LIFESPAN AND FAECAL IRON LOSS USING FERRIC-59-CITRATE

(i) The net counts per minute for each plasma sample were plotted against time in minutes as abcissa on semilogarithmic paper. The best straight line was fitted by computer and the half-

life (t1/2) determined.

(ii) Plasma iron turnover rate (PITR) of each animal was calculated using:

PITR (mg/day/100 ml blood) =

$$\frac{\text{serum iron (mg/100 ml)} \times 0.693 \times 1440^{*}}{\text{t1/2 (min)}} \times \frac{(100 - \text{Hct})}{100}$$

*1440 = minutes per day.

If, as is preferable, the plasma volume (V_P) is estimated simultaneously, the total PITR may be calculated from:

$$\text{PITR (mg/day)} = \frac{\text{serum iron (mg/ml)} \times 0.693 \times 1440 \times V_P}{\text{t1/2 (min)}}$$

(iii) From the counts/min/ml of each blood sample and its haematocrit the counts/min/ml red blood cells were calculated and then plotted against time in days as abcissa on semilogarithmic paper.

(iv) The percentage iron utilisation by red cells from the maximum ^{59}Fe activity/ml red cells and the total red cell volume V_{RC}:

$$\% \text{ utilisation} = \frac{100 \times V_{RC} \times {}^{59}\text{Fe max}}{\text{total infected activity}}$$

(v) The red cell iron incorporation rate (RCIIR) was calculated by:

RCIIR = % utilisation x PITR (mg/day)

(vi) For each 24 hour period the faecal clearance of blood and red cells was calculated by dividing the total counts per minute of the faecal collection by the counts/min/ml plasma at the beginning of that period. Similar calculations were made based on urinary activity.

4.2.5 Statistical Analysis

Student t-test were used to evaluate the results. P values of less than 0.05 were considered significant.

4.3 RESULTS AND DESCRIPTION OF TRENDS IN THE DATA

The results will be presented along with a description of the data. This will be derived directly from, and will contain no interpretation of, the results.

4.3.1 Erythrokinetics

The erythrokinetics data is presented in Table 4.1.

In the infested calves the circulating red cell volumes were not significantly reduced compared to the controls. Both had mean values of 23 ml (kg bw)$^{-1}$. Plasma volumes were relatively unchanged with the infested calves having a mean value of 61 ml (kg bw)$^{-1}$ compared to 55 ml (kg bw)$^{-1}$ for the lice free calves. These values were not significantly different. As a result of these figures the total blood volumes of the infested calves of 84 ml (kg bw)$^{-1}$ was not significantly different from the controls 79 ml (kg bw)$^{-1}$.

TABLE 4.1

Eryyhtokinryic studies on calves infested with Linognathus vituli

Animal number		Hct	RCV (^{51}Cr)	PV (^{125}I)	TBV	$t_{1/2}$ ^{51}Cr red cells	Faecal clearance of ^{51}Cr red cells
		litre litre^{-1}	ml (kg bw)$^{-1}$	ml (kg bw)$^{-1}$	ml (kg bw)$^{-1}$	h	ml d^{-1}
S9	INF	0.35	25	63	88	233	2.7
S10	INF	0.37	24	65	89	249	2.3
S11	INF	0.35	19	55	74	227	2.3
	mean =	0.36	23	61	84	236	2.4
	s.e. =	0.01	3	4	7	9	0.2
S12	C	0.32	20	62	82	193	2.5
S14	C	0.35	24	45	73	204	2.1
S15	C	0.38	24	59	83	200	2.2
	mean =	0.35	23	55	79	199	2.3
	s.e. =	0.02	2	7	5	4	0.2
Significance		NS	NS	NS	NS	P<0.01	

INF = infested
C = control

Hct = haematocrit
RCV = red cell volume
PV = plasma volume
TBV = total blood volume
NS = not significant

Red cell survival was significantly increased in the infested group, with a mean value of 236 hours as compared to the non-infested group 199 hours.

Red cell losses into the gastrointestinal tract as measured by ^{51}Cr red blood cell faecal clearance were very low in both groups and no significant differences were found.

4.3.2 Ferrokinetics

Ferrokinetics data is shown in Table 4.2.

The infested calves had slightly increased plasma iron levels as compared to the controls but not significantly so. Although not significantly different, the louse infested animals had slower ^{59}Fe plasma disappearance rates at 63.6 minutes, compared to 44.3 minutes for the controls and slightly reduced plasma iron turnover rates with 1.15 mg d^{-1} (kg bw)$^{-1}$ and 1.41 mg d^{-1} (kg bw)$^{-1}$.

4.3.3 Albumin Metabolism

Data for albumin metabolism is shown in Table 4.3.

Serum albumin levels and the intravascular (CA) pools showed no marked difference between infested and lice free groups. However, the extravascular (EA) pools were significantly elevated in the infested animals which had a mean value of 1.62 g (kg bw)$^{-1}$ compared to 1.23 g (kg bw)$^{-1}$ for the control group. Although significantly elevated the EA result was not sufficient to cause a significant difference in the EA/CA ratio.

TABLE 4.2

Ferrokinetic studies on calves infested with Linognathus vituli

Animal number		Plasma iron	^{59}Fe $t_{1/2}$	Total plasma iron turnover
		ug dl^{-1}	min	mg d^{-1} (kg bw)$^{-1}$
S9	INF	91	50.2	1.15
S10	INF	133	58.9	1.47
S11	INF	125	81.6	0.84
	mean =	116	63.6	1.15
	s.e. =	18	13.2	0.26
S12	C	115	45.1	1.57
S14	C	93	38.5	1.22
S15	C	119	49.2	1.43
	mean =	109	44.3	1.41
	s.e. =	11	4.4	0.14
Significance		NS	NS	NS

INF = infested
C = control
NS = not significant

TABLE 4.3

Albumin metabolism in calves infested with Linognathus vituli

Animal number		Albumin g litre^{-1}	PV ml (kg bw)$^{-1}$	CA g (kg bw)$^{-1}$	EA g (kg bw)$^{-1}$	EA/CA	$t_{1/2}$ ^{125}I-albumin h	F(CA)
S9	INF	28.00	63.00	1.77	1.89	1.07	394.00	0.068
S10	INF	29.75	65.00	1.94	1.52	0.78	321.00	0.047
S11	INF	28.00	55.00	1.54	1.45	0.94	300.00	0.060
	mean =	28.58	61.00	1.75	1.62	0.93	338.33	0.058
	s.e. =	0.82	4.32	0.16	0.19	0.12	40.29	0.09
S12	C	29.00	62.00	1.79	1.22	0.68	292.00	0.042
S14	C	28.75	45.00	1.30	1.23	0.95	317.00	0.049
S15	C	29.00	59.00	1.72	1.23	0.72	313.00	0.061
	mean =	28.92	55.33	1.60	1.23	0.78	307.33	0.051
	s.e. =	0.12	7.41	0.22	0.00	0.12	10.96	0.008
Significance		NS	NS	NS	$p<0.01$	NS	NS	NS

INF = infested
C = control

PV = plasma volume
CA = intravascular albumin pool
EA = extravascular albumin pool
F(CA) = fractional catabolic rate of albumin
NS = not significant

There were no significant differences in the plasma ^{125}I-albumin half-lives or the fractional catabolic rates between the two groups of calves.

4.3.4 Blood Content of Lice

Blood content of lice results are shown in Table 4.4.

All the lice removed from the infested group of calves were assumed to have fed due to the distended appearance of their abdomen.

Both ^{51}Cr and ^{59}Fe radioactivity measurements gave similar results when the blood content of the lice was determined. Using ^{51}Cr data from red cells and from the lice, the red cell content for 1 g weight of lice was found to be approximately 0.333 ml compared with 0.252 ml when ^{59}Fe data was used for the evaluation. The average weight of one louse was determined as 4.75×10^{-4} g, therefore the blood content of one louse was approximately 0.443 ul, determined by ^{51}Cr-red cells and 0.350 ul, using ^{59}Fe red cells. Using the ^{125}I-albumin data the mean plasma content of 1 g weight of lice was calculated as 3.316 ml and of one louse 1.570 ul, whilst in terms of albumin content the ^{125}I radioactivity represented 198.94 mg per 1 g weight of lice and 0.984 ug per louse.

4.3.5 Daily Liveweight Gain

The daily liveweight gains are shown in Table 4.5.

The infested calves gained weight at a lower rate than the controls during the radioisotope study, but not significantly so. Food and water intake throughout the trial was not adversly affected

TABLE 4.4

Blood, red blood cell and plasma content of *Linognathus vituli*

ISOTOPE: ^{51}Cr

Animal number	RBC Content		Blood Content	
	ml per 1 g lice	ul per louse	ml per 1 g lice	ul per louse
S9	0.259	0.123	0.736	0.300
S10	0.202	0.087	0.584	0.260
S11	0.539	0.260	1.635	0.770
mean =	0.333	0.157	0.985	0.443
s.e. =	0.147	0.075	0.464	0.232

ISOTOPE: ^{59}Fe

Animal number	RBC Content		Blood Content	
	ml per 1 g lice	ul per louse	ml per 1 g lice	ul per louse
S9	0.255	0.120	0.750	0.360
S10	0.246	0.120	0.664	0.320
S11	0.255	0.120	0.772	0.370
mean =	0.252	0.120	0.729	0.350
s.e. =	0.004	0.000	0.047	0.022

ISOTOPE: ^{125}I

Animal number	Albumin Content		Plasma Content	
	ml per 1 g lice	ul per louse	ml per 1 g lice	ul per louse
S9	209.16	0.099	3.486	1.650
S10	220.14	0.104	3.669	1.740
S11	167.52	0.079	2.792	1.320
mean =	198.94	0.984	3.316	1.570
s.e. =	22.66	0.011	0.378	0.181

TABLE 4.5

Weight changes in calves infested with Linognathus vituli

Animal number		Weight at day 1 kg	Weight at day 8 kg	Weight gain/loss kg	Daily weight gain/loss kg
S9	INF	47.00	47.00	0.1	0.000
S10	INF	52.00	57.50	5.5	0.786
S11	INF	52.00	51.00	-1.0	-0.143
	mean =	50.33	51.83	1.5	0.214
	s.e. =	2.86	4.33	2.9	0.410
S9	C	58.00	61.00	3.0	0.429
S10	C	52.00	59.00	7.0	1.000
S11	C	53.00	57.00	4.0	0.571
	mean =	54.33	59.00	4.7	0.670
	s.e. =	2.62	1.63	1.7	0.240
Significance		NS	NS	NS	NS

INF = infested
C = control
NS = not significant

and in the case of one louse infested calf it was above the mean values for the louse free animals.

4.4 DISCUSSION

In this study the effects of **L. vituli** on red cell and plasma protein turnover of calves was measured using ^{51}Cr-red blood cells, ^{59}Fe-transferrin and ^{125}I-albumin along with the uptake of blood by the lice.

Circulating red cell volumes and plasma volumes were estimated from the blood ^{51}Cr and ^{125}I activities 15 minutes after injection by application of the dilution principle (see Appendix 15). The apparent red cell half-life was determined from the red cell disappearance rate.

Plasma iron turnover rates were calculated from the ^{59}Fe-transferring disappearance rate by the method of Finch, Deubelbeiss, Cook, Eschbach, Harker, Funk, Marsaglia, Hillman, Slichter, Adamson, Ganzoni and Giblett (1970). An index of erythropoiesis was also obtained from the rate of red cell utilisation of the radioiron.

Albumin pools were calculated from plasma volume, serum albumin concentration and graphic analysis of the plasma ^{125}I radioactivity curve, the latter also providing the plasma ^{125}I-albumin half-life. The catabolic rate of albumin was obtained by dividing the daily excreted ^{125}I activity by the total plasma radioactivity during the preceding 24 hour period (Campbell, Cuthbertson, Matthews and McFarlane, 1956). The calves were dosed orally each day with 10 ml of 0.75% (w/v) potassium iodide to ensure rapid excretion of ^{125}I

released by catabolism of ^{125}I-albumin.

At the moderate level of infestation present in this study the pathophysiological effects were mild. However, a number of changes were detected. In particular, it was found that red cell survival was significantly increased and the plasma iron turnover rate slightly decreased in louse-infested animals relative to the controls. Serum albumin levels, plasma ^{125}I-albumin half-life and the fractional catabolic rate in the louse-infested calves were not significantly different from the controls. Similarly the louse-infested calves showed no significantly reduced values for circulating red cell volume and plasma volumes or total blood volumes compared with the controls. However, the extravascular pool of albumin was significantly greater in the infested cattle.

The number of lice feeding on the host animal on a regular basis will have a bearing on the quantities of red cells and plasma proteins being lost from the host and also the host's erythropoietic response. With a light infestation the amount of blood ingested by the lice may be insignificant and detrimental effects on the host negligible, but if the infestation is severe then the degree of blood lost may be at a rate faster than the host can compensate for and consequently it becomes anaemic. Heavy infestations of **H. eurysternus** (Collins and Dewhirst, 1965; Nelson **et al**, 1970 and Peterson **et al**, 1953) and **L. vituli** (Mehrotra and Singh, 1986) do cause anaemia if the infestations are classed as being severe. However, it must be noted that **H. eurysternus** is a much larger louse than **L. vituli** and consequently has the capacity to remove more blood from its host than the smaller species. Using standard

haematological techniques Nelson **et al** (1970) found, with cattle infested with **H. eurysternus,** decreased red cell, haemoglobin and P.C.V. values indicating anaemia. Similar results were found by Peterson **et al** (1953) and Shemanchuk **et al** (1960) again studying cattle infested with **H. eurysternus.** Mehrotra and Singh (1986) working with **L. vituli** infested cattle reported that there were depressed haemoglobin levels with increased P.C.V. values indicating that the animals were anaemic. These studies, however, involved six calves sampled four times over a ten month experimental period.

In the present experiment the calves bore a moderate infestation. This was determined using the number of lice removed on the last two days of the experiment and from counts made from coat-partings throughout the experiment. As a guideline the number of lice on each infested animal was approximately two thousand. This quantity of lice did not cause anaemia and no changes were detected in the haematocrit of the louse infested calves and the circulating red cell volumes. However, the red cell survival rate was longer in the lice infested calves than the controls. This is a surprising finding since blood loss would normally be associated with a decrease in red cell survival. Further studies are required with **L. vituli** and other ectoparasites to confirm this effect. The ferrokinetic data also demonstrated that the lice infested animals were showing a slightly depressed erythropoietic response as they had lower plasma iron turnover rates compared to the control animals although the differences were not significant. A higher turnover rate would be expected where substantial blood loss was occurring.

The albumin levels were not reduced to a significant degree and there was no change in the distribution of albumin between extravascular and intravascular compartments although the extravascular pool was elevated in the infested calves. In contrast, in gastrointestinal parasitism, losses of plasma proteins into the gastrointestinal tract are frequently sufficiently high to cause hypoalbuminanaemia, changes in the EA/CA ratio and an elevation in the catabolic rate of albumin (Holmes **et al**, 1986; Bremner, 1969; Holmes and Maclean, 1971 and Dargie, 1975).

Although no anaemia was detected in the infested calves the amount of radioactivity determined for the lice using ^{51}Cr-red cells and ^{59}Fe-transferrin, both of which gave comparable results, demonstrated that the lice were withdrawing blood from the host. These measurements, derived from the radioactivity counts, reflect the amount of red cells ingested by the louse during the experimental period. However, they give no indication of the total blood content, in terms of volume, within the louse or the rate at which this quantity of blood was ingested. In the louse there is a concentrating effect due to water loss via transpiration from the cuticle resulting in a much smaller volume of plasma being present than is revealed by the measured radioactivity. Lees (1946) found that argasid ticks were able to concentrate their blood meal by ultra-filtration of excess plasma water by the production of coxal fluid. Ticks appear to have great concentrating abilities as Kitaoka (1961) found in a 24 hour period a tick of **Boophilus microplus** had a blood meal three times its engorged body weight.

In order to determine the daily blood uptake of a louse it would be necessary to place unfed lice onto a host with radiolabelled red cells and plasma, leave for 24 hours, remove and the radioactivity in the lice determined. The difficulties involved in this type of study include possible damage on transfer of lice from one host to another and the uncertainty whether the lice would feed on the new host. As yet a supply of unfed **L. vituli** is unavailable as the parasite has not been successfully maintained in the laboratory. Indeed sucking lice are very difficult to rear artificially, the only success to date being the rearing of the human head louse, **Pediculus humanus capitus**, on rabbits (Maunder, 1984).

An interesting finding was observed in the plasma uptake using ^{125}I labelled albumin. Using the data the plasma content was calculated to be an unrealistic figure of 3.316 ml per 1 g weight of lice or 1.570 ul per louse. When the ^{125}I radioactivity of the lice was expressed as millilitres of albumin the albumin content of 1 g weight of lice was found to be 198.94 ml or 0.984 ul per louse. There are one or more possible explanations for this finding of a higher relative content of ^{125}I-albumin than ^{51}Cr- and ^{59}Fe-labelled red cells in lice. Firstly, the lice may selectively ingest plasma in preference to red cells. Secondly, the lice feed from intradermal capillaries which have a much lower haematocrit and therefore more plasma is ingested relative to red blood cells than would be expected from the haematocrit of blood samples collected from the jugular vein. Thirdly, once blood is concentrated the albumin may be retained but some of the labelled red cells may be passed out as faecal material. The last proposal is supported by the observation

that lice excrete dark, dry, dust-like faeces suggesting they are retaining the plasma proteins and excreting the red blood cells or labelled portion of those cells.

The present study showed no significant loss of liveweight gain in calves infested with **L. vituli**. This is in agreement with previous studies in Australia and New Zealand (Kettle, 1974; Tweddle **et al**, 1977 and Cummins and Graham, 1982). However, Collins and Dewhirst (1965) found that moderate and heavy infestations of **H. eurysternus** significantly increased winter weight loss in heifers. Freer and Gahan (1968), also studying cattle infested with **H. eurysternus**, noted significant liveweight gains in cattle following treatment to erradicate their lice. However, as stated earlier, **H. eurysternus** is a much larger louse than **L. vituli** and so may ingest larger quantities of blood.

Throughout the trial there was no loss of appetite in any of the calves, indeed, one louse infested calf had an above average food and water intake. A reduction in voluntary food intake is one of the common features associated with gastrointestinal parasitism and is accepted as a major factor in the pathogenesis of such infections. In these infections the degree of inappetance is usually in proportion to the level of parasitic infection. The degree of inappetance in lambs infected with **Haemonchus contortus** has been shown to vary not only with the level and duration of infection but also with the level of protein nutrition (Abbott, Parkins and Holmes, 1985 and Holmes **et al**, 1986). Despite the apparent importance of inappetance in parasitised ruminants, the reasons for its occurrence are still uncertain (see review by Symons, 1985). Inappetance in

ectoparasites could also have a different sequel to that of endoparasites. Pigs heavily infested with the hog louse, **Haematopinus suis**, had increased growth rates and better feed efficiences than pigs with fewer or no lice (Davis and Williams, 1986). Since uninfested pigs spent more time at feeders, Davis and Williams (1986) suggested that a decrease in activity caused by the lice may reduce muscle use and thereby make more metabolic energy available for body growth. However, the pigs in this study were not chosen at random and they suggested alternatively that faster growing pigs can support more lice. Lice are usually first noticed among poor, unthrifty animals. However, it is often uncertain which is the cause and which the effect. Ely and Harvey (1969) suggested that although rations are important in determining the condition of cattle they may indirectly affect the numbers of lice. Their results merely indicated that the type of ration fed to animals seems to influence the degree to which animals may become infested with **H. eurysternus.** Animals on a high plane of nutrition were better able to control their lice populations than animals on a low plane of nutrition (Cummins and Graham, 1982). This confirms the findings by Utech **et al** (1969) and Cummins and Tweddle (1977). Similarily, in gastrointestinal nematode infections such as **H. contortus** it has been found that those lambs fed on a low protein diet had more severe clinical signs than infected lambs on a high protein diet (Abbott **et al**, 1985).

The present experiment was conducted under stringent conditions using healthy animals introduced to the experimental diet well in advance of the radioisotope studies and they were maintained on this

diet throughout the duration of the experiment. The fact that the animals which were infested with lice were healthy and maintained on a good diet may have reduced the possibility of the lice causing significant disturbances to the physiological mechanisms of the host. Had this present experiment been carried out on animals in poor condition and given a sub-optimal diet, different results may have been obtained. Mehrotra and Singh (1986), for example, found that calves infested with **L. vituli** did become anaemic although they made no mention of the health of the animals throughout the experimental period, the level of appetite of the calves or the diet used in the experiment. It is possible that lice infested animals on a poor or restricted diet may firstly have higher lice burdens and secondly they may show sub-optimal physiological responses to the blood loss brought about by the lice. Further studies could be conducted to evaluate these two potential features of the pathogenesis of the lice infestations.

CHAPTER 5

ANALYSIS OF SALIVARY GLANDS FROM LINOGNATHUS VITULI

5.1 INTRODUCTION

Until recently, the function of the saliva of haematophagus arthropods was open to debate. Coagulation seemed to be the sole potential impediment to ingestion of blood, and so evidence of some anticoagulant in saliva became the focal point for research.

Recent studies demonstrated anti-haemostatic and anti-inflammatory components in the saliva of blood feeding arthropods (Ribeiro, Makoul, Levine, Robinson and Spielman, 1985; Ribeiro and Spielman, 1986 and Ribeiro, 1987). These substances appear to act by preventing the host from reacting to the trauma associated with the insects mouthparts (Ribeiro, 1987). Mustard and Packham (1977) found that aggregation of platelets established haemostasis much more rapidly than does coagulation and arthropod saliva is capable of inhibiting such platlet activity.

The vector-host interface can be described as vectors wishing to neutralise their host's anti-haemostatic and anti-inflammatory factors, while the host at the same time seeks to recognize salivary antigens and also oppose their effects by eliciting local inflammatory reactions. An understanding of the attributes of this interface may help in the understanding of how these two parties have co-evolved.

As saliva is normally the first insect secretion to which the blood meal is exposed there have been a number of investigations into the effects saliva may have on blood. It was Bates (1949) who suggested that it was the anticoagulins and haemagglutins present in the salivary glands of mosquitoes which assisted in the initial

breakdown of the blood meal. Fisk (1950) suggested that coagulation or aggultination of the blood meal denatures the proteins present sufficiently to allow attack by mosquito proteinases.

Devine, Venard and Myser (1965) noted that **Aedes aegypti** feeding upon young mice left on average 4.7 ug of saliva in the mice during consumption of a meal. It appears likely that because of the minute amount of saliva secreted by blood sucking insects, most investigators have used homogenates of salivary glands to test for a variety of enzymes, haemolysins, agglutins and anticoagulins (Gooding, 1972).

Isoelectric focusing, sometimes called electrofocusing, is based on a moving boundary. Amphoteric substances such as amino acids and peptides are separated in an electric field across which there are both voltage and pH gradients. The anode region is at a lower pH than the cathode region and a stable pH gradient is maintained between the electrodes. A pH range is chosen such that the samples being separated will have their isoelectric points within this range. Substances which are initially at pH regions below their isoelectric point will be positively charged and will migrate towards the cathode, but as they do so the surrounding pH will be steadily increasing until it corresponds to their isoelectric points. They will then be in the zwitterion form with no net charge so further movement will cease. Likewise, substances which are initially at pH regions above their isoelectric points will be negatively charged and will migrate towards the anode until they reach their isoelectric points and become stationary. Amphoteric substances thus become focused into narrow stationary bands. As the samples will always

move towards their isoelectric points it is not crucial where they are applied. Samples can thus be separated with very high resolution.

Sodium dodecylsulphate (SDS) polyacrylamide gel electrophoresis is one of the most widely used techniques for separating protein mixtures and determining their molecular weights. SDS is an anionic detergent which as it binds strongly to proteins causes them to be denatured. In the presence of excess SDS approximately 1.4 g binds to each gramme of protein thus giving the protein a constant negative charge per unit mass. Protein-SDS complexes therefore all move towards the anode during electrophoresis, and because of the molecular-sieving properties of the gel, their mobilities (and so the distances they migrate in any period of time) are inversely proportional to the $\log_{10}$ of their molecular weights. If standard proteins of known molecular weight are also run, the molecular weights of the sample proteins can be determined.

Enzyme-linked immunosorbant assay, sometimes referred to as enzyme immunoassay, or ELISA, combines the specificity of antibodies with the sensitivity of simple spectrophotomeric enzyme assays by using antibodies or antigens conjugated to an easily assayed enzyme which also possesses a high turnover number. ELISA is replacing radioimmunoassay (RIA) since it is relatively inexpensive to run, lacks radiological hazards of RIA and is suitable for use in small laboratories which lack gamma radioactive counting facilities.

5.2 MATERIALS AND METHODS

The data presented was obtained from studies undertaken at the Veterinary Investigation and the Nutrition and Microbiology Departments of the West of Scotland College, Auchincruive, Ayr and the Bacteriology Department of Stobhill Hospital, Glasgow.

These studies investigated the protein content of salivary glands from **Linognathus vituli** lice, and the isoelectric focusing point of these glands. Polyacrylamide gel electrophoresis was used to discern the major molecular weight components of these salivary glands. Also studied was the detection of antibodies against lice in calf sera by means of an enzyme linked immunosorbent assay (ELISA) technique.

5.2.1 Estimation of Protein Content of Salivary Glands

5.2.1.1 PREPARATION OF SAMPLES

Salivary glands were removed from recently obtained **L. vituli** lice. Fifty glands were placed in separate, plastic microcentrifuge tubes with lids (Sigma, Poole, England) containing 50 ul of distilled water and stored at -20^{o}C until required.

5.2.1.2 PROTEIN ASSAY

The assay was carried out using Pierce BCA Protein Assay reagent supplied by Pierce, Cambridge, England. Procedure for carrying out the assay is described in Chapter 2, section 2.4.

5.2.2 Isoelectric Focusing of Salivary Glands

5.2.2.1 PREPARATION OF SAMPLES

Salivary glands were removed from recently obtained **L. vituli** lice and placed in Ringers solution as described in Chapter 2, section 2.5.1.

5.2.2.2 ISOELECTRIC FOCUSING PROCEDURE

The technique employed for isoelectric focusing of the salivary glands using a flat-bed apparatus supplied by Pharmacia Ltd., Milton Keynes, England is described in Chapter 2, section 2.5.2.

5.2.3 Polyacrylamide Gel Electrophoresis

5.2.3.1 PREPARATION OF SAMPLES

Salivary glands from one hundred recently obtained **L. vituli** lice were dissected out and placed in 50 ul of distilled water in separate plastic microcentrifuge tubes with lids (Sigma, Poole, England) and stored at -20^{o}C until required.

5.2.3.2 ELECTROPHORESIS PROCEDURE

The equipment, solutions, buffers, techniques required to prepare the gels and application of samples onto the gels are described in Chapter 2, section 2.6.

5.2.4 Enzyme Linked Immunosorbent Assay (ELISA)

5.2.4.1 PREPARATION OF SALIVARY GLANDS

Salivary glands from one hundred and fifty **L. vituli** lice were removed and placed in 50 ul distilled water in separate, plastic microcentrifuge tubes with lids (Sigma, Poole, England) and stored at -20^{o}C until required.

5.2.4.2 ELISA PROCEDURE

The techniques employed in initiating the ELISA assay, including titration of sera and conjugate to discern their working dilutions, are described in Chapter 2, section 2.7.

When the optimum dilutions of sera and conjugate were known four plates were used to analyse sera from each of the four sampling days chosen. Each plate was equally divided between infested and lice free sera. Once read on the ELISA plate reader a mean figure for both infested and control sera wells on each plate was determined.

5.2.5 Statistical Analysis

In all the experiments carried out one way analysis of variance was used to test for significant differences in:

(i) protein concentration determined.

(ii) isoelectric point (the mid-point of the range being used in the calculation).

(iii) molecular weights of the main bands produced by polyacrylamide gel electrophoresis.

(iv) optical densities observed by ELISA analysis between infested and lice free sera on four different sampling days, between infested sera on the same four sampling days and between lice free sera on those same sampling days.

5.3 RESULTS

This section will present the protein concentration estimation of salivary glands from **L. vituli** lice, the isoelectric point of these glands, the molecular weights of the major bands observed through gel electrophoresis of the glands and the optical density values determined by ELISA of infested and lice free sera. There will also be a description of trends in the data. These will be derived from, and contain no interpretation of, the results.

5.3.1 Protein Concentration Estimation of Salivary Glands

The estimation of protein concentration is shown in Table 5.1.

The mean concentration of protein from the salivary gland samples was found to be 106.12 ug/ml (s.e. 4.19). Statistical analysis of the data using one way analysis of variance revealed no significant differences between each of the experimental runs.

5.3.2 Isoelectric Focusing

The data obtained by isoelectric focusing is shown in Table 5.2.

Table 5.1

Absorbance Readings to Give Protein Concentration of Salivary Glands

Sample number	Run A	Run B	Run C	Run D	Run E
1	0.234	0.174	0.198	0.218	0.185
2	0.181	0.192	0.201	0.204	0.157
3	0.196	0.210	0.173	0.190	0.190
4	0.215	0.159	0.168	0.228	0.207
5	0.204	0.191	0.181	0.187	0.170
mean	0.206	0.185	0.184	0.195	0.182
s.e.	0.018	0.017	0.013	0.018	0.017
s.d.	0.020	0.019	0.015	0.019	0.019
Protein content ug-ml	113.75	103.75	103.13	107.50	102.50

s.e. = standard error
s.d. = standard deviation

Table 5.2

Isoelectric Point of Salivary Glands

Sample number	Run A range	Run B range	Run C range
1	3.2 - 3.5	3.1 - 3.4	3.2 - 3.4
2	3.1 - 3.3	3.2 - 3.6	3.1 - 3.5
3	3.2 - 3.6	3.1 - 3.4	3.1 - 3.4
4	3.1 - 3.4	3.1 - 3.5	3.2 - 3.3
5	3.2 - 3.5	3.3 - 3.5	3.1 - 3.3
mean	3.29	3.32	3.26
s.e.	0.07	0.07	0.04
s.d.	0.08	0.08	0.04

s.e. = standard error
s.d. = standard deviation

The mean isoelectric point was found to be at pH 3.29. Statistical analysis of the data, using the mid-point of each of the ranges observed, indicated no significant difference for the isoelectric values observed between any of the gels.

5.3.3 Polyacrylamide Gel Electrophoresis

The mean molecular weights for the four main bands present on the gels are shown in Table 5.3.

From the five gel runs carried out, each with four lanes of sample material and three of molecular weight standards, four bands were seen consistently in the sample lanes. The mean molecular weights of the four bands were determined, using a calibration curve produced by the molecular weight standards, as 80320, 58665, 42310 and 32320.

Statistical analysis of the data, using the meal value for each of the four bands on each gel, revealed no significant difference in the values found for each of the four bands between any of the gels.

5.3.4 ELISA

5.3.4.1 GENERAL TRENDS

The optimum working dilution of the sera was found to be 1:5 and that of the conjugate 1:100 (see Figures 5.1 and 5.2 and Plate 4). It was at these dilutions that the sera from different sampling days and the conjugate were used.

Table 5.3

Molecular Weights Determined from PAGE

Gel number	Band A mean	Band B mean	Band C mean	Band D mean
1	80350	58750	42275	32425
2	80350	58625	42400	32300
3	80450	58625	42200	32225
4	80250	58575	42400	32225
5	80200	58750	42275	32425
mean	80320	58665	42310	32320
s.e.	87.18	71.76	78.42	90.00
s.d.	97.47	80.23	87.68	100.62

s.e. = standard error
s.d. = standard deviation

Lice Infested Sera
Optical Densities

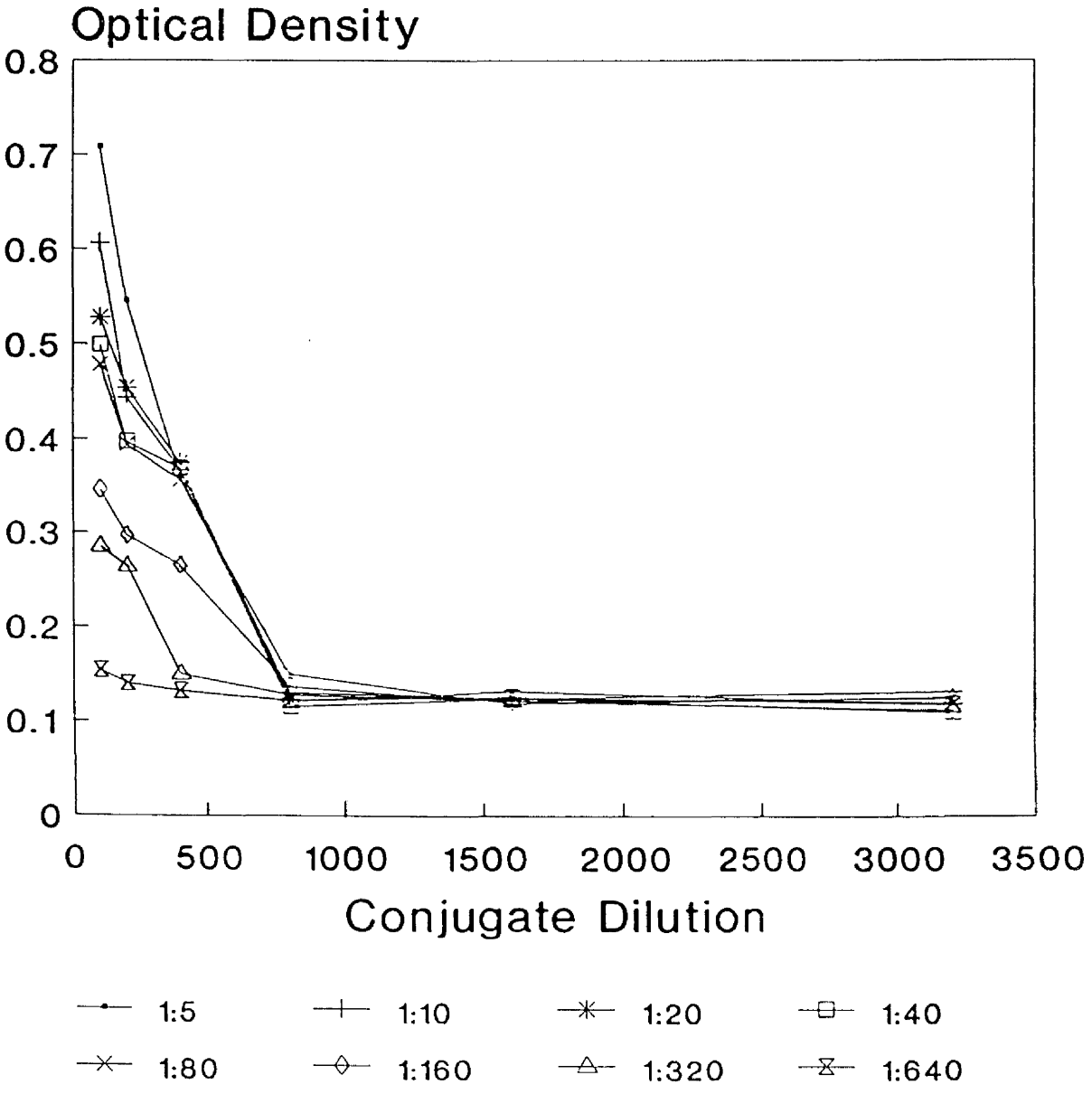


Figure 5·1

Lice Free Sera
Optical Densities

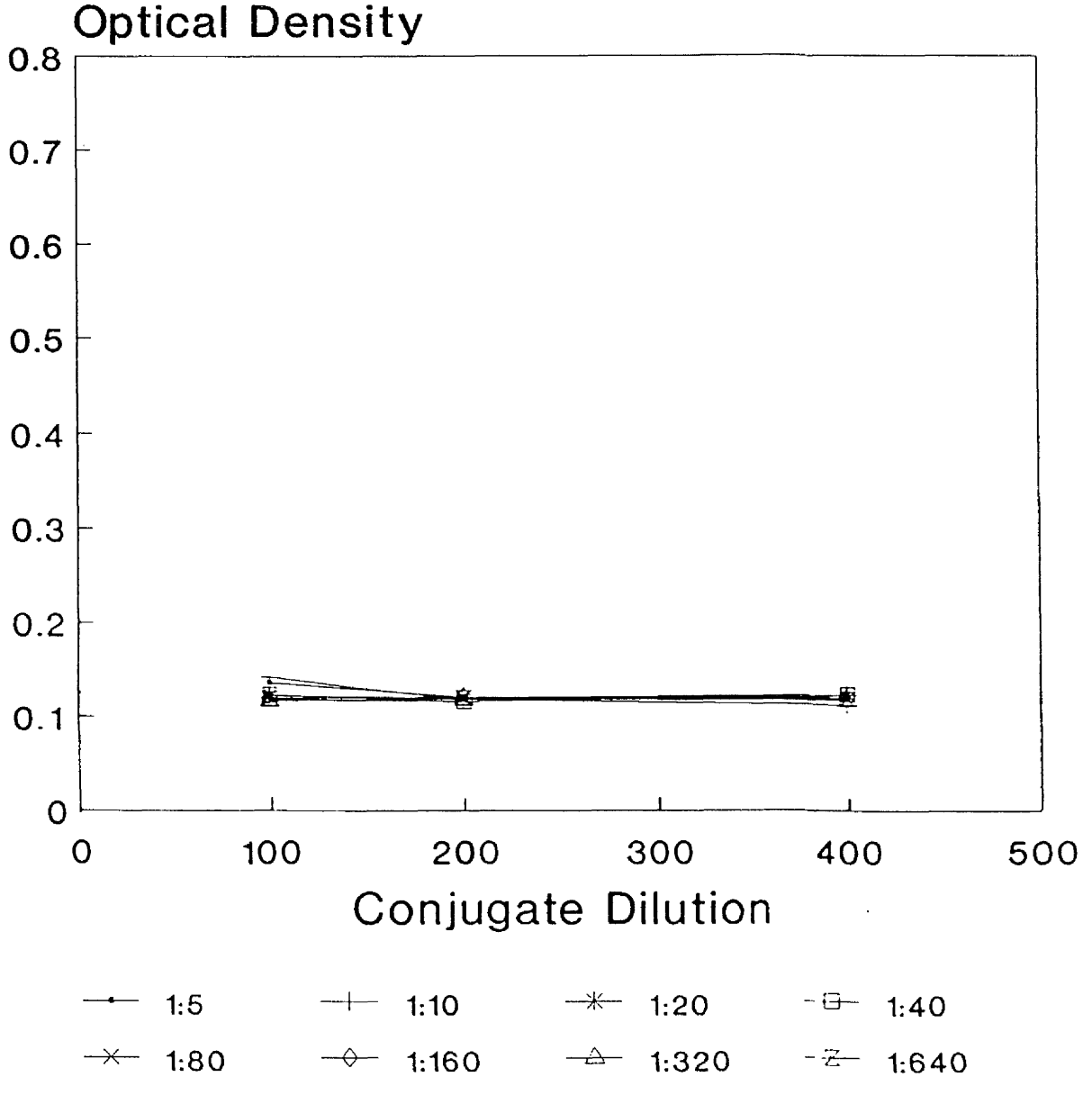


Figure 5·2

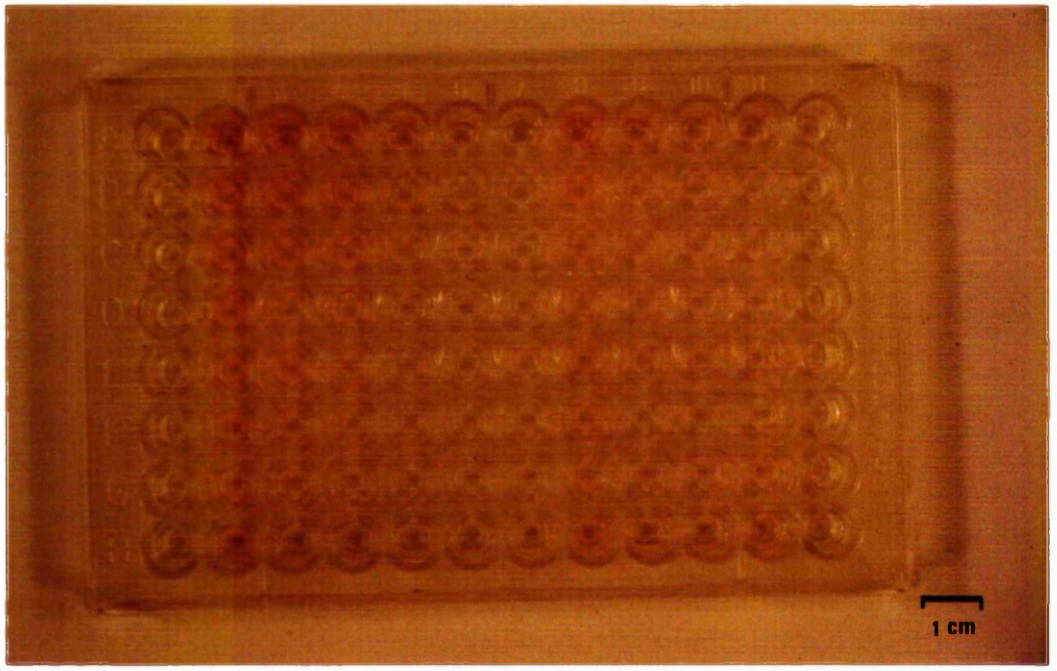


Plate 4 ELISA plate indicating antibody levels present in calf sera at differing conjugate and sera dilutions.

Columns 2-6: lice infested sera applied to wells.

Columns 7-11: lice free sera applied to wells.

The values for the sera from both infested and lice free animals are shown in Table 5.4. At eight weeks of age the calf sera showed antibody levels approximately three times that of the lice free sera (0.348 and 0.125 optical density (O.D.) respectively). At 16 weeks the difference between infested and lice free sera was slightly over five times greater for the infested sera (0.704 and 0.123 O.D. respectively). At 20 and 28 weeks the O.D. values for the infested sera was similar and both were approximately seven times greater than the lice free sera.

5.3.4.2 STATISTICAL EVALUATION

Statistical analysis of the data revealed that on each of the sampling days chosen the infested sera had significantly greater optical density values than the control sera ($P<0.05$).

In addition, the infested sera at 20 and 28 weeks had significantly greater O.D. values than both 8 and 16 weeks with the values for 16 weeks being significantly greater than those for 8 ($P<0.05$). There were no significant difference between the values obtained at 20 and 28 weeks. No significant difference was found between the lice free sera on any of the sampling days.

Table 5.4

ELISA Optical Density Values for Lice Infested and Lice Free Sera

	Age of calves (weeks) 8	16	20	*28
Level of infestation	Slight	Moderate	Severe	Receding severe
Infested sera				
Plate 1	0.352	0.700	0.871	0.878
2	0.342	0.708	0.894	0.890
3	0.349	0.714	0.887	0.891
4	0.348	0.692	0.882	0.878
mean	0.348	0.704	0.884	0.884
s.d.	0.004	0.101	0.010	0.007
s.e.	0.004	0.008	0.008	0.006
Control sera				
Plate 1	0.128	0.122	0.128	0.128
2	0.123	0.124	0.129	0.122
3	0.126	0.123	0.127	0.126
4	0.124	0.124	0.130	0.125
mean	0.125	0.123	0.129	0.125
s.d.	0.002	0.001	0.001	0.003
s.e.	0.002	0.001	0.001	0.002
Significance	P<0.05	P<0.05	P<0.05	P<0.05

s.d. = standard deviation
s.e. = standard error

*The sera used at 28 weeks was obtained from experiment A calves only since experiment B ceased sampling at 24 weeks (see Chapter 2).

5.4 DISCUSSION

Isoelectric focusing is a very high resolution technique for the separation of proteins (or other ampholytes) according to their isoelectric properties. Kolin (1955) considered that the behaviour of a protein uniformally distributed through a column of electrolyte solution, with a pH gradient, will vary with its position in the gradient and for any given protein molecule there will be a characteristic pH (the isoelectric point) at which the net charge on the molecule is zero. If an electric field of constant intensity is applied across the pH gradient protein ions converge or focus towards the isoelectric point. When salivary gland extract was applied to the isoelectric gel the protein ions migrated to give an acidic pH of 3.29.

Since it was first introduced by Davis (1964), polyacrylamide gel electrophoresis has become widely used as a technique for separating complex mixtures of proteins or nucleic acids. Early workers, in making qualitative comparisons of tissue extracts, have used disc electrophoresis (Davis, 1964). For more complex studies, involving detailed and quantitative comparison of stained bands in different samples, and localization of enzymes or radioactive proteins, a flat matrix is much more suitable. The method employed by Raymond and Weintraub (1959) required rather thick blocks of gel, with attendant problems of heating and slow entry of fixatives, stains and enzyme reagents. Reid and Bieleski (1968) avoided such problems by designing an apparatus that used much thinner sheets of gel.

When proteins solubilized by the detergent sodium dodecyl sulphate (SDS), long known to be one of the more potent protein denaturants and solubilizing agents (Tanford, 1968), are electrophoresed through SDS acrylamide gels, an extremely good correlation is obtained between migration distance and molecular weight (Shapiro and Maizel, 1969; Dunker and Reuckert, 1969 and Weber and Osborn, 1969). In practically all cases the molecular weight can be estimated to within 10%, the method is inexpensive, rapid and simple, and it is possible to separate and determine the molecular weights of mixtures of proteins in one gel (Swank and Munkres, 1971). Generally, all silver staining techniques are up to one hundred fold more sensitive than those using Coomassie Brilliant Blue (R-250) and are therefore indispensible for the detection of small amounts of proteins which cannot be stained by Coomassie Blue (Heukeshoven and Dernick, 1985). However, the experimental realization of silver staining, with the many reaction steps it requires, raised some difficulties in the present study, especially in the timing of each step. Background staining was sometimes intense and not uniform. Therefore when consistency in appearance of the major components of salivary glands from **L. vituli** was desired the silver staining technique was not able to compete with the simple and reliable Coomassie Blue staining. This method of staining indicated that there were four main molecular weight components to the salivary gland extract used in this present study which were observed as 80320, 58665, 42310 and 32320 daltons. Characterizing guinea pig exocrine pancreatic proteins Scheele (1975) stated that proelastase had a molecular weight of 28700 with procarboxypeptidase observed at 45000 and amylase at 54000 daltons. However, as the molecular

weights in the present study were determined using homogenates from a number of whole salivary glands it is not possible to define the substance that each weight signifies. In order to clarify the situation gel electrophoresis could be carried out on salivary secretions only. These would be extremely difficult to obtain since such small quantities would be involved and care would be essential if the samples were not to include any cell wall debris. Alternatively, Western Blotting could be carried out on the gel bands produced by the homogenates. The individual bands could then be applied to separate lanes of subsequent gels and electrophoresed in order to separate out any impurities.

Two dimensional resolution, termed **iso-dalt** systems since they are based on **iso**electric focusing in the first dimension followed by slab electrophoresis molecular weight separations (expressed in **dal**tons) in the second, was first introduced by Anderson and Anderson (1978). The technique was developed for the separation of soluble proteins larger than 10000 daltons (Scheele, 1975). This technique requires loading of the first dimension isoelectric gel portions containing the focused proteins onto the second dimension electrophoresis gel. In the present study as only one isoelectric focusing point was established fresh samples were applied to the electrophoresis gel rather than the focused protein portion from the isoelectric gel.

Enzyme immunoassays have been developed as alternatives for radioimmunoassays for the detection of antigens and antibodies in body fluids and was first introduced by Engvall and Perlmann, 1971. Quantitation of specific antibodies is obtained by adding enzyme-

labelled anti-immunoglobulin to antigen coated tubes. The methodology for ELISA is relatively simple, uses small amounts of reagents making it inexpensive, is very sensitive and can be automated. Engvall and Perlmann (1971) carried out the assay using polystyrene tubes. The enzyme remaining in the tubes after washing provided a measure of the amount of specific antibodies in the serum. The assay has potential for serodiagnosis and has been applied for the detection of antibodies against **Salmonella** (Carlsson, Lindberg and Hammarstrom, 1972), **Trichnella spiralis** (Ljungstrom, Engvall and Ruitenberg, 1974), **Plasmodium** (Voller, Huldt, Thors and Engvall, 1975), **Treponema** (Veldkamp and Visser, 1975) and has been employed successfully in the detection of antibodies to many diseases of veterinary importance (Ruitenberg and van Knapen, 1977).

One advantage of the enzyme immunoassay is its sensitivity. Specific antibodies to **Trichinella spiralis** could be detected in experimentally infected specific pathogen free pigs from three days after infection onwards (Ruitenberg, Steerenberg, Brosi and Buys, 1974).

With the introduction of an eradication scheme for cattle warble flies in Britain, a simple and reliable serological test for the detection of antibodies to **Hypoderma bovis** in cattle was established by Sinclair and Wassall (1983). This involved modifying the method of Ruitenberg, Steerenberg, Brosi and Buys, (1976) for ELISA in polystyrene tubes for flat bottomed polystyrene microtitre plates and an extra antiglobulin stage inserted between the adhesion of the test serum and the conjugate. Sinclair and Wassall (1983) noted that preliminary tests on sera from cattle known to be infected with **H.**

bovis showed no positive reactions when an antiglobulin/perocidase conjugate was added immediately after bovine serum. However with the introduction of the extra step of adding an unconjugated antiglobulin to bovine IgG, after the application of the serum to the absorbed antigen and followed by a suitable conjugated antiglobulin, strong specific reactions were produced when the sera from infected animals was tested. Without this intermediate antiglobulin these researchers failed to produce an acceptable result even when the concentrations of the antigen were increased by factors of 20 and 40. After testing the activity of all the reagents on other systems and finding consistent and acceptable results Sinclair and Wassall (1983) suggested that the reason the normal form of ELISA failed was because the bovine globulins which were attached to the Hypoderma antigen on the walls of the microtitre plate were not presented in a way that allowed an antiglobulin conjugate to act upon the substrate. A point to note from study is that although only a small number of cattle were available for testing, the ELISA technique appeared to give a definite diagnosis of infection by Hypoderma species even when the cattle had only one detectable larva. These researchers suggested that future work could be carried out to clarify the period over which the assay would detect circulating antibodies in relation to the age of the infection and the period over which detectable antibodies are present after the elimination of the larvae.

Boulard (1975) estimated the antibody titres in calves less than a year old which had been naturally infected with Hypoderma species from December to June. For the first five months the titre remained at a high level but dropped during the final month when it was

probable that the third instar larvae emerged. The timing of this decrease is compatable with the results of Sinclair, Tarry and Wassall (1984) who investigated the persistence of antibody in calves after an infection with **H. bovis.** These workers proposed that since eight cattle were found with only one observable warble this did not preclude the possibility that many more instar larvae began their migration and stimulated the host's immunological mechanisms. They also noted that as these animals were experiencing their first infection it was unlikely that the migrating larvae encountered any form of resistance from the host's immunological mechanisms. Sinclair **et al** (1984) therefore suggested that the migration of a single larva was sufficient to produce a response which was of the same magnitude as that produced by 10 or 20 warbles which had completed their migration.

It is unknown what effect previous infections and the increasing age of the cattle have on the rate of decline of the circulating antibody concentrations. The main factors in resistance to Hypodermis in cattle, noted by Evstafjev (1980), were intensity of infestation, the number of recurrences and the use of insecticides. Under field conditions Evstafjev (1980) showed that cattle which had heavy infections one year were much less susceptible to attack in the following year. Conversly, he noted, animals lightly infected or treated with insecticide experienced heavier infections the next year.

The present study, not requiring the intermediate step introduced by Sinclair and Wassall (1983), showed that there was an increase in antibody levels correlating to an increase of infestation

of **L. vituli** on young calves. However when the infestation was receding after being at a peak, the antibody level at this point was still that which had been observed when sera from peak infestation sampling days were tested. These results indicate that the host response to the lice infestation was one of paralleling the infestaton as time progressed but remaining at the highest level seen even when the louse population had begun to decline. As these calves had been monitored from one week of age this infestation of lice was the first experienced by them. Future work could include monitoring the antibody levels after a louse population had been lost, either naturally or with the help of a commercial preparation, in order to discern whether or not the levels of antibody decline or remain at a plateau level when no lice are present. Another interesting experiment could include observing antibody levels in calves infested with lice, treated to erradicate the infestation and then reinfested in order to establish the effect of previous exposure to the lice on the antibody levels produced by the host's immune response mechanism.

There are two pairs of salivary glands in lice; one pair is elongate and tubular, the other compact and reinform; their ducts apparently combining to form the salivary canal whose opening is regarded as the modified middle stylet accommodated in the trophic sac, a well developed pouch opening off the ventral side of the cibarium (Richards and Davis, 1977).

The composition and function of salivary secretions may vary with the gland and with the insect. They may be used to moisten mouthparts, to provide a solvent for food, or to carry digestive enzymes (Rockstein, 1974). Hyaluronidase probably acts as a

spreading agent to break down intracellular matrix and enables penetration of digestive enzymes into prey tissues (Edwards, 1964). It was Metcalf (1945) who discovered anticoagulins and agglutins in female mosquitoes only several hours after emergence and suggested that their appearance may determine when the first blood meal is taken. Hellman and Hawkins (1966) found that an antithrombin in the mid gut differed from that of the salivary anticoagulin in **Triatoma maculata.** The salivary glands of blood sucking insects apparently do not secrete demonstrable amounts of digestive enzymes such as proteases, carbohydrases or lipases (Gooding, 1972).

Studying the salivary glands of **Rhipicephalus appendiculatus,** the brown ear tick of cattle, Walker, Fletcher and Gill (1985) noted that the paired salivary glands were similar in both sexes with a large central duct present through most of the length of each gland which appeared to have several major branches at the end and a number of branching lobular ducts throughout the length of the glands. Walker **et al** (1985) noted four types of acini present in the gland structure. They stated that the type 1 acini were confined to the main ducts, in females about 250 and males 150 of these acini per single gland. The type 2 acini were seen both on the main duct and on the inner sections of lobular ducts leading from the main duct, numbering 300 in females and 200 in males per single gland. The third type of acini were found at the distal portions of all the lobular ducts with females having 850 and males around 600 per single gland. The fourth type of acini, as described by Walker **et al** (1985), were only observed in males who had about 400 per single gland and were found in conjunction with the third type. Walker **et**

al (1985) then proceeded to describe in detail the structural and histochemical changes of each type of acini during feeding as the salivary glands were observed to occupy a large proportion of the haemocele of feeding ticks. They concluded that the type 1 acini were unlikely to be involved with the secretions of any salivary components which had an active role in feeding. The **a** cells of the type 2 and the **e** and **d** cells of the type 3 acini were all observed by Chinery (1973) to be involved in the production of attachment cement. It was therefore proposed by Walker **et al** that the glyproteins and other possible constituents of the **b** and **c** cells found in the type 2 acini were possibly involved in influencing the host responses such as increased vascular permeability, immunosuppression and inflammatory which have been proposed as saliva induced (Willadsen and Riding, 1980; Wikel, 1981 and Higgs, Vane, Hart, Potter and Wilson, 1976). Gill and Walker (1987) were the first to observe that the granule secretion of the **b** and **c** cells seemed to synthesise and secrete their products throughout feeding, these secretions being anticoagulants, enzymes and other pharmacologically active substances which were required for the tick to continue to feed successfully.

Histochemical observations carried out by Gill and Walker (1988) showed a strong homogenous reaction for aminopeptidase and weak reaction for acid phosphatase, which are present in the cement cone of attachement. The presence of these enzymes in the saliva of **Hyalomma anatolicum anatolicum**, confirms the suggestion of Gill, Boid and Ross (1986) that they were secreted into the host as active constituents of salivary secretions.

Ribeiro and Garcia (1981) showed that salivarectomized blood sucking bugs **Rhodnius prolixus** could feed with difficulty on a rabbit, ingesting less blood than intact insects when a 'normal' length of time was allowed for the meal. The difficulty appeared to be related to the beginning of feeding since there were repeated puncture marks of the rabbits skin indicating unsuccessful feeding attempts. Normal insects however, unlike the salivarectomized produced more or less extensive haemorrages during probing. The anti-haemostatic action of the salivary secretion, along with the observations of Lavoipierre, Dickerson and Gordon (1959) that **R. prolixus** is a vessel feeder, and the probing behaviour of salivarectomized insects, suggest that the salivary secretion is important in enhancing the production of large haemorrages, therefore making blood available for ingestion during the probing phase. This appears to indicate to the insect that a blood vessel exists in the probing area and so encourages the insect to continue its maxillary probing in that particular area and eventually locate a blood vessel. It was suggested by Ribeiro and Garcia (1981) that the anti-haemostatic activity in the salivary excretion may also be of importance in the maintenance of feeding by maintaining a continuous blood flow.

Ribeiro, Rossignol and Spielman (1984) explained salivary function from observations on mosquito mouthparts within living tissue. They noted that after puncturing the skin, mosquitoes thrust their stylets back and forth before locating the blood vessel and beginning to feed. While Griffiths and Gordon (1952) observed that during this probing phase mosquitoes salivate copiously. It was

indicated by Ribeiro and Garcia (1984) that this extravascular secretion could not inhibit coagulation of ingested blood and so creating a paradox. Consequently, they posulated that the main function of mosquito saliva is to enhance vessel location. Mosquito saliva reduces the amount of probing required for locating a blood vessel. They showed that saliva inhibits platlet aggregation and identified apyrase activity in saliva that could carry out such a function. It was therefore suggested by these researchers that saliva may promote haematoma formation around blood vessels and so increase the possibility of finding such vessels. The presence of a haematoma would increase the volume of blood in the probed tissue and so prevent a larger target area for blood vessel detection.

Gillet (1967) noted that mosquitoes must engorge rapidly on their hosts with slow feeding mosquitoes perhaps being unable to complete engorgement before irritating its host. Ribeiro **et al** (1984) proposed that brief periods of host contact increase parasite survival with anti-haemostatic components of saliva involved in such a role by enhancing blood vessel location and therefore shortening the duration of vector-host contact.

Saliva produced by **Ixodes dammini** contains anti-haemostatic, anti-inflammatory and immunosuppressive components that appear to enhance blood finding success of this particular tick during its prolonged period of host attachment (Ribeiro, Makoul, Levine, Robinson and Spielman, 1985). These workers were the first to denote platlet anti-aggregating activity in the saliva of a tick. Salivary apyrase may be responsible for other effects important to a ticks successful feeding in addition to inhibiting haemostasis by degrading

ADP. It may be important in preventing those inflammatory processes stimulated by ATP (Ischikawa, Hayashi, Minami and Tomita , 1972), inducing mast cell degranulation (Bloom, Diamant, Hagermark and Ritzen, 1970) and aggregation of neutrophils (Ford-Hutchison, 1982).

Ribeiro **et al** (1985) found that **I. dammini** saliva is immunosuppressive. This action of the tick saliva may delay, reduce or abolish the hosts response to the ticks salivary antigens, thus reducing immune mediated inflammatory responses at the ticks feeding site. Saliva of the tick **I. dammini** contains an enzyme that degrades ATP and ADP to AMP and orthophosphate (apyrase), prostoglandin E_2, a kinase and an anaphylatoxin inactivator (Ribeiro **et al**, 1985; Ribeiro and Spielman, 1986). These activities confer anti-haemostatic, anti-inflammatory and immunosuppressive properties to the saliva of the tick and might explain why ticks can feed repeatedly on their natural hosts without significant rejection (Trager, 1939; Ribeiro **et al**, 1985; Ribeiro and Spielman, 1986 and Ribeiro, 1987). Since inflammation is a phenomenon involving several mediators each playing different relative roles in different species, Ribeiro (1987) suggested that a tick adapted to a particular host may have evolved a salivary defence mechanism. He went on to explain that the mechanism of action of **I. dammini** salivary anti-complement remains to be explored and it may be possible that other ectoparasites contain salivary anti-complement activity such as has been isolated from **Boophilus microplus** eggs and larvae (Willadsen and Riding, 1980).

CHAPTER SIX

HISTOLOGY OF SKIN SECTIONS AND EXAMINATION OF TANNED SKINS FROM CALVES INFESTED WITH LINOGNATHUS VITULI AND DAMALINIA BOVIS

6.1 INTRODUCTION

Arthropods can elicit a response from the host in a number of ways:

(i) Injection of substances into the host's body (e.g. bees, wasps, saliva of fleas).

(ii) Invasion of tissues (e.g. mites).

(iii) Contact (e.g. some caterpillars).

(iv) Ingestion (e.g. shrimps, lobsters).

(v) Inhalation (e.g. house dust mites).

Host immunity to arthropods can be divided under three general headings:

(i) Immunity associated with the neutralization of toxic compounds introduced into the hosts body by Arthropods (e.g. venoms of different arthropods).

(ii) Hypersensitivity to antigens of Arthropod origin (e.g. reactions to Arthropod bites, stings or spines).

(iii) Immunity to infestation or invasion by arthropods (e.g. tick infestations).

The types of reaction of the host to haematophagus Arthropods has been examined by various researchers with the response of the host being variable and dependant upon the presence or absence of previous exposure. Prior sensitization and the presence of humoral

antibodies by the positive Prausnitz-Kustner (P-K) reaction was proposed by Hecht (1933) who used the sera of patients who had reacted severely to mosquito bites. Other workers have also obtained positive P-K reaction, namely Riek (1954) using horse sera sensitive to **Culicoides robertsi** and Baker and O'Flanagan (1964) using sera of dogs sensitive to fleas.

Allan and West (1964) observed circulating antibodies in guinea pigs which had delayed reactions to mosquito bites, using leucocytes removed from sensitive animals which had been injected intra-peritoneally into guinea pigs with no previous exposure to mosquitoes, subsequent challenge of the skin of those animals with mosquito bites giving positive reactions.

It was Lester and Lloyd (1928) who removed the salivary glands of Tse-Tse flies and found these salivarectomized flies no longer elicited a reaction in sensitized skin. They also noted that sectioning of the salivary ducts of mosquitoes was effective in preventing reactions and therefore concluded that salivary gland secretions contained types of irritating and sensitizing substances.

Repeated exposure of the skin to haematophagous insects was proposed by Mellanby (1946) as initiating four stages in the skin reactivity of the host. These were firstly, delayed reaction; secondly, delayed and immediate; thirdly, immediate only and finally, non-reactive. Work carried out by McKiel (1955); Benjamini, Feingold and Kartman (1961) and Larrivee **et al** (1964) confirmed Mellanby's work.

Goldman, Rochwell and Richfield (1952) described the reaction of sensitized human skin to mosquitoes whilst French (1972) observed the dermal reaction of guinea pigs sensitized to **Aedes aegypti**. Both of these authors described neutrophils involved in the reacton, however, Larrivee **et al** (1964) described a specific relationship between delayed skin reaction and lymphocyte infiltration and between the immediate reaction and eosinophilia. Investigating the histopathology of **Sarcoptes scabiei** in pigs Sheahan (1975) stated that intense infiltration of eosinophils occurred due to their antihistaminic nature. He proposed that the accumulations of perivascular lymphocytes in pigs, 24 hours intra-dermal post challenge with whole mites, indicated that delayed hypersensitivity was associated with immediate reactions.

It was shown experimentally by Nelson and Bainborough (1963) that sheep can develop resistance to the sheep ked, **Melophagus ovinus** with dermal vasoconstriction preventing feeding. A similar reaction was shown to occur in the skin of mice infested with the louse **Polypax serrata** in a series of experiments carried out by Nelson **et al** (1972) reporting on the histopathology of the reaction. When observing the histological changes of hare host tissues caused by tick bites Saito, Ohara and Unagami (1960) noted that the wound contained masses of necrotic tissues which were made up of leucocytes, erythrocytes and nuclear debris. In the deep areas of the wound haemorrhages were generally observed along with extensive cellular infiltrations which consisted mainly of large mononuclear cells and leucocytes in the tissue surrounding the haemorrhage. Saito and Ohara (1961) followed up these studies by proposing, like

Nelson **et al** (1972), that there were two types of tissue reaction. The first, named the 'Ixodes' type, was found to be a lesion which showed a heavy haemorrhage with a large amount of destructive effects to the host tissue. The 'Haemophysalis', or second type, was characterized by mild, diffuse haemorrhages and negligible effects on the host tissue.

Developing hypersensitivity was observed by Walker and Fletcher (1986) studying, histologically, the attachment sites of adult **Rhipicephalus appendiculatus** on rabbits and cattle. They noted larger intra-dermal pustules in cattle than rabbits and also a greater degree of necrotic tissue in rabbits. These pustules consisted of liquid exudate forming vesicles between keratinocytes and were usually tightly packed with neutrophils and a smaller number of mononuclear cells. These researchers suggested that these early responses showed a developing inflammatory response but was more likely to be a rapidly developing hypersensitivity to the cement or other saliva components.

In the United Kingdom the main bulk of leather is produced from skins of cattle and sheep. The most commonly used heavy skins ('hides') are from cattle and the most common light skins ('skins') are from calf, sheep and goat (Dempsey, 1961). Most of the grain defects in leather occur during the life of the animal on the farm and indeed, the quality of the leather lies very much in the husbandry of the animals by the farmer but he is usually unaware of the extent of the damage or the financial losses involved (Haines, 1978). There are variable opinions as to the economic effects of pediculosis. For example, Baker (1973) considers that lice are

responsible for the numerous scratches seen on tanned hides and consequently believes them to be of considerable economic importance. Tancous, Roddy and O'Flaherty (1959) suggested that stable flies, horn flies and sucking flies caused leather damage but produced no experimental evidence, however, Everett, Miller, Gladney and Hannigan (1977) provided experimental evidence in the United States which showed hard ticks as the main cause of damage to hides. However, as will be shown, some workers have not indicated the extent or the nature of pediculosis cases or examined infested hides sufficiently closely after slaughter and at the tanned stage when damage is clearly visible.

6.2 MATERIALS AND METHODS

The data presented were obtained from studies carried out in the Environmental Sciences Department, The West of Scotland College, Auchincruive, Ayr; the Electron Microscopy Department, Moredun Research Institute, Edinburgh and at the British Leather Corporation, Northampton, England.

These investigated the damage, if any, of **Linognathus vituli** and **Damalinia bovis** on the skin of young calves using histopathology and electronmicroscopy. Also investigated were the effects of these lice on skins sent for tanning.

6.2.1 Histological Examination of Skin Samples

6.2.1.1 SAMPLES AND TREATMENTS

Skin blocks (1.0 x 1.0 x 0.5 cm) were obtained at post mortem from Ayrshire calves infested with **L. vituli** which had been used in the radioactive studies. Skin was also obtained from Friesian calves infested with **D. bovis** and uninfested controls which had been part of a drug efficacy experiment 12 months previously.

6.2.1.2 HISTOLOGICAL TECHNIQUES

The skin sample blocks were embedded in either paraffin wax (see Chapter 2, section 2.8.1) or plastic resin (see Chapter 2, section 2.8.2). The stains used for both types of embedded material are described in Chapter 2, sections 2.8.1.3 and 2.8.2.4 respectively.

6.2.2 Electronmciroscopy Techniques

6.2.2.1 SAMPLE MATERIAL

As described in 6.2.1.1.

6.2.2.2 PROCESSING PROCEDURE

The skin samples were processed using the Thiocarbohydrazide method described in Chapter 2, section 2.9.

6.2.3 Leather Analysis

6.2.3.1 ANIMALS AND TREATMENTS

Ayrshire bull calves were purchased from local farms. They were housed in individual pens and kept separate from one another by straw

bales. All calves were examined at weekly intervals for the presence of developing louse infestations. The calves were slaughtered for veal at approximately 50 kg. The skin was removed, salted and stored at -20°C. Batches of skins were sent for leather processing. A total of seventeen skins were sent for tanning and comprised:

- skins from animals with no evidence of louse infestation.
- skins from animals infested with **L. vituli** or **D. bovis** or a mixed infestation of these two species.
- skins from animals treated with pour-on (Coopers Animal Health, Berkhamstead, England) to prevent infestation.

6.2.3.2 TANNING OF SKINS

The skins were taken to the British Leather Confederation, Northampton, England where they were processed into leather. The finished leather was examined for any marks.

6.3 RESULTS AND DESCRIPTION OF TRENDS IN THE DATA

This section will present the skin histology and electronmicroscopy results along with observations on the tanned hides. A description of trends will also be included. This will be derived from, and contain no interpretation of, the results. For the purpose of data analysis and discussion, the following criteria will be applied to the level of infestation present on the calves:

mean count >50 lice = very severe infestation

mean count of 20-50 lice = severe infestation

mean count of 10-20 lice = moderate infestation

mean count of 5-10 lice = slight infestation

mean count <5 lice = very slight infestation

(after Titchener, 1985).

6.3.1 Histological Examination

6.3.1.1 PARAFFIN WAX EMBEDDED SAMPLES

It was found that this form of embedding for the skin samples was not entirely satisfactory. Difficulty was experienced when the skin sections were being cut. To try and overcome the breaking up of the thin sections the microtome was set to cut at an increased width. Unfortunately, although at 12 um the sections remained intact they were found to be too dense for microscopic examination. At lower cutting widths the sections crumbled and failed to ribbon. From sections reasonably intact and cut at 5 um it was found that the entire stratum corneum was removed from the skin samples of calves infested with either **L. vituli** or **D. bovis** and also uninfested calves which acted as controls.

6.3.1.2 PLASTIC RESIN EMBEDDED SAMPLES

This type of embedding medium was found to be relatively quick, easy to use and cutting sections was much easier than with paraffin wax embedded samples. The sections were cut at 1 um and although they did not ribbon the majority cut were intact. In order to cut the sections satisfactorily it was necessary to replace the glass

knife frequently, more so than was required for paraffin wax embedded sections.

On examination of the sections microscopically it was found that the stratum corneum remained intact on the uninfested control skin samples unlike those which had been wax embedded where the stratum corneum was almost totally removed. Skin sections from calves which had been infested with **L. vituli** appeared to have no damage to the stratum corneum or the underlying layers of the epidermis or in the dermis itself (Plates 5 to 8).

Contrastingly, the sections from calves infested with **D. bovis** were observed to be depleted of the stratum corneum in some areas (Plates 9 to 12). This depletion extended to approximately two thirds when compared to uninfested control skin sample sections.

6.3.2 Electronmicroscopy Studies

The electron micrographs taken of both **L. vituli** and **D. bovis** lice along with those of the skin samples from infested calves are shown in Plates 13 to 19.

The surface view (Plate 15) along with the cross section through a piece of **L. vituli** infested calf skin (Plate 16) show holes penetrating to the bottom of the dermal layer of the skin, a distance of approximately 30 um. No such holes were apparent in **D. bovis** infested or uninfested control skin samples.

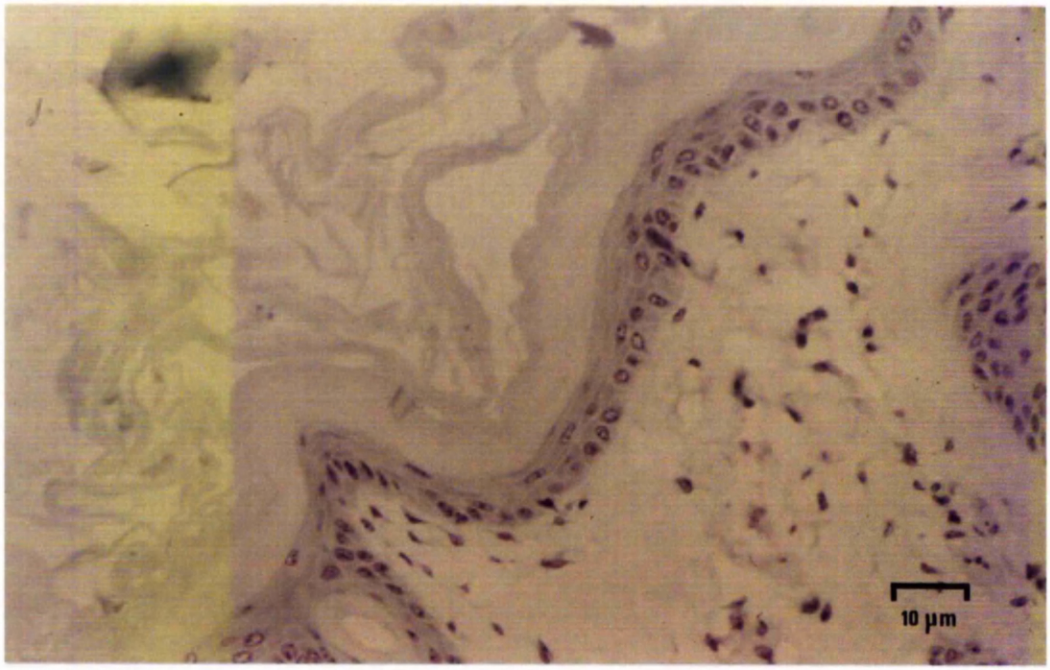


Plate 5 L. vituli infested skin section indicating no damage to stratum corneum.

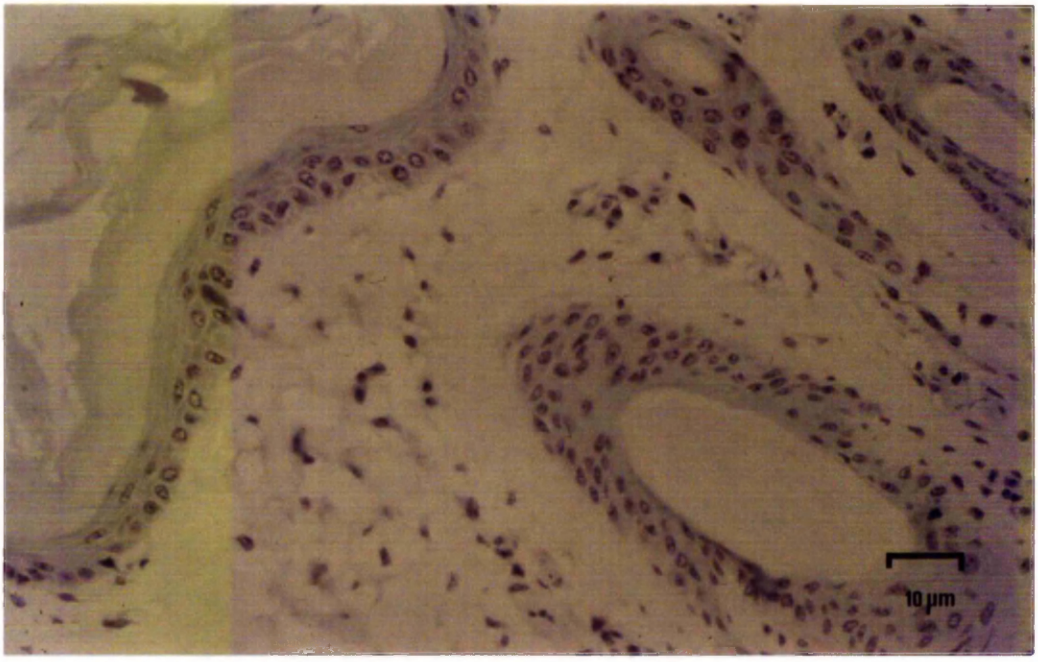


Plate 6 L. vituli infested skin section with no damage to stratum corneum.

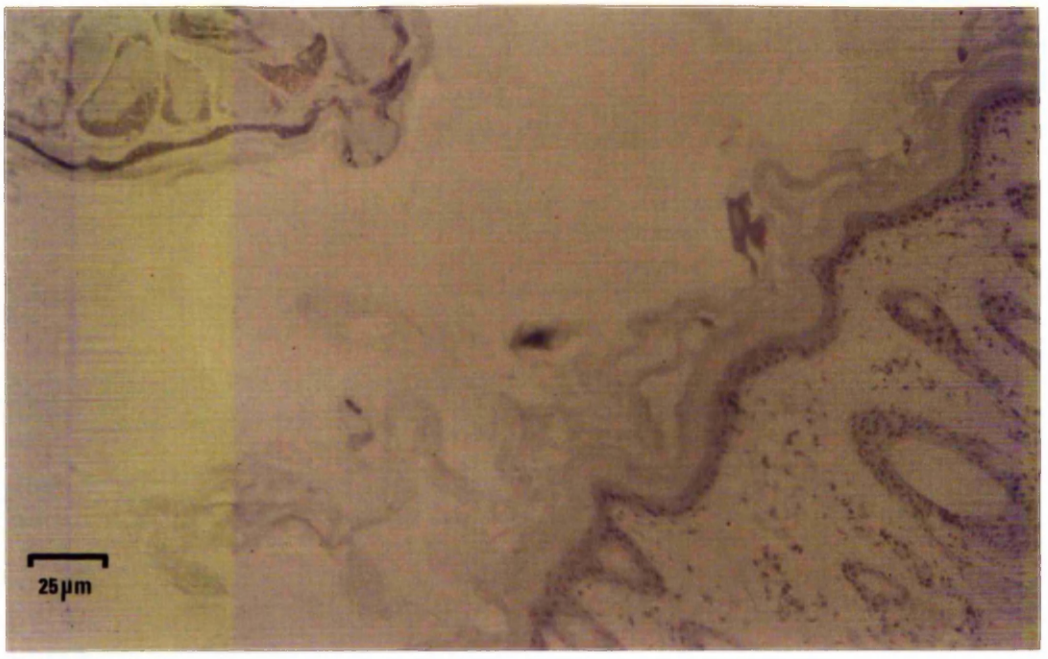


Plate 7 L. vituli infested skin section showing no damage to stratum corneum.

Plate 8 Skin section from lice free group of calves acting as controls with intact stratum corneum.

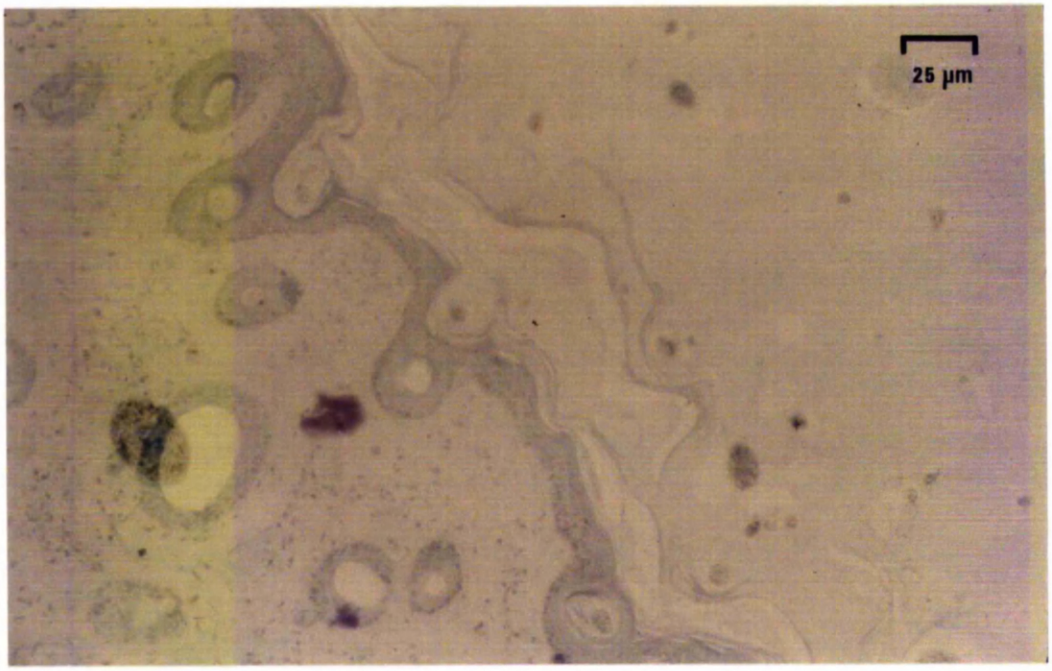


Plate 9 Skin section from D. bovis infested calf indicating depletion of stratum corneum.

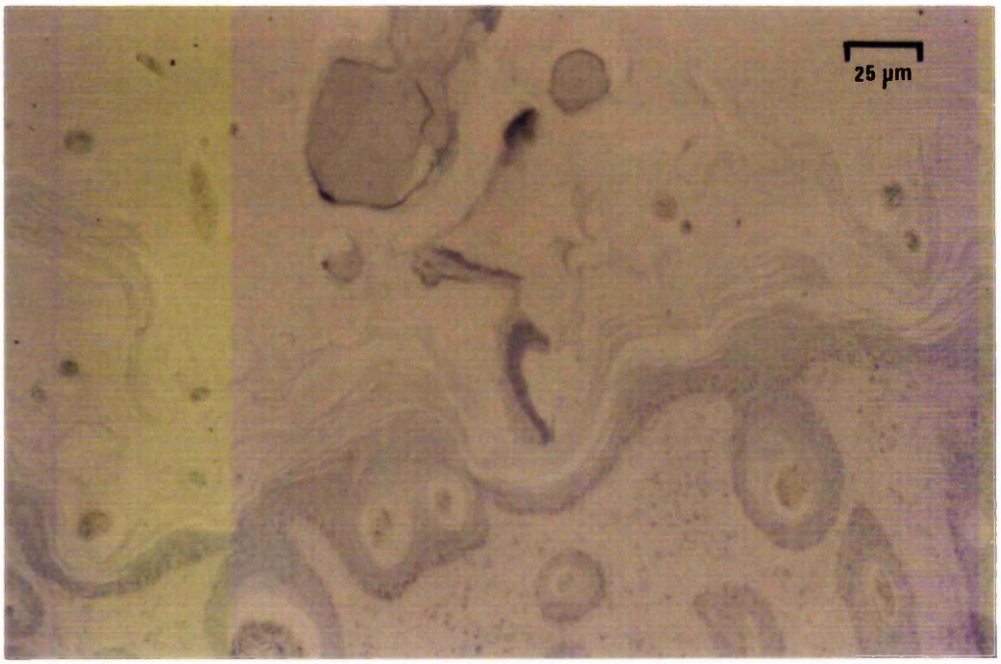


Plate 10 D. bovis infested skin section with breakdown of stratum corneum.

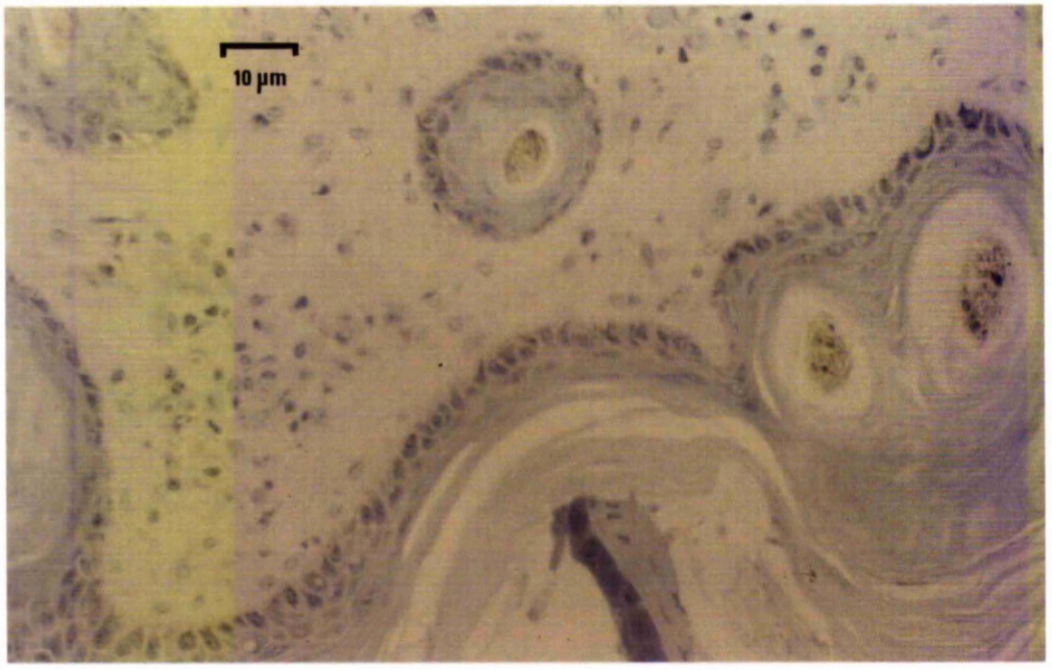


Plate 11 D. bovis infested skin section. Close up of Plate 10.

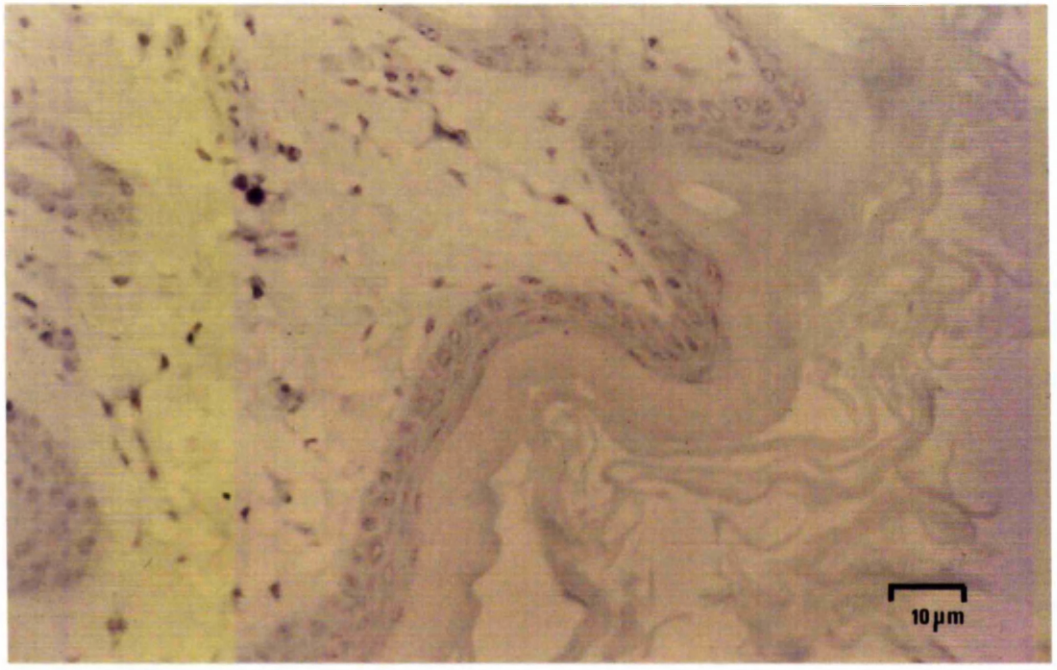


Plate 12 Lice free control skin section with intact stratum corneum.

Plate 13 Scanning electron micrograph of L. vituli.

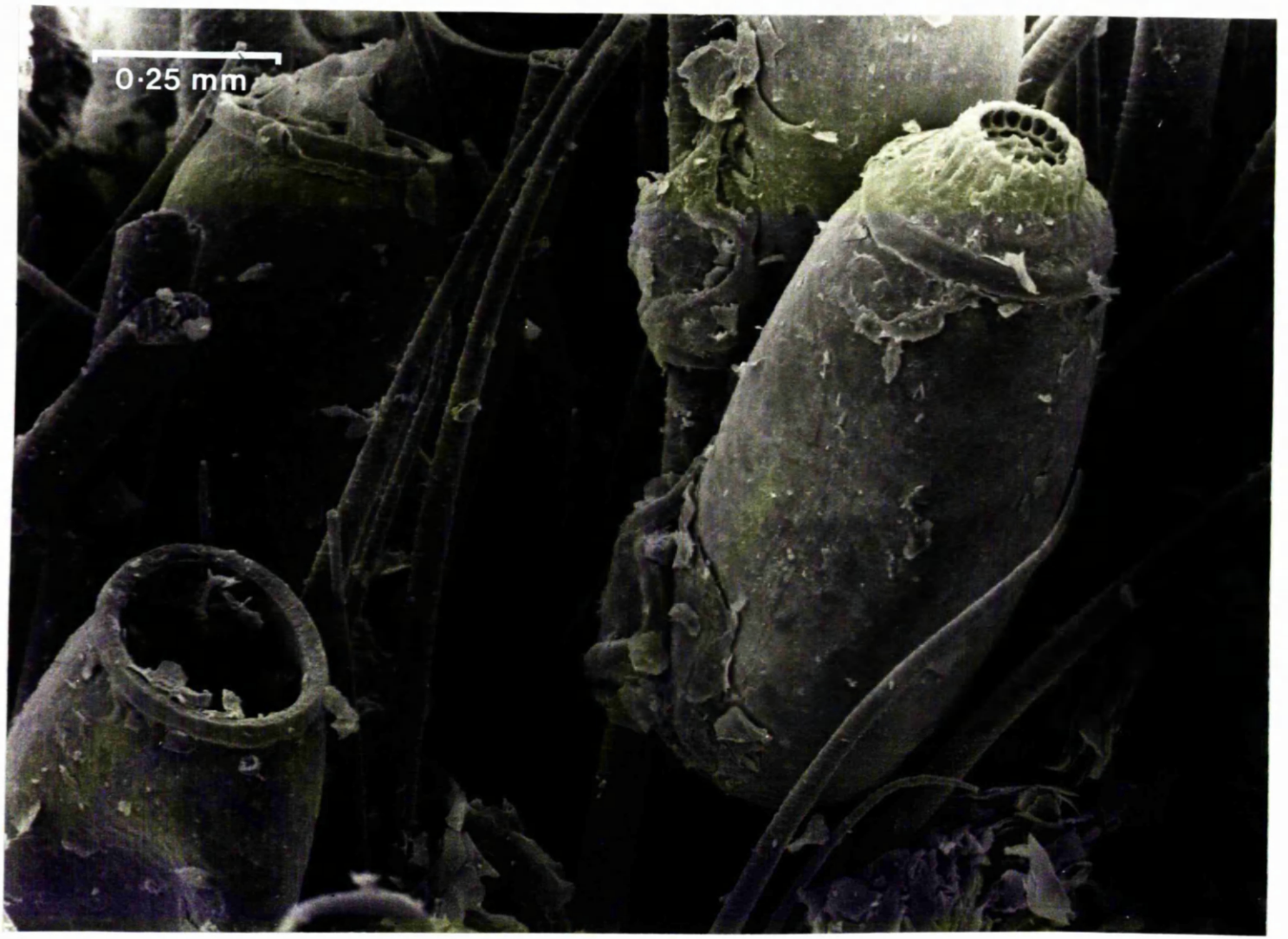


Plate 14 Scanning electron micrograph of L. vituli egg cases.

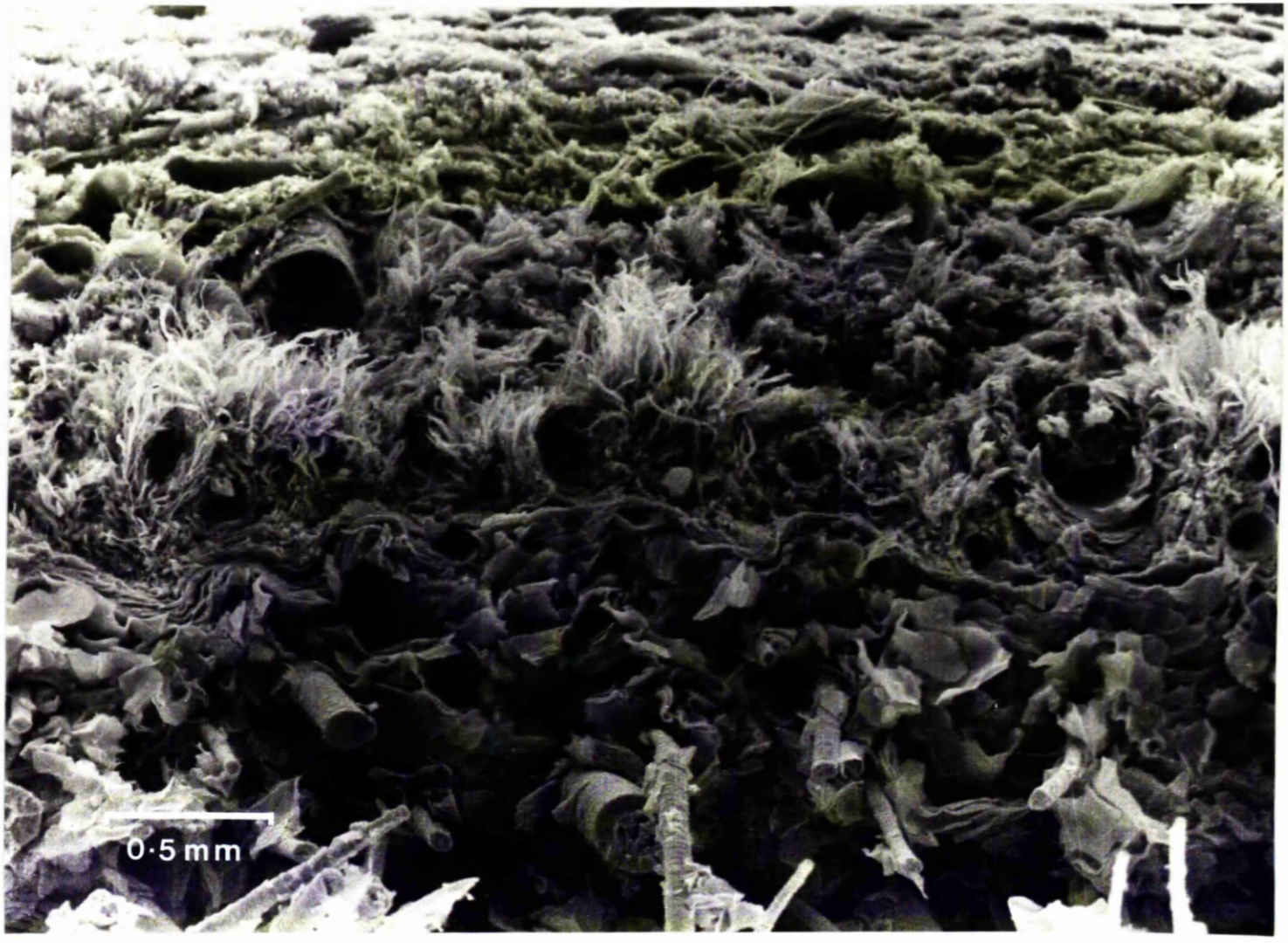


Plate 15 Scanning electron micrograph of calf skin which was infested with **L. vituli** indicating puncture holes made by the lice.

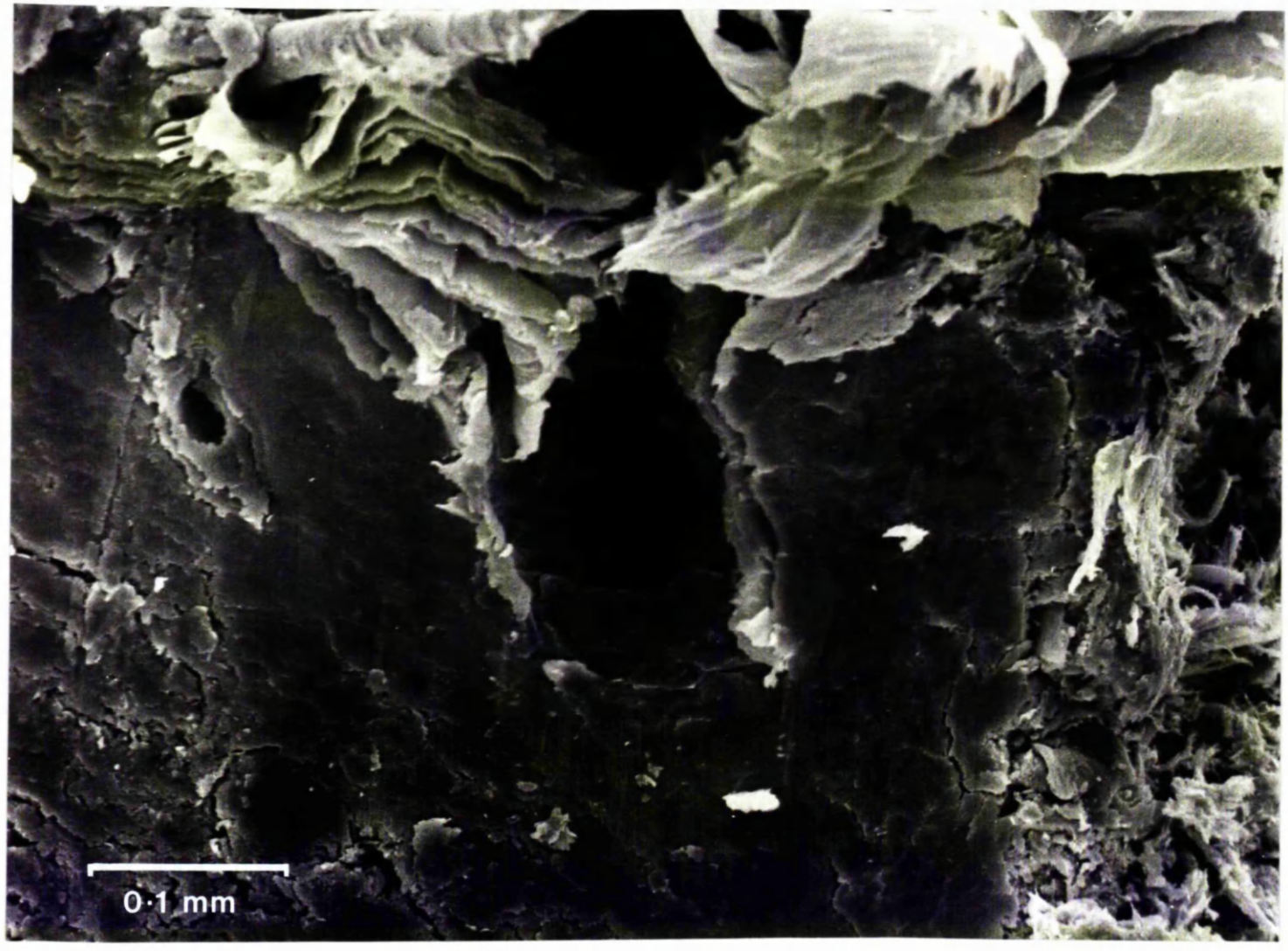


Plate 16 Scanning electron micrograph close up of puncture holes made by L. vituli.

Plate 17 Scanning electron micrograph of D. bovis.

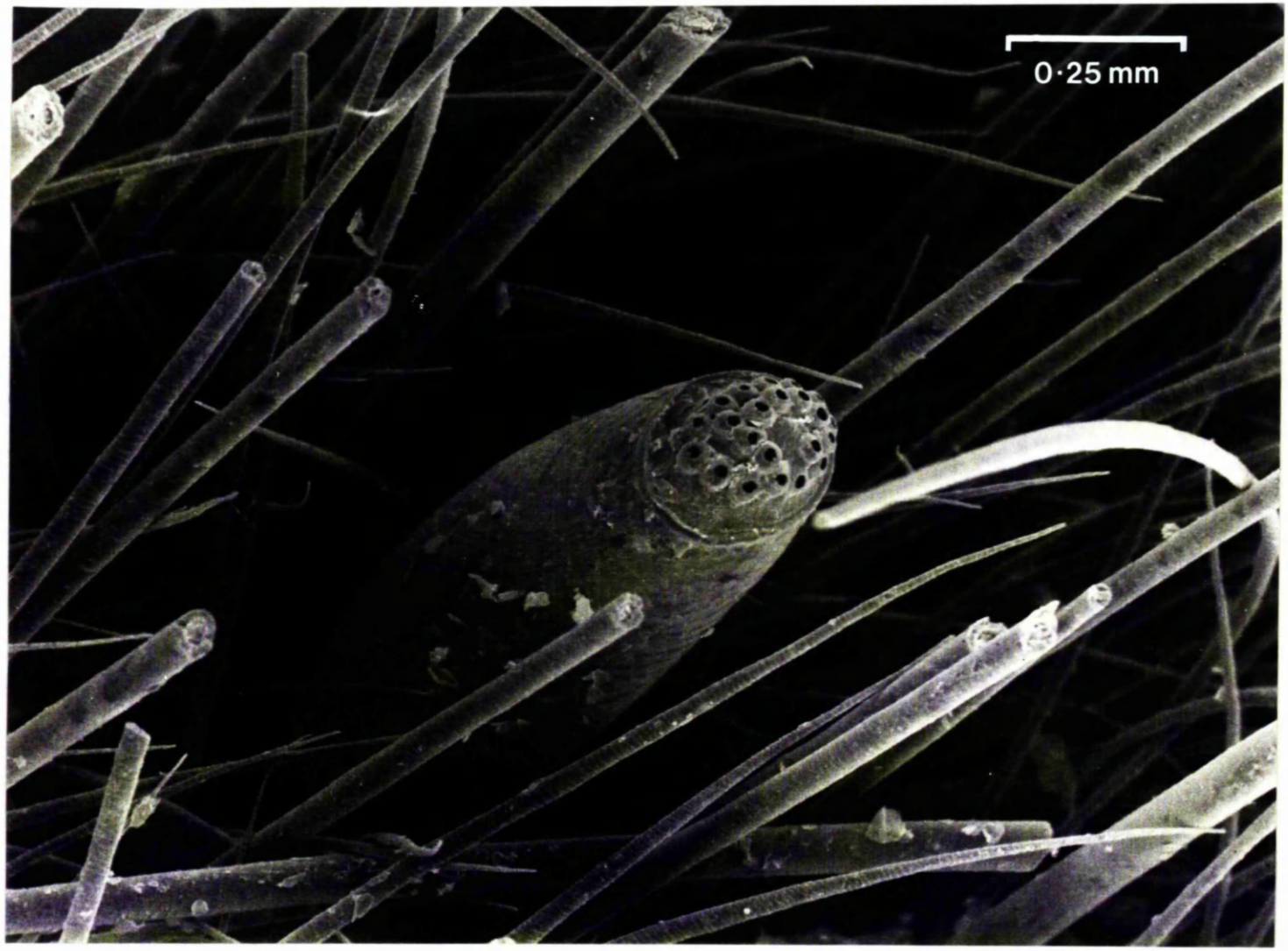


Plate 18 Scanning electron micrograph of D. bovis egg case.

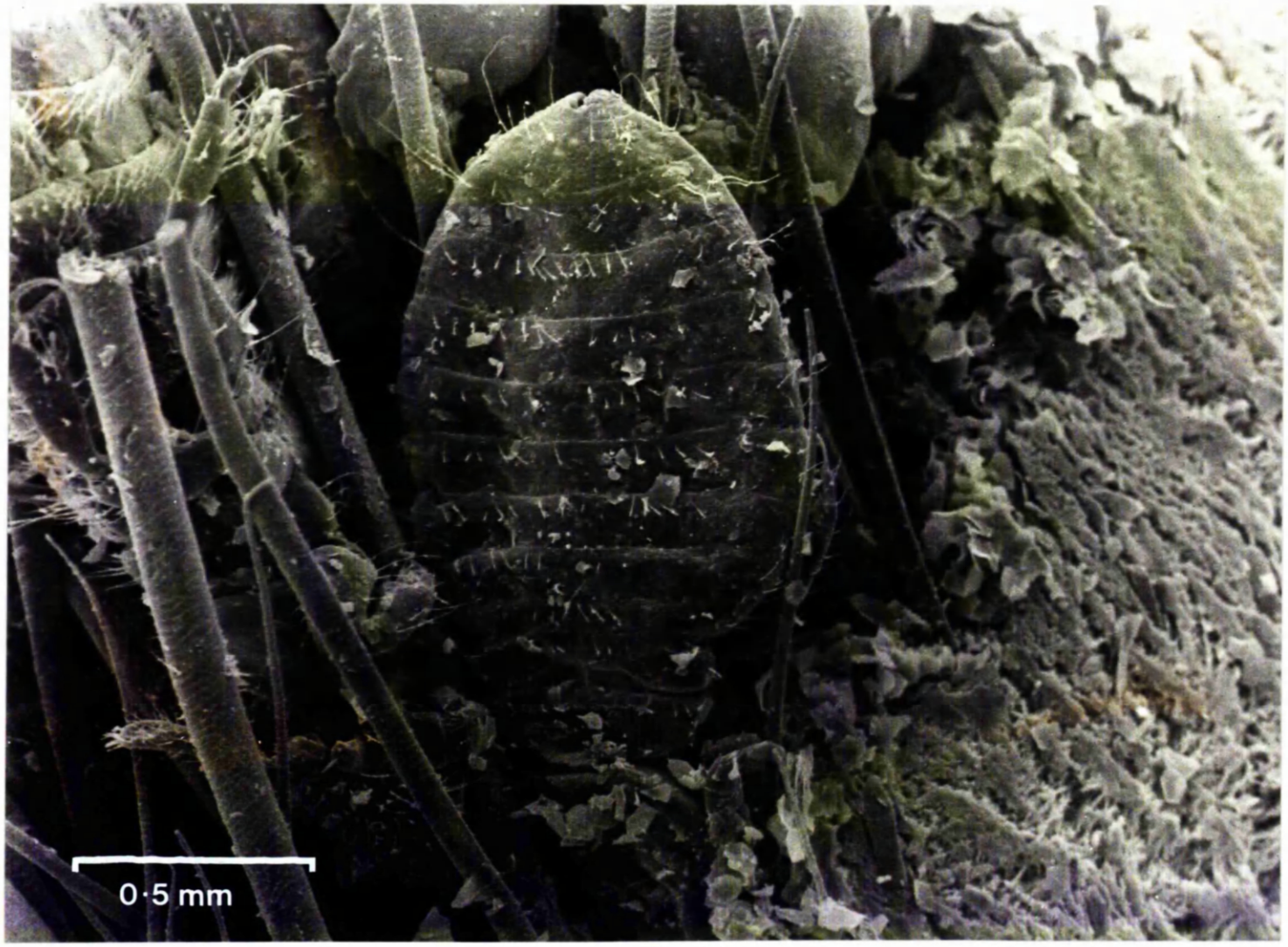


Plate 19 Scanning electron micrograph of browsing D. bovis with L. vituli.

6.3.3 Hide Damage After Tanning

The levels of infestation throughout the lives of the calves along with the damage seen on the tanned hides are shown in Tables 6.1 to 6.3.

Looking at the levels of infestation seen in the animals it appears to take on average 13.67 days to establish a slight infestation of **L. vituli**, 27.50 days to become moderate and 42 days to reach a severe infestation level. For an infestation of **D. bovis** the length of time for the infestation to be regarded as slight was 11.75 days, 26.33 days for moderate and a severe infestation was found after approximately 48 days.

For calves numbers 4 and 7 which both harboured a slight infestaton of **L. vituli** no damage was observed on the finished, tanned hides. When severely **L. vituli** infested skins (numbers 9 and 10) were examined a number of dark pin head sized marks were noticeable over the shoulders and on the sides of the neck region. The hides of **L. vituli** infested calves which harboured a severe infestation that had receded to a moderate level (numbers 2 and 3) showed dark pin head sized marks, again seen on the sides of the neck area (Plate 21).

Hide number 13, which was slightly infested with **D. bovis**, showed no observable damage on post-tanning examination. Calf skin number 17, a moderate **D. bovis** infestaton, revealed a large amount of fleck on the neck, across the shoulders and on the backbone (Plates 22 and 23). Some fleck along with a lot of white spot was observed along the backbone with more being seen in the saddle region and a

Table 6.1

Calf number	Infestation type	Age in days from birth with infestation level			Age at slaughter	Damage
4	**L. vituli**	19 ++			19	No damage.
7	**L. vituli**	13 ++			13	No damage.
9	**L. vituli**	15 ++	31 +++	46 ++++	57	Dark on shoulders, neck area.
10	**L. vituli**	15 ++	31 +++	46 +++	57	Dark marks on shoulders, neck area.
2	**L. vituli**	10 ++	24 +++	38 +++	62	Dark marks on shoulders and neck area.
3	**L. vituli**	10 ++	24 +++	38 +++	62	Dark marks on shoulders, neck area.

Infestation levels: ++ = slight
+++ = moderate
++++ = severe

Table 6.2

Calf number	Infestation type	Age in days from birth with infestation level					Age at slaughter	Damage
6	**D. bovis**	12 ++	27 +++	48 ++++	62 +++	83 ++++	92	Some fleck (2 mm along backbone). Lot of white spot (>2 mm). (Mostly across saddle area near base of tail too.)
8	**D. bovis**	12 ++	27 +++	48 ++++	62 +++	83 ++++	92	Some fleck along backbone. Lot of white spot. (Mostly across saddle area with small amount around base of tail.)
13	**D. bovis**	14 ++					30	No damage.
17	**D. bovis**	9 ++	25 +++				29	Lot of fleck.
11	**L. vituli** and **D. bovis**	10 ++ 72 ++	19 +++ 84 +++	43 ++++ 100 ++++	64 ++++		110	Light spot over neck, shoulders and backbone. Some scratching on neck. Dark marks on shoulders.
12	**D. bovis** and **L. vituli**	6 ++ 52 ++	20 +++ 63 +++	44 +++ 80 ++++			91	Light spot on neck, shoulders and along backbone. Scratching on neck region. Dark marks down shoulders.

Infestation levels: ++ = slight
+++ = moderate
++++ = severe

Table 6.3

Calf number	Infestation type	Infestation level throughout experiment	Age at slaughter	Damage
1	Untreated control - no lice	0	13	No damage.
5	Untreated control - no lice	0	8	No damage.
15	Untreated control - no lice	0	13	No damage.
18	Untreated control - no lice	0	13	No damage.
20	Treated control - no lice	0	12	Treated at 2 days of age with 20 ml Ryposect. No damage.

Infestation level: 0 = no lice

Plate 20 Top quality leather is used in the production of expensive lounge suites.

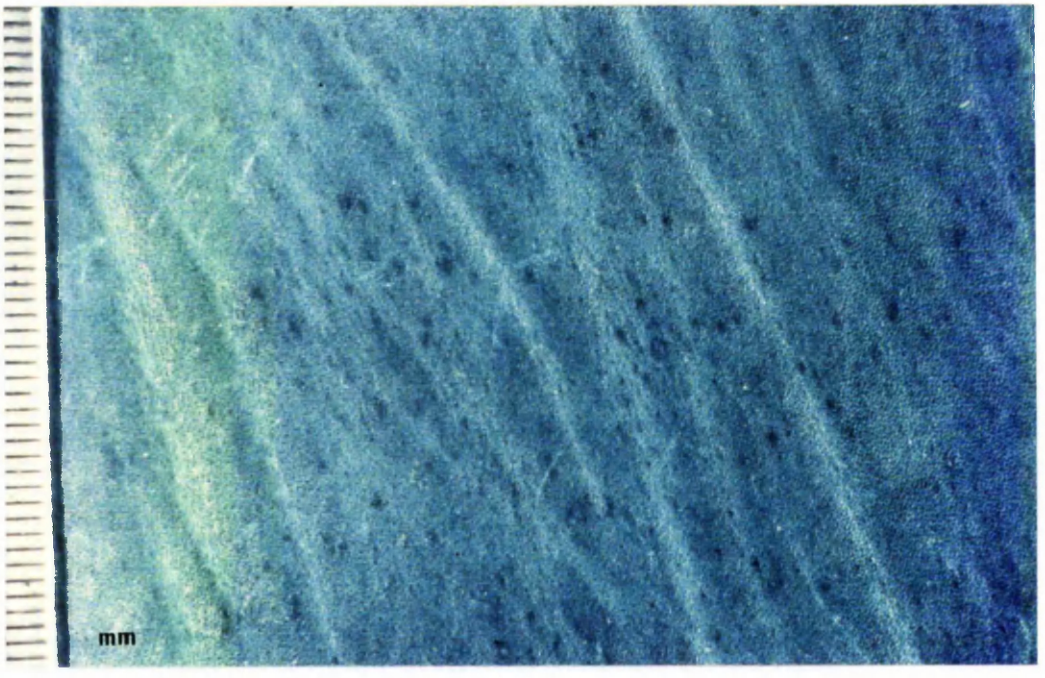


Plate 21 Dark marks caused by L. vituli still present in finished leather.

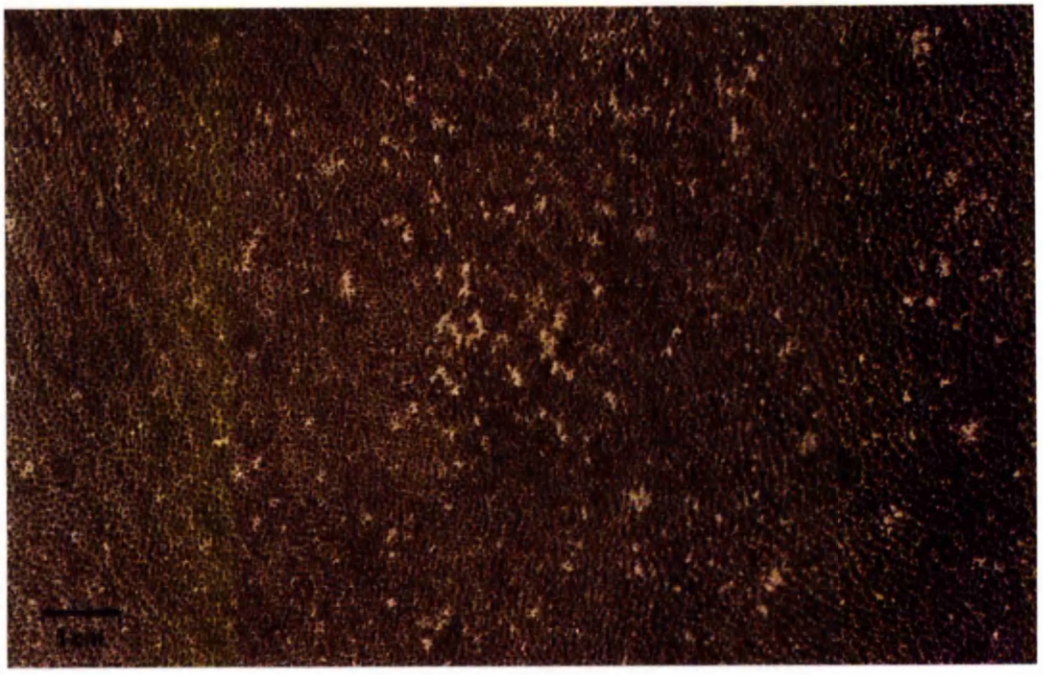


Plate 22 White fleck caused by D. bovis present in finished leather.

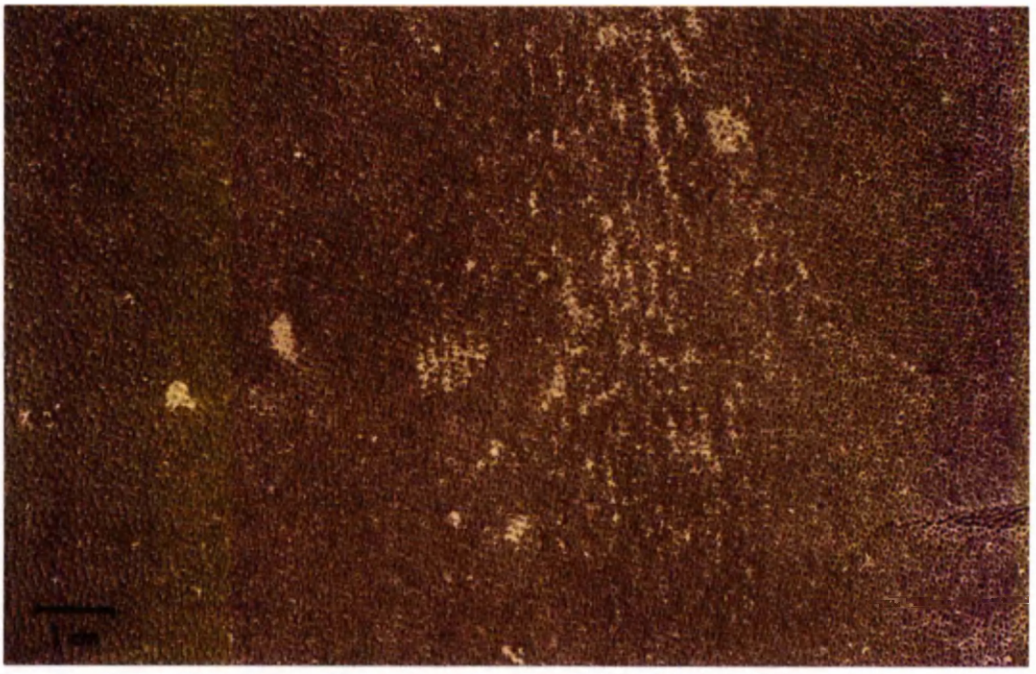


Plate 23 White spot caused by D. bovis present in finished leather.

small amount around the base of the tail was seen on hides 6 and 8 which were both infested with **D. bovis**. These infestations, however, had reached peak severity and then receded to a moderate level once more before culling took place.

Two skins were examined which had a mixed infestation of **L. vituli** and **D. bovis**. Skin number 11 was infested initially with **L. vituli** and when this reached severe proportions at 64 days **D. bovis** infested calves were mixed with the same group and a severe infestation of this louse species was seen at 100 days. Conversely, skin 12 was infested with **D. bovis** initially which recorded as severe at 44 days after which time **L. vituli** infested calves were introduced into the same stall. At 80 days a severe infestation of **L. vituli** was noted. Both of these skins showed similar results when tanned. White spot was present on both skins over the neck and shoulder regions with a small amount along the backbone. There was also a large amount of dark pin head sized marks down both shoulders and at the base of the neck. Scratching was also seen on the neck areas of both hides (Plates 24 and 25).

On the uninfested and the uninfested treated control skins (numbers 1, 5, 15 and 18 to 20 respectively) there was no apparent damage on any of the tanned hides.

Plate 24 Scratch marks resulting from D. bovis infestation.

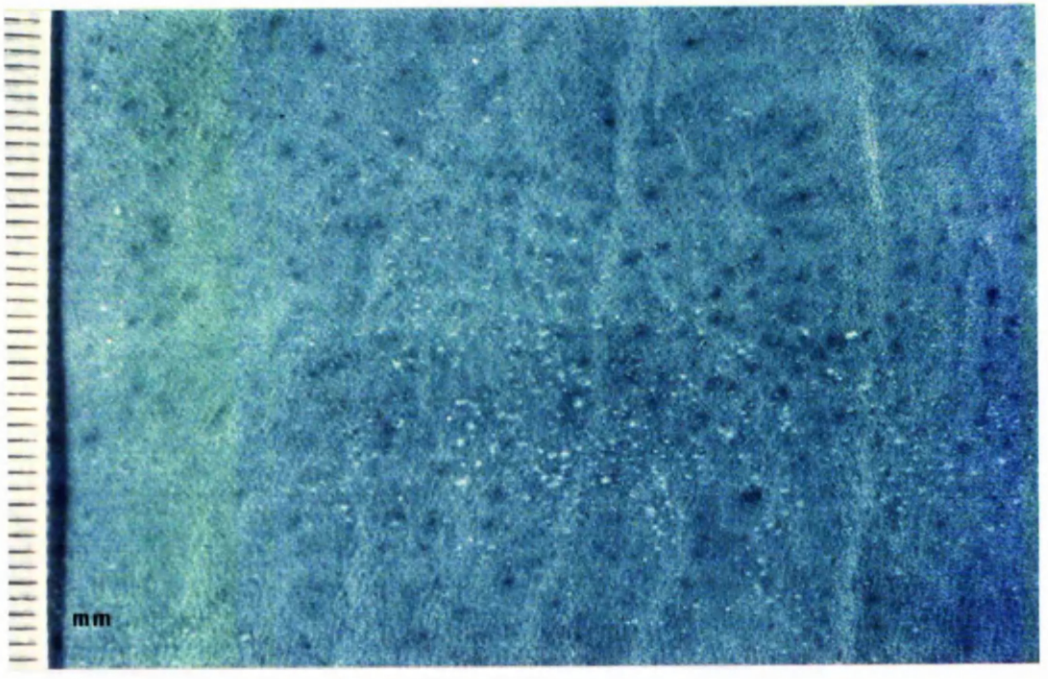


Plate 25 White spot and dark marks resulting from mixed infestation of D. bovis and L. vituli.

6.4 DISCUSSION

The living epidermis and stratum corneum in cattle were both found to be approximately 30 um thick when measured on cryostat sections (Lloyd, Dick and McEwan Jenkinson, 1979). However, the corneum was found to consist of about 30 cell layers whereas the living epidermis was composed of only four. As was shown in the present study, Lloyd **et al** (1979) noted that paraffin wax embedding resulted in depletion of the stratum corneum and proved to be unsuitable as a medium in which to study this layer. Other researchers have also noted significant tissue shrinkage with paraffin wax as the embedding medium as well as experiencing loss of the outer layers of the stratum corneum (Nay and Hayman, 1963; Pan, 1963 and Strickland, 1975).

Although no observable damage was seen on the skin sections from calves infested with **L. vituli** it does not totally preclude any damage from ever being present. It could be said that no damage was apparent because the skin sections were not taken from infested areas. This was not the case, however, as the skin samples were taken from known louse infested areas of the calves and were examined for lice upon removal from the host. However, it may have been that the lice seen on these small samples had just migrated to this area with the skin being removed before the lice had opportunity to probe and successfully locate a blood meal. Alternatively, as the louse populations had been present on the calves for around 10-12 weeks this may have resulted in the host immune response gradually disappearing as in the case of mosquito bites (Rockwell and Johnson, 1952) so that when the samples were taken the immune response had

reduced to unnoticeable levels. Had the sections been taken at the beginning of the infestation an immediate reaction may have been visible. Ideally, in order to gauge the host response, samples would have been required to be taken at regular intervals from the beginning of the infestation. Economically this was not possible during the present study.

The removal, in places, of two-thirds of the stratum corneum, seen in sections from **D. bovis** infested skin, indicates the sporadic browsing nature of this louse. As the stratum corneum layer is composed of keratinized cells and is not totally removed there is no real harm to the host except a mild irritation causing the animal to scratch, therefore the immune system does not need to be brought into operation and hence no reaction being observed in the skin sections.

There are a number of reports on the hosts response to ticks, fleas and mosquitoes but only a few refer to histopathological investigations. Rockwell and Johnson (1952) noted that immediate and late reactions to mosquito bites may be due to primary irritation and suggested that these reactions are small and gradually disappear with regular exposure. Haemorrhages were observed in tissues removed from guinea pigs 24 hours after mosquitoes had fed (Gordon and Crewe, 1948). There was also a distinct swelling at the side of the bite. They stated that there appeared little doubt that it was the presence of saliva that was responsible for the irritation associated with the bite.

Man's reaction to bites of mosquitoes are of two kinds (Gordon and Crewe, 1948). First there is an immediate reaction which is

initiated whilst the mosquito is still biting with secondly, the delayed reaction, which usually commenced 24 hours after a bite had been inflicted. The delayed reaction was found to disappear in individuals which were exposed to the insect bites over a long period of time (Gordon and Crewe, 1948). They noted that where irregular exposure to insect bites was seen to result in the immediate reaction, irregular exposure over long periods of time, either in the tropics or in this country, caused the disappearance of the delayed reaction. Regular exposure, in contrast, caused the disappearance of the immediate reaction. Under such conditions of regular exposure, Gordon and Crewe (1948) noted that the delayed reaction diminished progressively with the reaction gradually resulting in shorter duration and its intensity becoming so slight as to be hardly noticeable. They concluded that, as with the immediate reaction, in the delayed reaction the substance responsible to elicit the response was contained in the saliva. The delayed reaction is much less severe than the immediate one and usually appears independantly of previous exposure to the bites of the insect concerned, while unlike the immediate reaction, it disappears after both regular or irregular feeding. It was Peck **et al** (1943) who observed that no one before them had considered the possibility of louse faeces being a factor in the pathogenesis of skin irritation. Indeed, their experiments demonstrated that following exposure to lice, the reaction appeared to be more intensified when a considerable quantity of faeces was present on the skin. They also stated that the earliest manifestations of the louse bites were minute, red non-inflammatory points which were flush with the skin but when large numbers of lice were involved, the area of the bite sites were red, oedematous and

raised above the surface of the skin. Goldman **et al** (1952) stated that the persistence of the insect bite reaction is dependant to a degree upon the amount of tissue response. However, they proposed that certain external factors such as scratching and rubbing could influence the persistency of the bite site reaction.

Studying the histopathology of the skin of louse infested (**Polyplax serrata**) mice Nelson **et al** (1972) noted that the acquired resistance of mice to lice was due to vasoconstriction with the resultant decreased dermal blood flow thus depriving the ectoparasites of the available blood supply. Within the skin there was a well defined diphasic cellular reaction, the first occurring in the first four weeks with the second during the remainder of the cycle. In the first of two defined phases of cellular reactivity by Nelson **et al** (1972) inflammatory reaction was observed between 8-14 days in the majority of the mice which was in agreement with the findings of Peck **et al** (1943); McKiel and West, 1961 and Benjamini **et al** (1961). Phase two was distinguished by an increase in lymphocytes, monocytes and mast cells although after a peak at the fifth and sixth weeks the lymphocytes and monocytes decreased to levels slightly above those noted for control animals. These results differ from those of Larrivee **et al** (1964) in two noticeable ways. Firstly, Larrivee **et al** (1964) did not find any neutrophils amongst the inflammatory cells during the period of reactivity, although eosinophils were found. Nelson **et al** (1972) suggested that these findings could be interpretated in one of two ways. Either neutrophils were not present or that they were not easily distinguishable from eosinophils. The second difference between the

two studies was in the number of stages of cellular reactivity said to be taking place. Five stages of reactivity were suggested by Larrivee **et al** (1964), as previously stated, whereas Nelson **et al** (1972), proposed only two.

The effects of pediculosis on tanned bovine skin have not been investigated to such a degree as is shown in the present study. Any effects of pediculosis on the skin can only be thoroughly determined by close examination of the hide with the hair coat removed by shaving, or after the skin has been tanned. So, although Kettle (1974) stated that the skin of beef cattle infested with either **L. vituli** or **D. bovis** did not show any damage, his conclusions were based merely on the usual examination of an unprocessed skin.

These studies, using processed skins, indicate that infestations of **L. vituli** caused dark, pin head sized marks in the areas which the lice inhabit, namely the neck, shoulders, along the backbone and around the base of the tail. Similarly, **D. bovis** infestations were found in these regions and appeared to cause small white circular areas of damage termed white spot (diameter >2 mm) and fleck (diameter <2 mm) amongst the commercial members of the leather industry.

Where a mixed infestation skin was processed and examined, both types of damage were observed. Although there was an overlap in the damage, dark pin head sized marks were seen more specifically down each shoulder with more at the base of the shoulders and the base of the neck whereas the white fleck was more prevalent at the top of the shoulders and along the backbone. This indicates that either

symbiosis was occurring between the two lice species so that they could both mutually occur on the same host animal or competition was present ensuring that each species retained an area on the host or were restricted to a particular area due to the competitive dominance of the other louse species.

A condition referred to as 'white spot' by Naghski (1971), in his 1970 John Arthur Wilson Lecture, which was apparently not related to curing since it also appeared on leather produced from fresh hides prompted Everett **et al** (1977) to carry out investigations using some of the important ectoparasites on the grain quality of cattle hide leather. Naghski (1971) found that the defect was difficult to conceal with a clear finish and so had important economic consequences. He noted that the upper shoulder and rump areas of the skin were most frequently affected. Under low magnification the white spots resembled minor abrasions exposing fraying fibres. Naghski (1971) concluded that the spots may be representative of wounds or scars inflicted by biting insects. It was because of this work that Everett **et al** (1977) studied bloodsucking ectoparasites which must puncture the skin surface in order to obtain a blood meal and so would seem likely to leave scars. However, Everett **et al** (1977) concluded that there was no significant grain damage to leather resulting from prolonged exposure of cattle to horn flies, stable flies or mosquitoes despite the application of massive infestations under controlled conditions. They also found similar negative results from natural, uncontrolled infestations by the short nosed cattle louse, **Haematopinus eurysternus.** They did find that moderate to severe grain damage occurred on two experimental hides as

the result of infestation by the demodectic mange mite **Demodex bovis.** They proposed the theory that this mite is transmitted by cows to very young calves during nursing which also may be applied to the initial transmission of the two lice species observed on the calves in the present study. Tancous **et al** (1959) considered that stable flies, horn flies and sucking flies caused leather damage but produced no experimental evidence to confirm this statement.

George, Wright, Guillot and Buechler (1986) began studying the possible relationships between psoroptic mange of cattle and white spot damage on leather after a previously unreported problem with cattle hides was being experienced by the leather indusutry over the preceding few years. Large numbers of apparently normal hides had been arriving at the tanneries which underwent the early stages of processing satisfactorily, but when they reached the blue (chrome-tanned) or the brown crust stage, a number of small white spots (2-3 mm in diameter) were observed on the grain surface of the leather. The spots did not accept dye and although the cause of the problem was unknown it was not restricted to one tannery. These white spots had been observed on fresh and brined hides so excluding curing or processing as the cause of the problem. It was also noted that the problem was more prevalent on winter hides.

George **et al** (1986) suggested **Psoroptes ovis,** the causative agent for bovine psoroptic mange, outbreaks of which are rare in the United Kingdom, as the possible cause of the damage. This was further confirmed by a study carried out by Beuchlar, Hannigan, Carroll, Dahms and Feairheller (1984) who found hyperkeratosis of cattle that indicated a similarity in cross-section between the

appearance of white spot damage and the earliest signs of psoroptic mange. Supporting the hypothesis that the two were related was a seasonal correlation between the peak incidence or outbreaks of **P. ovis** and the appearance of white spot.

George **et al** (1986) found that the hide from an uninfested control animal was completely free of white spot damage. They also observed that the white spot damage was sporadic. White spots were not apparent on the blue stock from cows exposed to a small number (small was not numerically specified) for 3, 7, 10 and 21 days but were visible on blue stock hides of infested cows which had 50 and 200 mites for 14 days. There was no white spot damage on brown crust hides from cattle infested with 50 mites except for the animals infested for 10 or 14 days, the damage being more extensive on the 14 day infested skin. A 10 day infestation of 200 mites showed a small amount of damage but white spots were distributed over most of the hide of a cow infested for 14 days with the same numbeer of mites.

From their work George **et al** (1986) suggested that **P. ovis** could be a cause of white spot although their results were inconclusive. The most extensive white spot damage occurred on the hides of cows infested with 200 mites and George **et al** (1986) stated that there appeared to be a direct relationship between the length of time an animal was infested and the severity of the white spot damage. These researchers concluded that there is considerable variation in the response of cattle to psoroptic mites in terms of factors such as the level of acquired immunity, the rate at which mites multiply and the extent of damage caused by an infestation.

Passman and Sumner (1987) stated that white spot was the term used to describe a pelt defect which appears as dull 2-5 mm diameter spots randomly distributed over the grain surface. This and other experiments (Passman and Dalton, 1982 and Passman and Sumner, 1983) have shown significant breed differences in aspects of leather quality. However, present monetary returns from skins are insufficient to justify farmers choosing a specific breed or slaughter time solely on the grounds of skin attributes.

Sykes, Webster and Bugby (1988) stated that in spite of the Ministry of Agriculture, Fisheries and Foods' initial view that white spot on cattle hides was essentially a mild allergic reaction to some unknown causal agents such as the presence of ectoparasites or ingestion/inhalation of toxins in the diet, their studies implicated lice as the principal cause of white spot/fleck. Sykes **et al** (1988) stated that it was both sucking (**L. vituli**) and biting (**D. bovis**) lice which were involved. Because of the possible involvement by psoroptic mites (George **et al**, 1986) Sykes **et al** (1988) concluded that a treatment for cattle which would eliminate all ectoparasites would be highly desirable.

An alternative hypothesis to the lice as sole causative agents of the damage observed in tanned skins was proposed by Baker (1973) studying 278 hides which were part of a consignment which had been selected for processing into top quality leather. He graded the hides into one of three groups. Group one contained hides which had no defects. Grade two hides showed damage confined to the less valuable areas of the hides while grade three had numerous defects over the whole hide area. Scratches formed the major fraction of the

defects observed. Ticks bites were present in large numbers on some hides, some contained the broken off hypostome from the tick which could be felt on palpitation with the fingertips whereas others had healed and were surrounded by white scar tissue for a short distance. Baker (1973) concluded that as scratches accounted for the majority of the defects, it may have been that such scratches were the result of the host being irritated by the presence of lice and so rubbed its skin against a fence post, for example, for relief and thus caused the damage. This point of the presence of the lice causing the initial irritation and thus causing the host to rub itself resulting in damage is carried on by Baker and Oormadzi (1981) when investigating the probable cause of the multiple linear scratch defect of cattle hides in Ireland. At six weeks of age a group of five calves was experimentally infested with **Bovicola bovis** and **L. vituli** with the infestation being maintained for 17 months. The commonest defect noted was that of innumerable scratches, their direction being mostly anteroposterior with some showing evidence of healing. The experimentally infested cattle were observed to scratch frequently and lick their coats unlike the uninfested cattle which were very seldom observed to be scratching.

Baker and Oormadzi (1978) concluded that multiple scratches result from ante-mortem damage consequent upon ectoparasitism, particularly pediculosis. They also noted that the distribution of scratches corresponded to those areas of the host body in which lice congregate and where the cattle were observed to scratch. The hides of uninfested cattle were free from blemishes even although scratching posts had been made available to the animals.

Contrastingly, the hides from cattle heavily infested had numerous scratch defects in the areas where lice congregate. Post-mortem processing trauma would have resulted in scratches distributed in a random manner. Haines (1978) noted scratch defects similar to those described by Baker and Oormadzi (1978) and considered that barbed wire scratches are the major cause of loss of quality in hides from British cattle. She proposed that wider use of systemic parasiticides would reduce lice and tick populations, thus reducing skin irritation.

Oormadzi (1976) noted that examination of hides at the chrome leather stage showed multiple scratches in hides processed from cattle infested with **L. vituli** and **D. bovis**. These scratches were located in the same areas where lice congregated and which infested cattle were observed to excoriate. Further support was given by the finding of multiple scratch marks observed on the hides of two cattle heavily infested by **H. eurysternus** whereas the uninfested animals had none. Oormadzi (1976) concluded that moderate infestations of **B. bovis** and **L. vituli** are of importance in damaging the hide. The neck, shoulder and back areas of cattle were observed to be the main predilection sites for **B. bovis, H. eurysternus** and **L. vituli** with the face and head for **Solenopotes capillatus** (Oormadzi, 1976). Spencer (1939) reported on the preference of other types of mammalian lice to the back of animals, in particular, in wild deer the back is the main site where lice and their eggs can be found. Oormadzi (1976) noted that in the very light and moderate infestations of **L. vituli** all the stages were found on the neck, shoulder and back, these regions having a skin temperature in the range of 30-33.5^{o}C.

It was noted that the hair coat is thicker in these areas and the variation of microclimatic factors are very small thus ensuring that these regions were more conducive for reproduction to take place. Unlike this study, a heavy infestation (heavy was not numerically defined) of **L. vituli** was found distributed all over the body except the inguinal and axillary areas whose skin temperature ranged from 34 to 37.5^{o}C and is unfavourable for the life cycle of the louse.

Scharff (1962) observed no specific body regions being preferred by **L. vituli**, however, he did note that they were more prevalent on the shoulder and rump of calves, a finding which was endorsed by Rich (1966) and was noted in the present study.

Tancous **et al** (1959) suggested it was the presence of the lice that caused the host irritation thus initiating the relief rubbing by the host. They also proposed that this relief rubbing either caused the damage or was instrumental in allowing bacteria to enter the bite area, with the presence of these bacteria causing the majority of the damage. This damage may have been a result of the actions of the bacteria and the rubbing of the host, the immune reaction of the host trying to repair itself at the wound site oer a combination of all or some of these.

D. bovis, the biting louse, moves across the hosts skin surface ingesting skin debris and the keratinized layer forming the stratum corneum. Being a browser, the louse tends to feed periodically. The irritation caused by the louse feeding may result in the host scratching causing scratch mark damage or relief rubbing which may allow bacteria into the bite area. The presence of bacteria along

with the hosts immune system carrying out repair work on the bite area may also influence the size of the damaged area.

No other study, apart from this present one, noted the presence of dark pin head sized marks on skins infested with **L. vituli**. This louse species is a blood feeder locating the blood vessels in the dermal layer of the skin. These marks may indicate bacterial activity along with necrotic tissue in the pierce holes after the louse has penetrated the skin in either an unsuccessful attempt to locate a blood vessel or after a blood meal. If a blood meal had been obtained blood may be left in the hole, which, in conjunction with bacteria and the host immune response system, may result in the marks observed.

CHAPTER 7

CONCLUSIONS

7.1 PEDICULOSIS ON CALVES

The results detailed in Chapter Three suggest that the presence of **Linognathus vituli** lice on calves had no overall significant effect with regard to blood profile and liveweight gains. Although there were significant differences in haemoglobin concentration, lymphocyte counts, monocyte counts and total albumin determination for infested calves in experiment A there were more significant differences for infested calves in experiment B, namely leucocyte count, lymphocyte count, monocyte count, erythrocyte count, reticulocyte count and total protein determination. This would, at first glance, appear to suggest that experiment B infested calves were suffering from anaemia to a larger degree than experiment A. However, four out of the six significant differences in experiment B and two out of four calves in experiment A showed levels greater in the infested calves for these parameters, contrary to what would have been expected if anaemia was detected. It must be remembered that both experimentally infested groups never reached severe proportions, remaining only at a slight level for most of the experimental period with only isolated instances of moderate and severe infestations being noted. Because of such a generally low louse population it can only be stated that the lice did affect various blood parameters to a significant degree but these cannot be directly related to causing the animal to be anaemic. Another point to note is that the infested calves in experiment B appeared to be more affected than those in experiment A which may be related to their age as experiment B calves harboured an infestation at a younger age and were blood sampled earlier than those in experiment A.

Determination of the blood content of lice using radioisotopes must be clearly explained regarding what exactly is being measured. This study, detailed in Chapter Four, showed that similar results were obtained for the "blood content" of lice using both ^{51}Cr and ^{59}Fe isotopes. It could not be stated that the lice "ingested" a particular quantity of blood as excretion is an ongoing process in the louse during feeding which could eliminate part of the blood meal and hence radioisotopes from the determination.

The blood content of one louse was determined as approximately 0.443 ul. If one considers this to be a blood meal and a calf with a very severe infestation of three thousand lice, not impractable as six hundred lice were removed from each calf on the last two days of the radioisotope study, then this population could, in theory, remove approximately 1.3 ml of blood from the host at each feeding. Unfortunately the figure of 0.443 ul gives no indication of the time period for this amount to have been accumulated. However, it appears to indicate, along with the results of experiment A and B that a severe infestation could have an effect on the host animal, perhaps causing it to become anaemic - especially if the host was very young.

Saliva appears to play an important role in the life of the louse. Four bands were distinguished by means of gel electrophoresis and antibodies were detected in sera from louse infested calves, against dissected salivary glands (Chapter Five). These antibody levels rose in parallel to the infestation level and remained at the greatest value even when the louse population had begun to decline. The saliva also appeared to have immunosuppressive actions at the bite sites of both **L. vituli** and **D. bovis** as no significant cellular

reactivity was observed in the skin sections taken from infested animals (Chapter Six). It seems, therefore, that the browsing lice (**D. bovis**) do not cause sufficient irritation or damage as they are found to remove at most the upper two-thirds of the keratinized layers of cells making up the stratum corneum. Contrastingly, as the sucking lice, **L. vituli**, penetrate blood vessels in order to obtain a blood meal and therefore introduce foreign substances into the host blood system an immune response in the form of antibody production is initiated in the host. These antibodies would be responsible at future penetrating sites for neutralizing the anticoagulent and immunosuppressive nature of the salivary secretions thus making it increasingly difficult for the lice to obtain a blood meal and so endangering their whole existence on that particular host animal.

When skins were processed into finished leather **L. vituli** and **D. bovis** were both found to cause damage to the host skin if a moderate to severe infestation was observed on the host animal (Chapter Six). Dark pin-head sized marks, caused by the probing attempts of **L. vituli** were observed on the neck, down the shoulders and along the backbone of the calf. **D. bovis**, seen over the neck region, top of the shoulders and along the backbone, due to their browsing nature caused white spot and white fleck on the finished, tanned skins. Where both types of lice were existing on the same host animal there was a definite separation of the two species. There was some overlap at the boundaries of the areas occupied by the lice but in general **D. bovis** lice were found at the base of the neck, top of the shoulders and along the backbone with **L. vituli** over the neck region and down the shoulders. Both types of damage caused by these lice did not

take up dyes, left visible marks on the finished leather and so downgraded the quality of the leather produced. Economically, therefore, lice do have an effect on the host animal in that they cause sufficient damage to the leather resulting in a lower market price.

From these studies it is apparent that moderate to severe infestations of **L. vituli** could have a significant negative effect on the blood profile and liveweight gains in young calves. The presence of lice also have a negative economic effect on the quality of leather produced from the skins of infested animals.

A scheme of treatment, perhaps using commercial pour-on preparations could be implemented at an early age to eradicate the lice and regular visual examinations made to ensure that lice were not present. Carrying out these measures would ensure that loss of body condition and weight could not be attributable to the presence of lice.

APPENDICES

APPENDIX 1

Mean Packed Cell Volume (%)

Age of Calves (Weeks)	Experiment A				Experiment B			
	Test		Control		Test		Control	
	x	s.d.	x	s.d.	x	s.d.	x	s.d.
3	-	-	-	-	33.89	0.60	34.29	0.95
6	32.77	3.67	33.75	2.96	-	-	-	-
7	33.15	3.05	34.13	3.14	34.33	0.71	33.71	1.11
8	34.54	2.18	34.25	3.24	34.22	0.97	34.00	0.82
10	33.92	1.32	32.88	2.64	34.22	0.83	34.43	0.98
12	33.15	1.14	33.38	2.97	34.56	1.01	34.29	0.76
14	32.00	1.87	31.75	2.38	33.44	0.53	34.14	1.07
16	33.92	3.10	32.75	3.24	34.11	1.05	34.29	0.76
18	35.46	2.63	33.13	3.23	34.44	0.73	33.71	0.76
20	33.00	2.45	33.13	3.60	33.44	0.53	34.00	1.00
22	32.77	2.09	34.13	2.99	34.33	1.00	34.14	0.90
24	33.62	2.14	34.13	2.64	34.00	1.00	33.86	0.69
26	30.23	1.48	32.13	2.99	-	-	-	-
28	31.85	1.28	32.25	2.05	-	-	-	-
30	31.31	0.95	31.75	1.91	-	-	-	-
	n = 13		n = 8		n = 9		n = 7	

x = mean; s.d. = standard deviation; n = number of calves sampled

APPENDIX 2

Mean Haemoglobin Concentration (g/dl)

Age of Calves (Weeks)	Experiment A				Experiment B			
	Test		Control		Test		Control	
	x	s.d.	x	s.d.	x	s.d.	x	s.d.
3	-	-	-	-	11.53	1.32	11.24	1.06
6	10.81	1.24	11.20	0.92	-	-	-	-
7	11.33	1.08	11.54	1.24	11.90	1.15	11.67	1.13
8	11.38	0.85	11.14	1.06	11.42	0.99	11.36	1.06
10	11.60	0.43	10.96	0.87	11.87	1.20	11.63	0.85
12	11.47	0.49	11.29	0.90	11.97	0.88	12.23	1.09
14	11.28	0.50	11.11	0.80	10.90	0.79	11.79	0.96
16	11.58	0.68	10.95	0.89	11.49	0.73	12.20	0.91
18	12.02	0.87	11.26	1.09	11.33	0.59	11.49	1.08
20	11.08	0.72	11.16	1.15	11.05	0.80	11.00	1.01
22	11.45	0.81	11.61	1.08	11.39	0.83	10.89	0.69
24	11.11	0.66	11.11	0.82	10.98	0.72	11.09	0.78
26	10.08	0.57	10.49	0.87	-	-	-	-
28	10.54	0.63	10.83	0.88	-	-	-	-
30	10.32	0.45	10.46	0.64	-	-	-	-
	n = 13		n = 8		n = 9		n = 7	

x = mean; s.d. = standard deviation; n = number of calves sampled

APPENDIX 3

Mean Total Leucocyte Counts (x 10^9/l)

Age of Calves (Weeks)	Experiment A				Experiment B			
	Test		Control		Test		Control	
	x	s.d.	x	s.d.	x	s.d.	x	s.d.
3	-	-	-	-	7.89	1.25	8.01	2.34
6	8.90	2.24	8.50	1.09	-	-	-	-
7	8.88	2.52	9.14	1.91	7.68	1.29	9.01	1.06
8	8.72	2.12	9.16	1.82	7.23	1.28	9.44	1.88
10	9.45	2.19	8.59	1.12	8.29	0.99	8.87	1.66
12	9.06	2.82	9.04	1.99	8.29	1.77	9.31	1.51
14	8.90	2.06	9.41	2.01	7.07	1.43	8.43	1.16
16	9.16	2.45	9.44	1.78	8.59	1.68	9.27	1.27
18	9.49	2.14	8.99	1.12	8.40	1.41	9.13	1.69
20	8.12	1.86	9.55	1.55	8.23	1.20	9.27	1.48
22	8.77	1.85	10.36	2.08	8.28	1.04	8.11	1.89
24	9.30	1.86	10.20	1.93	7.70	0.96	8.60	1.73
26	9.72	2.10	11.06	1.17	-	-	-	-
28	10.33	2.31	11.90	2.22	-	-	-	-
30	9.92	1.73	9.71	1.93	-	-	-	-
	n = 13		n = 8		n = 9		n = 7	

x = mean; s.d. = standard deviation; n = number of calves sampled

APPENDIX 4

Mean Lymphocyte Counts (% of total leucocyte count)

Age of Calves (Weeks)	Experiment A				Experiment B			
	Test		Control		Test		Control	
	x	s.d.	x	s.d.	x	s.d.	x	s.d.
3	-	-	-	-	59.78	7.61	64.29	8.69
6	62.54	6.50	56.75	7.44	-	-	-	-
7	57.00	5.32	63.13	8.10	62.00	7.70	60.71	9.52
8	61.92	5.12	57.63	8.42	59.89	4.62	62.29	9.45
10	61.92	7.30	59.88	9.46	62.78	7.87	65.43	4.16
12	62.15	10.18	58.63	8.26	65.11	8.18	64.00	10.30
14	62.38	6.79	61.75	10.08	67.67	4.77	65.14	6.96
16	62.08	4.94	57.88	9.09	67.00	12.77	66.14	3.18
18	63.00	4.85	63.88	9.20	64.67	9.07	59.57	6.88
20	68.15	6.52	66.13	7.55	64.44	5.27	63.71	6.92
22	67.69	7.05	69.88	7.62	64.00	7.05	65.29	6.78
24	64.77	10.16	63.38	7.01	65.33	3.71	70.71	4.61
26	59.85	6.34	59.50	5.13	-	-	-	-
28	66.77	4.57	62.75	5.99	-	-	-	-
30	63.00	5.94	66.25	7.03	-	-	-	-
	n = 13		n = 8		n = 9		n = 7	

x = mean; s.d. = standard deviation; n = number of calves sampled

APPENDIX 5

Mean Neutrophil Counts (% of total leucocyte count)

Age of Calves (Weeks)	Experiment A				Experiment B			
	Test		Control		Test		Control	
	x	s.d.	x	s.d.	x	s.d.	x	s.d.
3	-	-	-	-	30.89	6.15	27.14	8.91
6	30.31	6.26	33.13	6.83	-	-	-	-
7	33.15	5.29	28.00	7.17	29.89	8.46	31.29	7.50
8	28.31	4.73	31.25	7.89	27.44	3.32	29.00	6.19
10	28.15	5.73	31.75	6.96	26.67	7.04	26.57	6.08
12	28.85	9.28	33.25	7.46	27.44	5.70	27.86	9.48
14	30.08	6.06	30.13	9.17	25.22	5.52	26.14	6.52
16	27.54	4.07	32.50	7.69	27.00	9.38	25.29	5.25
18	28.38	4.11	25.88	6.15	25.67	9.00	30.71	9.55
20	24.77	4.85	24.38	5.50	24.11	3.41	26.86	6.49
22	23.54	6.49	22.75	6.80	24.11	4.81	22.00	4.58
24	26.77	7.91	26.00	8.30	23.56	4.50	18.57	4.04
26	30.15	6.49	30.00	2.88	-	-	-	-
28	21.31	6.51	25.88	3.98	-	-	-	-
30	25.54	5.94	25.88	5.99	-	-	-	-
	n = 13		n = 8		n = 9		n = 7	

x = mean; s.d. = standard deviation; n = number of calves sampled

APPENDIX 6

Mean Monocyte Counts (% of total leucocyte count)

Age of Calves (Weeks)	Experiment A				Experiment B			
	Test		Control		Test		Control	
	x	s.d.	x	s.d.	x	s.d.	x	s.d.
3	-	-	-	-	9.00	2.96	8.14	2.67
6	7.00	2.31	10.00	3.82	-	-	-	-
7	9.54	3.89	9.00	3.46	7.56	3.57	7.57	2.88
8	9.54	3.86	11.38	3.54	12.11	3.06	8.43	4.43
10	9.77	3.61	8.00	2.98	10.00	4.61	7.57	2.51
12	8.69	3.86	7.88	2.36	6.78	3.31	7.71	3.64
14	6.92	4.01	7.63	3.81	6.78	3.80	8.43	5.06
16	9.92	4.11	9.38	2.56	5.67	4.56	8.00	4.47
18	9.08	3.80	9.88	3.91	9.44	2.65	9.29	3.25
20	7.38	3.80	9.00	3.12	11.22	4.55	9.14	4.53
22	8.38	2.96	7.13	3.80	11.22	2.95	12.14	4.63
24	8.08	4.03	10.38	3.89	10.89	3.44	10.14	2.19
26	9.69	4.03	10.13	3.52	-	-	-	-
28	12.46	3.86	10.88	3.87	-	-	-	-
30	11.08	3.35	7.63	3.93	-	-	-	-
	n = 13		n = 8		n = 9		n = 7	

x = mean; s.d. = standard deviation; n = number of calves sampled

APPENDIX 7

Mean Eosinophil Count (% of total leucocyte count)

Age of Calves (Weeks)	Experiment A				Experiment B			
	Test		Control		Test		Control	
	x	s.d.	x	s.d.	x	s.d.	x	s.d.
3	-	-	-	-	0.111	0.333	0.143	0.378
6	0.231	0.439	0.250	0.463	-	-	-	-
7	0.077	0.277	0.250	0.463	0.111	0.333	0.143	0.378
8	0.231	0.439	0.250	0.463	0.111	0.333	0.429	0.535
10	0.154	0.376	0.111	0.333	0.111	0.333	0.143	0.378
12	0.154	0.376	0.125	0.354	0.111	0.333	0.143	0.378
14	0.154	0.376	0.375	0.744	0.222	0.441	0.143	0.378
16	0.154	0.376	0.250	0.463	0.222	0.441	0.286	0.488
18	0.231	0.439	0.250	0.463	0.111	0.333	0.143	0.378
20	0.231	0.599	0.250	0.463	0.111	0.333	0.286	0.488
22	0.077	0.277	0.250	0.463	0.222	0.488	0.286	0.488
24	0.154	0.376	0.250	0.463	0.222	0.441	0.143	0.378
26	0.077	0.277	0.250	0.463	-	-	-	-
28	0.154	0.376	0.375	0.744	-	-	-	-
30	0.231	0.439	0.250	0.463	-	-	-	-
	n = 13		n = 8		n = 9		n = 7	

x = mean; s.d. = standard deviation; n = number of calves sampled

APPENDIX 8

Mean Erythrocyte Counts (x 10^{12}/l)

Age of Calves (Weeks)	Experiment A				Experiment B			
	Test		Control		Test		Control	
	x	s.d.	x	s.d.	x	s.d.	x	s.d.
3	-	-	-	-	8.58	1.35	8.79	1.06
6	8.82	0.83	9.36	1.21	-	-	-	-
7	9.09	0.84	9.75	1.42	9.47	1.35	10.47	1.20
8	9.79	0.68	9.89	1.17	10.13	1.25	10.03	0.94
10	9.82	0.77	9.55	1.25	10.43	1.52	10.20	0.67
12	9.10	0.72	8.94	1.16	10.24	0.78	11.77	1.05
14	9.21	0.76	9.16	1.08	9.07	0.97	9.70	0.96
16	9.90	1.07	9.24	0.93	9.37	1.15	9.83	0.49
18	9.97	1.01	9.44	1.32	9.64	0.87	10.41	1.29
20	8.89	0.75	8.91	1.08	9.37	1.61	9.30	0.72
22	9.35	0.66	9.15	0.98	9.56	1.20	9.43	0.71
24	9.16	0.66	9.25	1.29	8.54	1.41	8.60	0.92
26	8.31	0.73	8.50	1.13	-	-	-	-
28	9.03	0.72	9.35	1.29	-	-	-	-
30	8.13	1.16	8.19	0.80	-	-	-	-
	n = 13		n = 8		n = 9		n = 7	

x = mean; s.d. = standard deviation; n = number of calves sampled

APPENDIX 9

Mean Reticulocyte Counts (% of total erythrocytes)

Age of Calves (Weeks)	Experiment A				Experiment B			
	Test		Control		Test		Control	
	x	s.d.	x	s.d.	x	s.d.	x	s.d.
3	-	-	-	-	0.44	0.53	0.29	0.49
6	0.08	0.28	0	0	-	-	-	-
7	0.38	0.51	0.13	0.35	0.56	0.53	0	0
8	0.23	0.44	0.63	0.74	0.33	0.50	0.29	0.49
10	0.23	0.44	0.13	0.35	0.56	0.88	0.14	0.38
12	0.23	0.44	0.13	0.35	0.22	0.44	0.43	0.53
14	0.31	0.48	0.25	0.46	0.33	0.50	0.14	0.38
16	0.38	0.65	0.38	0.52	0.22	0.44	0.29	0.49
18	0.31	0.48	0.25	0.46	0.44	0.53	0.29	0.49
20	0.15	0.38	0.25	0.46	0.33	0.50	0.29	0.49
22	0.31	0.48	0.38	0.52	0.33	0.50	0.14	0.38
24	0.23	0.44	0.25	0.46	0.22	0.44	0.29	0.49
26	0.23	0.44	0.38	0.52	-	-	-	-
28	0.31	0.48	0.13	0.35	-	-	-	-
30	0.23	0.44	0.25	0.46	-	-	-	-
	n = 13		n = 8		n = 9		n = 7	

x = mean; s.d. = standard deviation; n = number of calves sampled

APPENDIX 10

Mean Total Protein Determination (g/l)

Age of Calves (Weeks)	Experiment A				Experiment B			
	Test		Control		Test		Control	
	x	s.d.	x	s.d.	x	s.d.	x	s.d.
3	-	-	-	-	64.90	1.14	65.01	0.94
6	64.75	0.96	64.15	0.52	-	-	-	-
7	64.23	1.11	64.55	1.12	64.98	0.98	64.74	0.86
8	64.45	0.78	64.78	0.93	64.70	0.97	64.91	1.39
10	64.85	0.95	64.39	0.86	64.39	0.71	65.00	0.88
12	64.97	1.10	65.24	0.96	65.33	1.04	65.30	0.68
14	65.32	0.93	65.10	1.01	64.73	0.95	64.80	0.99
16	65.12	0.61	64.95	1.12	64.76	0.91	65.24	1.06
18	65.19	1.11	65.10	1.06	64.87	0.99	65.31	0.59
20	64.82	0.96	65.45	0.78	65.32	0.86	64.90	0.94
22	65.34	0.83	65.09	1.01	65.22	0.61	64.40	0.89
24	65.41	0.77	65.03	0.99	64.62	0.80	64.74	0.95
26	65.03	1.04	65.01	1.10	-	-	-	-
28	65.12	0.81	64.86	0.98	-	-	-	-
30	65.16	1.09	64.50	0.77	-	-	-	-
	n = 13		n = 8		n = 9		n = 7	

x = mean; s.d. = standard deviation; n = number of calves sampled

APPENDIX 11

Mean Total Albumin Determination (g/l)

Age of Calves (Weeks)	Experiment A				Experiment B			
	Test		Control		Test		Control	
	x	s.d.	x	s.d.	x	s.d.	x	s.d.
3	-	-	-	-	34.62	1.11	34.54	0.82
6	34.23	0.82	34.85	0.88	-	-	-	-
7	34.85	1.06	34.76	1.02	34.74	0.84	34.47	0.89
8	34.92	0.95	34.44	0.75	34.48	0.70	34.63	0.87
10	34.68	0.90	34.26	0.59	34.62	0.78	34.38	0.79
12	35.05	1.22	34.88	1.17	34.32	0.66	34.71	1.05
14	34.79	0.93	35.23	1.27	34.64	0.99	34.23	0.96
16	34.55	0.95	34.24	0.65	34.93	1.10	34.91	1.04
18	34.42	0.84	34.23	0.61	34.67	1.08	34.76	1.06
20	34.38	0.98	35.60	1.17	34.66	0.79	35.04	0.92
22	34.80	0.96	35.15	0.95	34.79	1.13	34.07	0.47
24	34.75	0.91	34.31	0.62	35.20	1.28	34.49	0.76
26	34.67	1.03	34.30	0.96	-	-	-	-
28	34.68	0.81	34.71	0.99	-	-	-	-
30	34.75	0.91	34.79	0.92	-	-	-	-
	n = 13		n = 8		n = 9		n = 7	

x = mean; s.d. = standard deviation; n = number of calves sampled

APPENDIX 12

Mean Liveweights (kg)

Age of Calves (Weeks)	Experiment A				Experiment B			
	Test		Control		Test		Control	
	x	s.d.	x	s.d.	x	s.d.	x	s.d.
1	64.42	3.86	44.38	3.94	45.39	7.61	50.00	5.80
2	-	-	-	-	59.00	12.20	67.14	8.92
3	-	-	-	-	63.67	13.98	73.57	10.49
4	-	-	-	-	68.89	15.81	81.29	11.49
6	61.62	6.65	56.75	6.25	-	-	-	-
8	73.92	8.92	65.38	8.54	-	-	-	-
10	86.85	10.79	78.88	12.05	-	-	-	-
12	98.38	11.33	91.75	13.63	88.00	19.18	104.57	14.79
14	111.15	11.39	103.88	15.07	99.11	19.87	113.57	15.08
16	-	-	-	-	107.78	19.56	123.43	17.79
18	136.46	12.24	128.00	16.78	119.33	20.69	137.86	20.72
20	145.77	13.33	139.50	18.44	133.00	22.19	149.14	20.24
22	157.54	14.55	150.75	19.17	-	-	-	-
24	166.92	13.97	163.13	22.98	160.33	25.89	179.71	22.99
26	182.31	13.37	175.50	27.50	-	-	-	-
30	212.23	16.01	208.13	33.51	-	-	-	-
	n = 13		n = 8		n = 9		n = 7	

x = mean; s.d. = standard deviation; n = number of calves sampled

APPENDIX 13

Lice Counts

Experiment A			Experiment B		
Number of Lice	Number of Counts	% of Total Counts	Number of Lice	Number of Counts	% of Total Counts
0	109	11.98	0	88	17.78
<5	293	32.20	<5	150	30.30
5 - 10	421	46.26	5 - 10	211	42.63
11 - 20	78	8.57	11 - 20	43	8.69
21 - 50	9	0.99	21 - 50	3	0.60

APPENDIX 14

Calculation of Total Injected Radioactivity (TIR)

1. Weigh a syringe containing about 1 ml of the labelled preparation and inject the contents into a 100 ml volumetric flask; weigh the empty syrings and dilute the contents of the flask to the mark using dilute NaOH; shake the flask gently. This solution is termed the 'standard'.

2. Determine the net counts/min/ml of several aliquots of the 'standard' solution.

3. From the weight of the labelled preparation used for preparing the standard, calculate the total standard radioactivity (TSR) thus:-

$$\text{TSR (cts/min)} = \frac{\text{mean radioactivity in 1 ml of the standard solution (cts/min)}}{100}$$

4. From TSR, calculate the total amount of radioactivity injected into each animal (TIR) thus:-

$$\text{TIR (cts/min)} = \frac{\text{Wt. of labelled preparation injected* (g)} \times \text{TIR}}{\text{Wt. of labelled preparation in standard (g)}}$$

*into the animal.

APPENDIX 15

Plasma Protein Metabolism

Most mathematical models describing plasma protein metabolism represent all the extravascular pools by one common pool communicating with the plasma through pores in the capillary wall, i.e. as a two-compartment system (Fig. 1). This system is open in that protein transfer from one pool to another can take place in either direction, k_1 reflecting the outward movement of the protein under study and k_2 the return via the lymphatic flow. However, it must be stressed that since extravascular protein is localised in an infinite number of compartments, each of which has a different exchange rate, k_2 represents the average return rate of protein from all the extravascular pools into the plasma.

All mathematical models used to determine turnover rates of labelled proteins assume that the animal is in a 'steady state'. In work with diseased animals this assumption is unlikely to be valid - particularly where marked loss of body weight or disturbances in fluid balance prevail. Nevertheless, much useful information - particularly of a comparative nature - may be obtained regarding the disturbances in protein metabolism caused by parasites to their hosts.

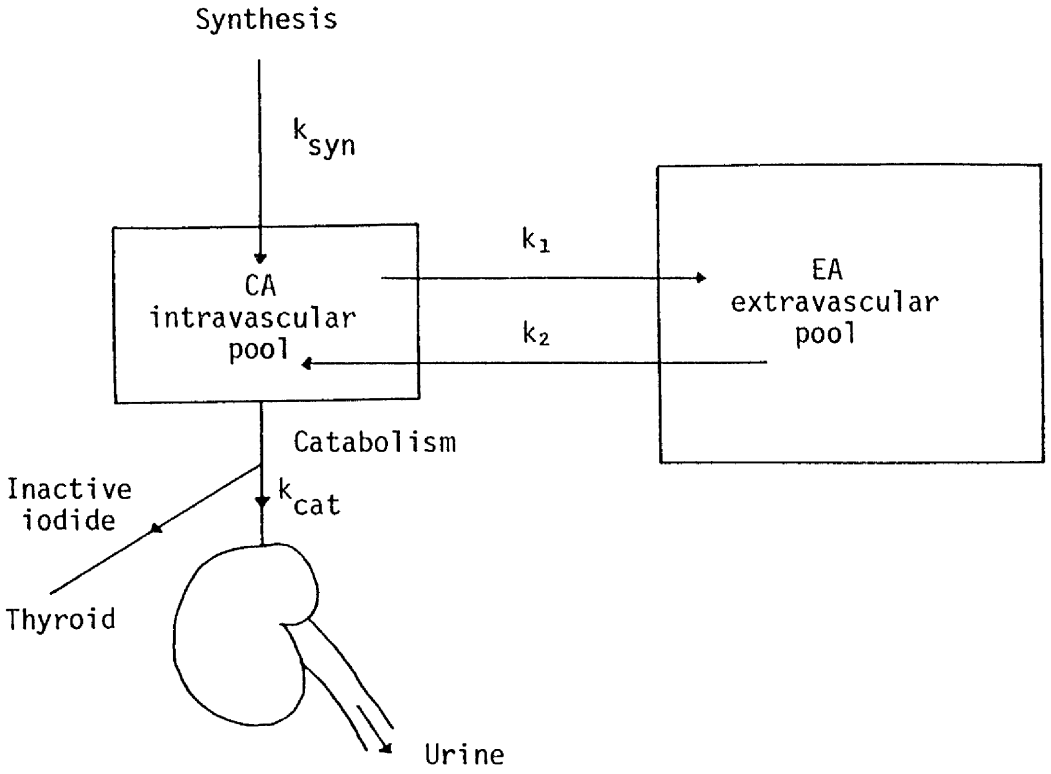


Figure 1 A two-compartment open model describing the distribution and metabolism of radioiodinated proteins.

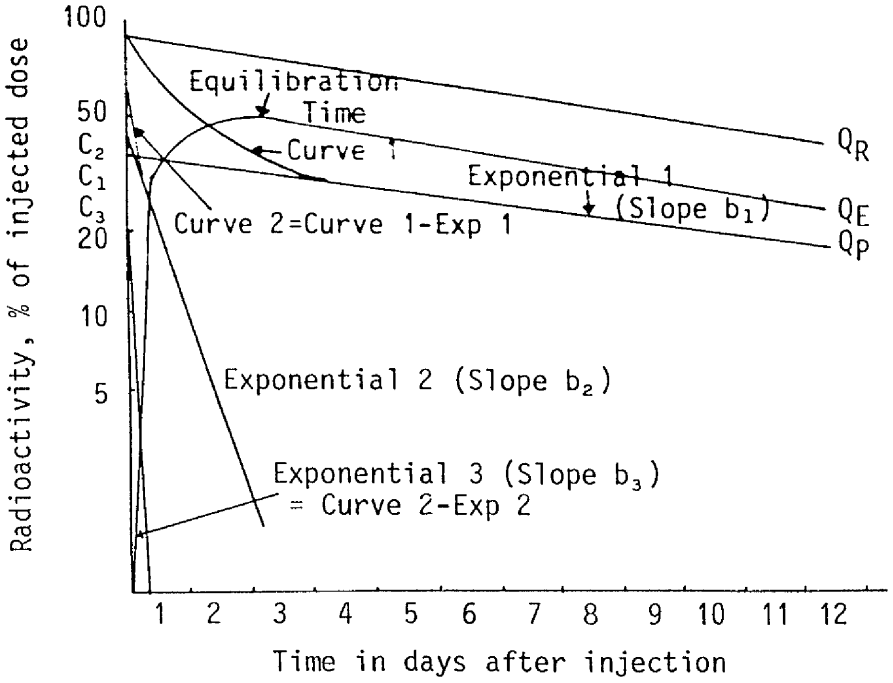


Figure 2 Activity in vascular and extravascular compartments after injection of radioiodinated protein.

1. Construction of Curves (Fig. 2)

1.1 Plasma activity (Q_P)

The count-rate of each plasma sample, corrected for radioactive decay by use of the standard, is expressed as a percentage of the 10-min post-injection sample and a semilogarithmic plot made of activity against time.

1.2 Retained activity (Q_R)

The activity retained in the body at the end of each 24-h period is obtained by subtracting from the injected dose the cumulative activity excreted in the urine and faeces. Curves are constructed by plotting daily retained activity as a percentage of the injected activity on a semi-log scale.

1.3 Extravascular activity (Q_E)

The activity present in the extravascular compartments is obtained as the difference between Q_R and Q_P at the end of each collection period.

2. Calculation of Results

2.1 Plasma volume (V_P)

The total activity injected into the animal is calculated from the counts.min^{-1}.ml^{-1} of the standard, the weight of labelled albumin solution used in preparing the standard and the weight of labelled albumin solution injected into the animal. Plasma volume is then calculated from:-

$$V_P(\text{ml}) = \frac{\text{total activity injected (counts/min)}}{\text{counts.min}^{-1}.\text{ml}^{-1}\ \text{plasma (10 min samples)}}$$

(a) Extrapolation method. Extrapolate the linear part of the plasma activity curve to zero time and note the intercept value (C_1):

$$\text{TA (grams)} = \frac{\text{CA (grams)}}{C_1}$$

Although theoretically less acceptable than method (b), this procedure does not require urine and faeces collection.

(b) Equilibrium-time method. The extravascular activity curve (Q_E) rises to a maximum and subsequently declines at a rate similar to Q_P. Where Q_E is maximal, the labelled albumin has completely equilibrated with the total exchangeable albumin pool. At this equilibrium time the ratio between extra and intravascular activities equals the ratio between pool masses and

$$\text{TA (grams)} = \frac{CA(Q_P + Q_E)}{Q_P}$$

2.4 **Extravascular albumin (EA)**

EA (grams) = TA - CA.

(Note: It is usual to express the size of all body protein pools on a body weight basis, i.e. g/kg).

2.5 **Albumin catabolism**

(a) By analysis of the plasma activity curve.

(i) From the linear part of the curve (i.e. from about day 4 onwards in Fig. 2), calculate the 'apparent

half-life' (t1/2) of the labelled protein, i.e. the time taken for the plasma activity to fall to 50%.

(ii) Calculate the slope constant (b_1), of the plasma activity curve using the equation

$$b_1 = \frac{\ln 2}{t1/2 \text{ (days)}}$$

(iii) Subtract the extrapolated part of the plasma curve from the original curve to yield a new curve with slope constant b_2 and intercept with the ordinate c_2. This peeling-off technique is continued until subtraction results in one single, linear curve. Thus, each exponential is characterised by a slope constant ($b_1 b_2 b_3$, etc.). and an intercept ($C_1 C_2 C_3$). The fraction of the intravascular pool catabolised, K, is now calculated from the equation:-

$$K = \frac{1}{\frac{C_1}{b_1} + \frac{C_2}{b_2} + \frac{C_3}{b_3} \ldots \frac{C_n}{b_n}}$$

(Note: In most cases only two exponentials are obtained. Conversion of fractional catabolic rates to absolute amounts of albumin degraded are then made from the equation:-

Albumin catabolised (grams/day) = CA (grams) x K.)

(b) By analysis of excreted activity. For each 24-hr collection period after the fourth day of injection, calculate the total activities excreted in the faeces (F) and urine (U). From the plasma volume and the counts.min^{-1}.ml^{-1} plasma at the beginning of each collection period, calculate the total plasma activity (P). The fractional catabolic rate, K, is then given by:-

$$K = \frac{U + F}{P}$$

2.6 Plasma and albumin leakage into the gut

Excessive losses of plasma and hence of albumin into the gut are a recognised feature of many parasitic infections. Some indication of the extent of such losses may be obtained by dividing the daily values for F by the activity/ml of plasma at the beginning of each collection period. The figure obtained, which is known as the daily 'faecal clearance of plasma', represents the volume of plasma which theoretically must have passed into the gut during that period to give rise to the faecal radioactivity. However, it should be borne in mind that such values grossly underestimate protein losses by this route and can therefore only be used as a qualitative guide, because substantial amounts of isotope from labelled protein degraded in the gut are reabsorbed and subsequently excreted in the urine.

Bibliography

McFARLANE, A.S. Mammalian Protein Metabolism I (Munro, Allison, Eds.), Academic Press, New York and London (1984).

ORATZ, M. Plasma Protein Metabolism (Rothschild, Waldmann, Eds.), Academic Press, New York and London (1970).

APPENDIX 16

Measurement of Body Fluid Volume Using Radioisotopically Labelled Materials

Body fluid volumes are normally measured by methods based on the 'dilution principal'. These are indirect methods which depend on the addition of a known weight of some marker substance to the fluid compartment of interest, allowing the substance to equilibrate with the fluid, removing a sample of the solution and finally determining the concentration of the marker. Knowing the total amount of marker added to the fluid, and also its concentration after complete mixing, the volume in which it has been diluted may be calculated as follows:

$$\text{Volume (ml)} = \frac{\text{Quantity of marker added (g)}}{\text{Concentration of marker after equilibration (g/ml)}}$$

Many of the techniques which are currently employed to measure the fluid compartments of animals depend on the use of radioactive marker substances, but the basic principal is still the same:

$$\text{Volume (ml)} = \frac{\text{Total radioactivity injected (cpm)}}{\text{Radioactivity of sample removed after equilibration (cts/min/ml)}}$$

Measurement of fluid compartments using radioactive markers has the advantage of speed, simplicity and accuracy. However, since all methods depend upon the introduction of a foreign substance into the blood and estimating the extent of dilution after a given interval of time, the substance used for the measurements must have certain characteristics. In the first place, it must be capable of becoming evenly distributed throughout the compartment of interest and not

'leak out' into other compartments. Thus, for example, a tracer for measuring plasma volume should not diffuse out of the blood stream, otherwise the concentration or radioactivity at equilibrium will be lower than it should be and hence the volume of the distribution overestimated; conversly, if the substance does not equilibrate with the total volume of plasma then the equilibrium concentration will be too high, and the volume of distribution underestimated. A second requirement is that the substance should not be broken down or excreted to any significant extent during the period required for mixing, otherwise the volume of distribution will again be overestimated. In measuring some fluid compartments, particularly those requiring fairly extended periods of equilibration (e.g. total body water) some losses are inevitable and have to be taken into account in the calculation.

REFERENCES

ABBOTT, E.M., PARKINS, J.J. and HOLMES, P.H. (1985). Influence of dietary protein on the pathophysiology of ovine haemonchosis in Finn Dorset and Scottish Blackface lambs given a single moderate infection. **Research in Veterinary Science, 38:** 54-60.

ALLEN, N.N. and DICKIE, R.J. (1952). Cattle lice control by clipping. **Journal of Economic Entomology, 45:** 970-973.

ALLAN, J.R. and WEST, A.S. (1964). Recent advances in studies on reactions to mosquito bites. **Proceedings of the 1st International Congress on Parasitology, 2:** 1091-1092.

ANDERSON, N.L. and ANDERSON, N.G. (1978). Analytical techniques for cell fractions. XXII Two-dimensional analysis of serum and tissue proteins: multiple gradient-slab gel electrophoresis. **Analytical Biochemistry, 85:** 341-354.

ANDREWS, J.R.H. (1971). Descriptions of the hitherto unknown males of **Damalinia longicormis** (Nitzch, 1818) and **Damalinia hemitragi** (Cummings, 1916) Trichodectidae Mallophaga. **Journal of Natural History, 6:** 153-157.

ANDREWS, J.W. (1942). Stomach worm (**Haemonchus contortus**) infection in lambs and its relation to gastric haemorrhage and general pathology. **Journal of Agricultural Research, 65:** 1-18.

ARCHER, R.K. (1960). The biology of the eosinophil leucocyte. **Veterinary Record, 72:** 155-158.

BAILEY, P.J., CARROL, W.A.D. and KNEE, B. (1984). Effects of lice infestation on the growth of beef heifers in Victoria. **Australian Journal of Experimental Agriculture and Animal Husbandry, 24:** 140-143.

BAKER, K.P. (1973). Some defects of hides. **Irish Veterinary Journal, 26:** 177-181.

BAKER, K.P. and O'FLANAGEN, J. (1964). Hypersensitivity of dog skin to fleas - a clinical report. **Journal of Small Animal Practice, 16:** 317-327.

BAKER, K.P. and OORMADZI, H. (1978). The probable cause of the multiple linear scratch defect of cattle hides in Ireland. **Journal of the Society of Leather Technologists and Chemists, 62:** 103-107.

BATES, M. (1949). The natural history of mosquitoes. **The Macmillan Co., New York,** 379pp.

BEAKLEY, W.R. and FINDLAY, J.D. (1955). The effect of environmental temperature and humidity on the ear temperatures of Ayrshire calves. **Journal of Agricultural Science, 45:** 373-379.

BELL, J.F. and CLIFFORD, C. (1964). Effects of limb disability on lousiness in mice. II Intersex grooming relationships. **Experimental Parasitology, 15:** 340-349.

BELL, J.F., CLIFFORD, C.M., MOORE, G.J. and RAYMOND, G. (1966). Effects of limb disability on lousiness in mice. III Gross aspects of acquired resistance. **Experimental Parasitology, 18:** 49-60.

BELL, J.F., JELLISON, W.L. and OWEN, C.R. (1962). Effects of limb disability on lousiness in mice. I Preliminary studies. **Experimental Parasitology, 12:** 176-183.

BENJAMINI, E., FEINGOLD, B.F. and KARTMAN, L. (1961). Skin reactivity in guinea pigs sensitized to flea bites: the sequence of reactions. **Proceedings of the Society of Experimental Biology and Medicine, 108:** 700-702.

BLOOM, G.D., DIAMANT, B., HAGERMARK, O. and RITZEN, M. (1970). The effects of adenosine-5'-triphosphate (ATP) on structure and amine content of rat peritoneal mast cells. **Experimental Cell Research, 62:** 61-75.

BOULARD, C. (1975). Evolution des anticorps circulants chez les bovines contre l'hypodermose. **Annals de Recherches Veterinaires, 6:** 143-154.

BREMNER, K.C. (1969). Pathogenic factors in experimental bovine oesophagostomosis. III Demonstration of protein-losing enteropathy with ^{51}Cr-albumin. **Experimental Parasitology, 24:** 364-374.

BUCHANAN, R.S. and COLES, P.G. (1971). Control of lice on cattle with Dursban. **New Zealand Veterinary Journal, 19:** 197-202.

BUECHLER, P.R., HANNIGAN, M.V., CARROLL, R.J., DAHMS, M.P. and FEAIRHELLER, S.H. (1984). Studies of hyperkeratosis of cattlehides attributable to psoroptic scabies. **Journal of the American Leather Chemists Association, 79:** 416-434.

BUSHLAND, R.C., RADELEFF, R.D. and DRUMMOND, R.O. (1963). Development of systemic insecticides for pests of animals in the United States. **Annual Review of Entomology, 8:** 215-238.

BUXTON, P.A. (1946). The Louse (second edition). Edward Arnold and Co., London, (viii) + 164pp.

CALLINAN, A.P.L. (1980). Effects of artificially induced infestations of the cattle louse **Linognathus vituli. Australian Veterinary Journal, 56:** 484-486.

CAMPBELL, R.M., CUTHBERTSON, D.P., MATTHEWS, C.M. and McFARLANE, A.S. (1956). Behaviour of ^{14}C- and ^{12T}I-labelled plasma proteins in the rat. **International Journal of Applied Radiation and Isotopes, 1:** 66-84.

CARLSSON, H.E., LINDBERG, A.A. and HAMMARSTROM, S. (1972). Titration of antibodies to salmonella O antigens by enzyme-linked immunosorbent assay. **Infection and Immunity, 6:** 703-708.

CHALMERS, K. and CHARLESTON, W.A.G. (1980a). Cattle lice in New Zealand: observations on the prevalence, distribution and seasonal patterns of infestation. **New Zealand Veterinary Journal, 28:** 198-200.

CHALMERS, K. and CHARLESTON, W.A.G. (1980b). Cattle lice in New Zealand: observations on the biology and ecology of **Damalinia bovis** and **Linognathus vituli. New Zealand Veterinary Journal, 28:** 214-216.

CHALMERS, K. and CHARLESTON, W.A.G. (1980c). Cattle lice in New Zealand: effects on host liveweight gain and haematocrit levels. **New Zealand Veterinary Journal, 28:** 235-237.

CHINERY, W.A. (1973). The nature and origin of the cement substance at the site of attachment of adult **Haemophysalis spinigeria. Journal of Medical Entomology, 10:** 355-362.

COLLINS, R.C. and DEWHIRST, L.W. (1965). Some effects of the sucking louse **Haematopinus eurysternus** on cattle on unsupplemented range. **Journal of the American Veterinary Medical Association, 146:** 129-132.

CRAUFURD-BENSON, H.J. (1941a). The cattle lice of Great Britain. Part I Biology, with special reference to **Haematopinus eurysternus. Journal of Parasitology, 33:** 331-342.

CRAUFURD-BENSON, H.J. (1941b). The cattle lice of Great Britain. Part II Lice populations. **Journal of Parasitology, 33:** 343-358.

CUMMINS, L.J. and GRAHAM, J.F. (1982). The effect of lice infestation on the growth of Hereford calves. **Australian Veterinary Journal, 58:** 194-196.

CUMMINS, L.J. and TWEDDLE, N.E. (1977). Influence of light infestations of **Linognathus vituli** on the growth of young cattle. **Australian Veterinary Journal, 53:** 591-592.

DARGIE, J.D. (1975). Applications of radioisotope techniques to the study of red cell and plasma metabolism in helminth diseases of sheep. In: Pathogenic Processes in Parasitic Infections, eds. Taylor, A.E.R. and Muller, R. Blackwell Scientific Publications, Oxford, pp. 1-26.

DAVIS, B.J. (1964). Disc electrophoresis. II Method and application to human serum proteins. **Annals of the New York Academy of Sciences, 121:** 404-427.

DAVIS, D.P. and WILLIAMS, R.E. (1986). Influence of hog lice, **Haematopinus suis,** on blood components, behaviour, weight gain and feed efficiency of pigs. **Veterinary Parasitology, 22:** 307-314.

DEMPSEY, M. (1961). Hides, skins and leather. **Veterinary Annual, 3:** 38-45.

DEVINE, T.L., VENARD, C.E. and MYSER, W.C. (1965). Measurement of salivation by **Aedes aegypti** (L.) feeding on a living host. **Journal of Insect Physiology, 11:** 347-353.

DUNKER, A.K. and RUECKERT, R.R. (1969). Observations on molecular weight determinations on polyacrylamide gel. **The Journal of Biological Chemistry, 244:** 5074-5080.

EDWARDS, J.J., ANDERSON, N.G., NANCE, S.L. and ANDERSON, N.L. (1979). Red cell proteins. I Two-dimensional mapping of human erythrocyte lysate proteins. **Blood, 53:** 1121-1132.

EDWARDS, J.S. (1961). The action and composition of the saliva of an assassin bug **Platymeris rhadamanthus** gaerst (Hemiptera, Reduviidae). **Journal of Experimental Biology, 38:** 61-77.

ELY, D.G. and HARVEY, T.L. (1969). Relation of ration to short-nosed cattle louse infestations. **Journal of Economic Entomology, 62:** 341-344.

ENGVALL, E. and PERLMANN, P. (1971). Enzyme-linked immunosorbent assay (ELISA). Quantitive assay of immunoglobin G. **Immunochemistry, 8:** 871-874.

EVERETT, A.L., MILLAR, R.W., GLADNEY, W.J. and HANNIGAN, M.V. (1977). Effects of some important ectoparasites on the grain quality of cattlehide leather. **Journal of the American Leather Chemists Association, 72:** 6-24.

EVSTAFJEV, M.N. (1980). The role of immunity during hypodermatosis of cattle. **Parasitologia, 14:** 197-205.

FINCH, C.A., DEUBELBEISS, K., COOK, J.D., ESCHBACH, J.W., HARKER, L.A., FUNK, D.D., MARSAGLIA, G., HILLMAN, R.S., SLICHTER, S., ADAMSON, J.W., GANZONI, A. and GIBLETT, E.R. (1970). Ferrokinectics in man. **Medicine, 49:** 17-53.

FINKEL, A. (1933). Obraz cytologicyzny krwi v ludzi, karmiacych wszy. **Polska Gazetta Lekarska, Krakow, 12:** 595-596.

FISK, F.W. (1950). Studies on proteolytic digestion in adult **Aedes aegypti** mosquitoes. **Annals of the Entomological Society of America, 43:** 555-572.

FORD-HUTCHINSON, A.W. (1982). Aggregation of rat neutrophils by nucleotide triphosphates. **British Journal of Pharmacology, 76:** 367-371.

FREER, R.E. and GAHAN, R.J. (1968). Controlling lice in beef herds - is it economic? **Agricultural Gazette of New South Wales, 79:** 308-309.

FRENCH, F.E. (1972). **Aedes aegypti:** histopathology of immediate skin reactions of hypersensitive guinea pigs resulting from bites. **Experimental Parasitology, 32:** 175-180.

GAAFAR, S.M. (1972). Immune response to arthropods. In: Immunity to animal parasites. Ed. Soulsby, E.J.L., Academic Press, New York and London. Chapter 10.

GEORGE, J.E., WRIGHT, F.C., GUILLOTT, F.S. and BUECHLER, P.R. (1986). Observations on the possible relationship between psorpotic mange in cattle and white spot damage on leather. **Journal of the American Leather Chemists Association, 81:** 296-304.

GIBNEY, V.J., CAMPBELL, J.B., BOXLER, D.J., CLANTON, D.C. and DEUTSCHER, G.H. (1985). Effects of various infestation levels of cattle lice (Mallophaga: Trichodectidae and Anoplura: Haematopinidae) on feed efficiency and weight gains of beef heifers. **Journal of Economic Entomology, 78:** 1304-1307.

GILL, H.S., BOID, R. and ROSS, C.A. (1986). Isolation and characterization of salivary antigens from **Hyalomma anatolicum anatolicum. Parasite Immunology, 8:** 11-25.

GILL, H.S. and WALKER, A.R. (1987). The salivary glands of **Hyalomma anatolicum anatolicum**: structural changes during attachment and feeding. **International Journal for Parasitology, 17**: 1381-1392.

GILL, H.S. and WALKER, A.R. (1988). The salivary glands of **Hylomma anatolicum anatolicum**: nature of salivary gland components and their role in tick attachment and feeding. **International Journal for Parasitology, 18**: 83-93.

GILLET, J.D. (1967). Natural selection and feeding speed in a blood sucking insect. **Proceedings of the Royal Society of London, Series B, 167**: 316-329.

GOJMERAC, W.L., DICKE, R.J. and ALLEN, N.N. (1959). Factors affecting the biology of cattle lice. **Journal of Economic Entomology, 52**: 79-82.

GOLDMAN, L., JOHNSON, P. and RAMSEY, J. (1952). The insect bite reaction. **Journal of Investigative Dermatology, 18**: 403-418.

GOLDMAN, L., ROCHWELL, E. and RICHFIELD, D.F. (1952). III Histopathological studies on cutaneous reactions to the bites of various arthropods. **Annual Journal of Tropical and Medical Hygiene, 1**: 514-525.

GOODING, R.H. (1972). Digestive processes of haematophagus insects. I: A literature review. **Quaestiones Entomologicae, 8**: 5-60.

GORDON, R.M. and CREWE, W. (1948). The mechanism by which mosquitoes and tsetse flies obtain their blood meal. The histology of the lesions produced and the subsequent reactions of the mammalian host; together with some observation on the feeding of **Chrysops** and **cimex. Annals of Tropical Medicine and Parasitology, 42:** 334-356.

GRANT, R.T. (1930). Observations on direct communications between arteries and veins in the rabbits ear. **Heart, 15:** 281-303.

GREATOREX, J.C. (1954). Studies on the haematology of calves from birth to one year of age. **British Veterinary Journal, 110:** 120-133.

GRIFFITHS, R.B. and GORDON, R.M. (1952). An apparatus which enables the process of feeding by mosquitoes to be observed in tissues of a live rodent, together with an account of the ejection of saliva and its significance in malaria. **Annals of Tropical Medical Parasitology, 46:** 311-319.

HAINES, B.M. (1978). Leather defects originating at the farm and at the abattoir. **Journal of the Society of Leather Technologists and Chemists, 62:** 25-47.

HECHT, O. (1933). Hautreaktionen auf die stiche blutsaugender insekten und milben als allergische erscheimungen. **Entralblatt fur Haut und Geschlechtskrankheiten, 44:** 421.

HELLMAN, K. and HAWKINS, R.I. (1966). An antithrombin (Maculatin) and a plasminogen activator extractable from the blood sucking hemipteran, **Eutriatoma maculatus. British Journal of Haematology, 12:** 376-384.

HEUKESHOVEN, J. and DERNICK, R. (1985). Simplified method for silver staining of proteins in polyacrylamide gels and the mechanism of silver staining. **Electrophoresis, 6:** 103-112.

HIGGS, G.A., VANE, J.R., HART, R.J., POTTER, C. and WILSON, R.G. (1976). Prostoglandins in the saliva of the cattle tick **Boophilus microplus. Bulletin of Entomological Research, 66:** 665-670.

HOLMAN, H.H. (1955). The blood picture of the cow. **British Veterinary Journal, 111:** 440-457.

HOLMAN, H.H. (1956). Changes associated with age in the blood picture of calves and heifers. **British Veterinary Jounal, 112:** 91-104.

HOLMES, P.H., ABBOTT, E.M. and PARKINS, J.J. (1986). Use of nuclear techniques to investigate the influence of host nutrition on the pathogenesis of ovine haemonchosis. In: Nuclear Techniques aimed at improving Meat, Milk and Wool Production from Ruminant Animals. Vienna: International Atomic Energy Agency.

HOLMES, P.H., DARGIE, J.D., MACLEAN, J.M. and MULLIGAN, W. (1968). The anaemia in fascioliasis: studies with ^{51}Cr-labelled red cells. **Journal of Comparative Pathology, 78:** 415-420.

HOLMES, P.H. and MACLEAN, J.M. (1971). The pathophysiology of ovine ostertagiasis: a study of the changes in plasma protein metabolism following single infections. **Research in Veterinary Science, 12:** 265-271.

HOPKINS, G.H.E. (1949). The host associations of the lice of mammals. **Proceedings of the Zoological Society of London, 119 Part II:** 387-604.

IMES, M. (1947). Cattle lice and how to eradicate them. **United States Department of Agriculture, Farmers' Bulletin, 909.**

INTERNATIONAL COMMITTEE FOR STANDARDISATION IN HAEMATOLOGY (1967). **British Journal of Haematology, 13:** (supplement) 71.

ISCHIKAWA, A., HAYASHI, H., MINAMI, M. and TOMITA, K. (1972). An acute inflammation induced by inorganic pyrophosphate and adenosine triphosphate, and its inhibition by cyclic 3'-5' adenosine monophosphate. **Biochemical Pharmacology, 21:** 317-331.

KEMPER, H.E., COBBETT, N.G., ROBERTS, I.H. and PETERSON, H.O. (1948). DDT emulsions for the destruction of lice on cattle, sheep and goats. **Amercian Journal of Veterinary Research, 9:** 373-378.

KETTLE, P.R. (1972). Pour-on insecticides for the control of **Linognathus vituli. New Zealand Veterinary Journal, 20:** 167.

KETTLE, P.R. (1974). The influence of cattle lice **Dammalinia bovis** and **Linognathus vituli** on weight gain in beef animals. **New Zealand Veterinary Journal, 22:** 10-11.

KETTLE, P.R. and LUKIES, J.M. (1979). The efficacy of some pour-on insecticides for the control of long-nosed sucking lice (**Linognathus vituli**) on cattle. **New Zealand Veterinary Journal, 27:** 78-79.

KETTLE, P.R. and PEARCE, D.M. (1974). Effect of the sheep body louse (**Damalinia ovis**) on host weight gain and fleece value. **New Zealand Journal of Experimental Agriculture, 2:** 219-221.

KITAOKA, S. (1961). Physiological and ecological studies on some ticks. V Nitrogen and iron excretion and amount of blood meal ingested during the blood sucking process in the tick. **National Institute of Animal Health, Tokyo, 1:** 96-104.

KOLIN, A. (1955). Isoelectric spectra and mobility spectra: a new approach to electrophoretic separation. **Proceedings of the National Academy of Science (Washington), 41:** 101-110.

LAMSON, G.H. (1918). Cattle lice and their control. **Storrs Agricultural Experimental Station Bulletin, 97:** 395-414.

LANCASTER, J.L. (1957). Cattle lice. Agricultural Experimental Station, University of Arkansas, Leyetteville. Bulletin 591.

LARRIVEE, D.H., BENJAMINI, E., FEINGOLD, B.F. and SHIMIZU, M. (1964). Histologic studies of guinea pig skin: different stages of allergic reactivity to flea bites. **Experimental Parasitology, 15:** 491-502.

LAVOIPIERRE, M.M.J. (1965). Feeding mechanism of blood-sucking arthropods. **Nature, 208:** 302-303.

LAVOIPIERRE, M.M.J. and BECK, J.A. (1967). Feeding mechanism of **Chiroptonyssus robustipes** on the transilluminated bat wing. **Experimental Parasitology, 20:** 312-320.

LAVOIPIERRE, M.M.J., DICKERSON, G. and GORDON, R.M. (1959). Studies on the methods of feeding blood sucking arthropods. I The manner in which Triatome bugs obtain their blood meal, as observed in the tissues of the living rodent, with some remarks on the effects of the bite on human volunteers. **Annals of Tropical Medical Parasitology, 53:** 235-250.

LEES, A.D. (1946). Chloride regulation and the function of the coxal glands in ticks. **Parasitology, 37:** 172-184.

LESTER, H.M.O. and LLOYD, L. (1928). Notes on the process of digestion in Tse-Tse flies. **Bulletin of Entomological Research, 19:** 39-60.

LEWIS, L.F. and CHRISTENSON, D.M. (1962). Induced build-up of populations of **Bovicola bovis** on cattle in Oregan. **Journal of Economic Entomology, 55:** 947-949.

LEWIS, L.F., CHRISTENSON, D.M. and GAINES, W.E. (1967). Rearing of long-nosed cattle louse and cattle biting louse on host animals in Oregan. **Journal of Economic Entomology, 60:** 755-757.

LIEBISCH, A. (1986). Bayticol pour-on: a new product and a new method for the control of stationary ectoparasites in cattle. **Veterinary Medical Review (Germany), 1:** 17-27.

LJUNGSTROM, I., ENGVALL, E. and RUITENBERG, E.J. (1974). ELISA, enzyme linked immunosorbent assay: a new technique for sero-diagnosis of trichinosis. British Society for Parasitology 69th Proceedings, **Parasitology, 69:** xxiv.

LLOYD, D.H. (1980). The inhabitants of mammalian skin surface. **Proceedings of the Royal Society of Edinburgh, 79B:** 25-42.

LLOYD, D.H., DICK, W.D.B. and McEWAN JENKINSON, D. (1979). Structure of the epidermis in Ayrshire bullocks. **Research in Veterinary Science, 26:** 172-179.

McEWAN JENKINSON, D. (1980). The topography, climate and chemical nature of the mammalian skin surface. **Proceedings of the Royal Society of Edinburgh, 79B:** 3-22.

McKIEL, J.A. (1955). Reactions to mosquito bites. Studies of causation and remedial measures. Ph.D. Thesis, Queen's University, Kingston, Ontario, Canada.

McKIEL, J.A. and WEST, A.S. (1961). Nature and causation of insect bite reactions. **Pediatric Clinics of North America, 8:** 795-816.

MADDER, D.J. and SURGEONER, R. (1979). Effect of permethrin, cypermethrin and chlorpyrifos on louse populations of beef cattle. **Proceedings of the Entomological Society of Ontario, 110:** 29-34.

MATTHYSSE, J.G. (1944). Biology of the cattle biting louse and notes on cattle sucking louse. **Journal of Economic Entomology, 37:** 436-443.

MATTHYSSE, J.G. (1946). Cattle lice: their biology and control. **Cornell University Agricultural Experimental Station Bulletin, 832.**

MAUNDER, J.W. (1984). Studies on the biology and control of human lice. Ph.D. Thesis, University of London.

MEHROTRA, P. and SINGH, T. (1986). Studies on some blood constituents of calves infested with **Linognathus vituli. Indian Journal of Animal Health, June 1986:** 29-32.

MELLANBY, K. (1946). Mans reaction to mosquito bites. **Nature (London), 158:** 554.

MELLANBY, K. (1988). Itching to study lice and mites. **The Scientist, 2:** 16.

METCALF, (1945). The physiology of the salivary glands of **Anopheles quadrimaculatus. Journal of the Malaria Society, 4:** 271-278.

MILLER, J.A., OEHLER, D.D. and KUNZ, S.E. (1983). Release of pyrethroids from insecticidal ear tags. **Journal of Economic Entomology, 76:** 1335-1340.

MUNRO, J.A. and TELFORD, H.S. (1943). Winter control of cattle lice. **North Dakato Agricultural Experimental Station Bulletin, 324:** 1-11.

MURRAY, M.D. (1957a). The distribution of the eggs of mammalian lice on their hosts. II Analysis of the oviposition behaviour of **Damalinia ovis. Australian Journal of Zoology, 5:** 19-29.

MURRAY, M.D. (1957b). The distribution of the eggs of **Damalinia ovis** on the sheep. **Australian Journal of Zoology, 5:** 173-182.

MURRAY, M.D. (1957c). The ecology of the louse **Polyplax serrata** on the mouse **Mus musculus. Australian Journal of Zoology, 9:** 1-13.

MURRAY, M.D. (1986). Arthropods - the pelage of mammals as an environment. Proceedings of the VI International Congress of Parasitology. **Australian Academy of Science, Canberra:** 191-195.

MURRAY, M.D. (1987). Effects of host grooming on louse populations. **Parasitology Today, 3:** 276-278.

MUSTARD, J.F. and PACKHAM, M.A. (1977). Normal and abnormal haematostasis. **Annual Review of Entomology, 32:** 463-478.

NAGHSKI, J. (1971). Leather to meet the needs of the consumer. **Journal of the American Leather Chemists Association, 66:** 408-429.

NAY, T. and HAYMAN, R.H. (1963). Some characters in five breeds of european (**Bos taurus** L.) dairy cattle. **Australian Journal of Agricultural Research, 14:** 294-302.

NELSON, W.A. and BAINBOROUGH, A.R. (1963). Development in sheep of resistance to the ked **Melophagus ovinus.** III Histopathology of sheep skin as a clue to the nature of resistance. **Experimental Parasitology, 13:** 118-127.

NELSON, W.F., BELL, J.F., CLIFFORD, C.M. and KEIRANS, J.E. (1977). Interaction of ectoparasites and their hosts: review article. **Journal of Medical Entomology, 13:** 389-428.

NELSON, W.A., CLIFFORD, C.M., BELL, J.F. and HESTEKIN, B. (1972). **Polyplax serrata:** histopathology of the skin of louse infested mice. **Experimental Parasitology, 31:** 194-202.

NELSON, W.A., KEIRANS, J.E., BELL, J.F. and CLIFFORD, C.M. (1975). Host-ectoparasite relationships. **Journal of Medical Entomology, 12:** 143-166.

NELSON, W.A., SCHEMANCHUK, J.A. and HAUFE, W.O. (1970). **Haematopinus eurysternus:** blood of cattle infested with the short nosed sucking louse. **Experimental Parasitology, 28:** 263-271.

NICKEL, W.E. (1971). The economical importance of cattle lice in Australia: advances in systemic control by pour-on method. **Veterinary Medical Review, 2:** 392-404.

NICKEL, W.E., HYLAND, J.H., GJUREKOVIC, I. and BRONDKE, D. (1970). The problem of cattle lice - systemic control by Fenthion. **New South Wales Veterinary Proceedings:** 38-40.

OORMAZDI, H. (1976). Studies on the biology of bovine **Phthiraptera** and an investigation of the economic importance of pediculosis in Ireland. Ph.D. Thesis, University of Dublin.

OORMAZDI, H. and BAKER, K.P. (1980). Studies on the effects of lice on cattle. **British Veterinary Journal, 136:** 146-153.

PAN, Y.S. (1963). Quantitative and morphological variation of sweat glands, skin thickness and skin shrinkage over body regions of Sahiwal Zebu and Jersey cattle. **Australian Journal of Agricultural Research, 14:** 424-437.

PASSMAN, A. and DALTON, D.C. (1982). Influence of Romney, Coopworth and Perendale breeds on lambskin leather quality. **New Zealand Journal of Experimental Agriculture, 10:** 7-13.

PASSMAN, A. and SUMNER, R.M.W. (1983). Effects of breed and level of feeding on leather production from 18 month old wethers. **New Zealand Journal of Experimental Agriculture, 11:** 47-52.

PASSMAN, A. and SUMNER, R.M.W. (1987). Effects of breed and age at slaughter on leather produced from export lambs reared on hill country. **New Zealand Journal of Experimental Agriculture, 15:** 309-316.

PECK, S.M., WRIGHT, W.H. and GRANT, J.Q. (1943). Cutaneous reactions due to the body louse, **Pediculus humanus. Journal of the American Medical Association, 123:** 821-825.

PETERSON, H.O., ROBERTS, I.H., BECKLUND, W.W. and KEMPER, H.E. (1953). Anaemia in cattle caused by heavy infestations of blood sucking louse **Haematopinus eurysternus. Journal of the American Veterinary Medical Association, 122:** 373-376.

PRICE, R.B., PRIGGE, W.C. and DICKE, R.J. (1949). Clipping as an aid to control of cattle lice. **Journal of Dairy Science, 32:** 724.

RAYMIOND, S. and WEINTRAUB, L. (1959). Acrylamide gel as a supporting medium for gel electrophoresis. **Science, 130:** 711.

REID, M.S. and BIELESKI, R.L. (1968). A simple apparatus for vertical flat sheet polyacrylamide gel electrophoresis. **Analytical Biochemistry, 22:** 374-381.

RIBEIRO, J.M.C. (1987). Role of saliva in blood-feeding by arthropods. **Annual Review of Entomology, 32:** 463-478.

RIBEIRO, J.M.C. and GARCIA, E.S. (1980). The salivary and crop apyrase activity of **Rhodnius prolixus. Journal of Insect Physiology, 26:** 303-307.

RIBEIRO, J.M.C. and GARCIA, E.S. (1981). Platelet anti-aggregating activity in the salivary secretion of the blood sucking bug **Rhodnius prolixus. Experimentia, 37:** 384-385.

RIBEIRO, J.M.C., MAKOWL, G.T., LEVINE, J., ROBINSON, D.R. and SPIELMAN, A. (1985). Antihemostatic, antiinflammatory and immunosuppressive properties of the saliva of a tick, **Ixodes dammini. Journal of Experimental Medicine, 161:** 332-344.

RIBEIRO, J.M.C., ROSSIGNOL, P.A. and SPIELMAN, A. (1985). **Aedes aegypti:** Model for blood finding strategy and prediction of parasite manipulation. **Experimental Parasitology, 60:** 118-132.

RIBEIRO, J.M.C. and SPIELMAN, A. (1986). **Ixodes dammini:** Salivary anaphylaxatoxin inactivating activity. **Experimental Parasitology, 62:** 292-297.

RICH, G.B. (1966). Pour-on systemic insecticides for the protection of calves from **Linognathus vituli. Canadian Journal of Animal Science, 46:** 125-131.

RICHARD, O.W. and DAVIES, R.G. (1977). Imms' General Textbook of Entomology, 10th edition, volume 2, classification and biology. Chapman and Hall, London.

RIEK, R.F. (1954). Studies on allergic dermatitus (Queensland itch) of the horse: the aetiology of the disease. **Australian Journal of Agricultural Research, 5:** 109-129.

RIEK, R.F. (1962). Studies on the reactions of animals to infestations with ticks. VI Resistence of cattle to infestation with the tick **Boophilus microplus** (Canestrini). **Australian Journal of Agricultural Research, 13:** 532-550.

ROBERTS, R.H.S. (1938). Cattle lice: their economic importance in Queensland. **Australian Veterinary Journal, 14:** 55-58.

ROCKSTEIN, M. (1974). In: The physiology of Insecta - 2nd edition. Academic Press, New York and London.

ROCKWELL, E.M. and JOHNSON, P. (1952). The insect bite reaction. II Evaluation of the allergic reaction. **Journal of Investigative Dermatology, 19:** 137-155.

RUITENBERG, E.J. and van KNAPEN, F. (1977). The enzyme linked immunosorbent assay and its application to parasitic infections. **The Journal of Infectious Disease, 136:** S267-S273.

RUITENBERG, E.J., STEERENBERG, P.A., BROSI, B.J.M. and BUYS, J. (1974). Serodiagnosis of **Trichinella spiralis** infections in pigs by enzyme-linked immunosorbent assays. **Bulletin of the World Health Organization, 51:** 108-109.

RUITENBERG, E.J., STEERENBERG, P.A., BROSI, B.J.M. and BUYS, J. (1976). Reliability of the enzyme-linked immunosorbent assay (ELISA) for the serodiagnosis of **Trichinella spiralis** infections in conventionally raised pigs. **Journal of Immunological Methods, 10:** 67-83.

SAITO, Y. and OHARA, S. (1961). Studies on ixodid ticks. Part V Further studies on the reactions of the skin of laboratory animals to the bites of immature ticks. **Acta Medica et Biologica NIIGATA, 9:** 1-32.

SAITO, Y., OHARA, S. and UNAGAMI, T. (1960). Studies on ixodid ticks. Part III Comparative observation on the histological changes of host tissue caused by tick bite. **Acta Medica et Biologica NIIGATA, 7:** 323-329.

SCHALM, O.W. (1961). Veterinary Haematology. Bouliere, Tindall and Cox, London.

SCHALM, O.W., JAIN, N.C. and CARROLL, E.J. (1975). Veterinary Haematology, 3rd edition. Lea and Febiger, Philadelphia. 807pp.

SCHARFF, D.K. (1962). An investigation of the cattle louse problem. **Journal of Economic Entomology, 55:** 684-688.

SCHEELE, G.A. (1975). Two-dimensional gel analysis of soluble proteins. **Journal of Biological Chemistry, 250:** 5375-5385.

SHAPIRO, A.L. and MAIZEL, J.V. (1969). Molecular weight estimation of polypeptides by SDS-polyacrylamide gel electrophoresis: further data concerning resolving power and general considerations. **Analytical Biochemistry, 29:** 505-514.

SHEAHAN, B.J. (1975). Pathology of **Sarcoptes scabiei** infection in pigs. II Histological, histochemical and ultrastructural changes at skin test sites. **Journal of Comparative Pathology, 85:** 97-110.

SHEMANCHUK, J.A., HAUFE, W.O. and THOMPSON, C.O.M. (1960). Anaemia in range cattle heavily infested with the short nosed sucking louse **Haematopinus eurysternus** (Anoplura: Haematopinidae). **Canadian Journal of Comparative Medical Veterinary Science, 24:** 158-161.

SHEMANCHUK, J.A., HAUFE, W.O. and THOMPSON, C.O.M. (1963). Effects of some insecticides on infestations of the short nosed cattle louse. **Canadian Journal of Animal Science, 43:** 56-64.

SHULL, W.E. (1932). Control of the cattle louse **Bovicola bovis** (Mallophaga, Trichondectidae). **Journal of Economic Entomology, 25:** 1208-1211.

SIMPSON, G.G. and BECK, W.S. (1965). Life: an introduction to biology, 2nd edition, Harcourt Brace and World Inc., New York, Chicago, 869pp.

SINCLAIR, I.J., TARRY, D.W. and WASSALL, D.A. (1984). Persistence of antibody in calves after an infection with **Hypoderma bovis. Research in Veterinary Science, 37:** 383-384.

SINCLAIR, I.J. and WASSALL, D.A. (1983). Enzyme-linked immunosorbent assay for the detection of antibodies to **Hypoderma bovis** in cattle. **Research in Veterinary Science, 34:** 251-252.

SMITH, C.L. and RICHARDS, R. (1955). Evaluations of some new insecticides against lice on livestock and poultry. **Journal of Economic Entomology, 48:** 566-568.

SMYTH, J.D. (1962). Introduction to animal parasitology. The English Universities Press Ltd., London, 470pp.

SNIPES, B.T. (1948). Beef cattle freed of lice in one treatment control. **Agricultural Chemicals, 3:** 30-34.

SPENCER, G.J. (1939). Ectoparasites of deer in British Colombia. **Proceedings of the Entomological Society of British Colombia, 35:** 15-19.

SPRENT, J.F.A. (1969). Evolutionary aspects of immunity in zooparasitic infections. In: Immunity to Parasitic Animals, eds. Jackson, G.J., Herman, R. and Singer, I., Appleton Century Crofts, New York, 292pp.

STOHLMAN, F. Jnr. (1961). Humoral regulation of erythropoiesis. VI Mechanism of action of erythropoietine in the irradiated animal. **Proceedings of the Society of Experimental Biology and Medicine, 107:** 751-753.

STRICKLAND, N.C. (1975). A detailed analysis of the effects of various fixatives on animal tissue with particular reference to muscle tissue. **Stain Technology, 50:** 255-264.

SWANK, R.T. and MUNKRES, K.D. (1971). Molecular weight analysis of oligopeptides by electrophoresis in polyacrylamide gel with sodium dodecyl sulphate. **Analytical Biochemistry, 39:** 462-477.

SYKES, R.L., WEBSTER, R.M. and BUGBY, A. (1988). Possible association between warble fly treatment and 'Light spot' damage on bovine hides. **British Leather Confederation Journal, 31:** 152-156.

SYMONS, L.E.A. (1985). Anorexia: occurrence, pathophysiology and possible causes in parasitic infections. **Advanced Parasitology, 24:** 103-133.

TANCOUS, J.J., RODDY, W.T. and O'FLAHERTY, F. (1959). Skin, hide and leather defects. The Western Hills Publishing Company, 3708 Davis Avenue, Cincinatti, Ohio, United States of America.

TANFORD, C. (1968). Protein Denaturation. **Advances in Protein Chemistry, 23:** 121-282.

THEIS, J.H. and BUDWISER, P.D. (1974). **Rhipicephalus sanguineus:** sequential histopathology at the host-parasite interface. **Experimental Parasitology, 36:** 77-105.

THEODOR, O. (1935). A study of the reaction to Phlebotomus bites with some remarks on harana. **Transactions of the Royal Society for Tropical Medicine and Hygiene, 29:** 273-284.

TITCHENER, R.N. (1983). Prevalence of cattle lice on calves. **Veterinary Record, 112:** 460.

TITCHENER, R.N. (1985). The control of lice on domestic livestock. **Veterinary Parasitology, 18:** 281-288.

TWEDDLE, N.E., CUMMINS, L.J. and GRAHAM, J.F. (1977). The prevalence of cattle lice and their effect on liveweight change of beef cattle. **Proceedings of the 54th Annual Conference of the Australian Veterinary Association:** 89-91.

UNDERHILL, B.M. (1923). Some parasites of hogs, sheep, cattle and poultry. **General Bulliten of Pennsylavania Department of Agriculture, 354:** 40.

UTECH, K.B.W., WHARTON, R.H. and WOODERSON, L.A. (1969). Biting cattle-louse infestations related to cattle nutrition. **Australian Veterinary Journal, 45:** 414-416.

VELDKAMP, J. and VISSER, A.M. (1975). Application of the enzyme-linked immunosorbent assay (ELISA) in the serodiagnosis of syphilis. **British Journal of Venereal Diseases, 51:** 227-231.

VOLLER, A., HULDT, D., THORS, C. and ENGVALL, E. (1975). New serological test for malaria antibodies. **British Medical Journal, 1:** 659-661.

WALKER, A.R. and FLETCHER, J.D. (1986). Histological study of the attachment sites of adult **Rhipicelphalus appendiculatus** on rabbits and cattle. **International Journal for Parasitology, 16:** 399-413.

WALKER, A.R., FLETCHER, J.D. and GILL, H.S. (1985). Structural and histochemical changes in the salivary glands of **Rhipicephalus appendicculatus** during feeding. **International Journal for Parasitology, 15:** 81-100.

WEBER, K. and OSBORN, M. (1969). The reliability of molecular weight determinations by dodecyl sulphate - polyacrylamide gel electrophoresis. **Journal of Biological Chemistry, 244:** 4406-4412.

WHITTEN, L.K. (1970). The control of external parasites of cattle, **New Zealand Veterinary Journal, 18:** 146-147.

WIKEL, S.K. (1981). The induction of host resistance to tick infestation with a salivary gland antigen. **American Journal of Tropical Medicine and Hygiene, 30:** 284-288.

WILLADSEN, P. and RIDING, G.A. (1980). On the biological role of a proteolytic enzyme inhibitor from the ectoparasitic tick **Boophilus microplus. Biochemical Journal, 189:** 295-303.

WOOD, J.C. (1970). Parasitic skin diseases of large animals. **Veterinary Record, 87:** 471-473.

YEATES, N.T.M. (1955). Photoperiodicity in cattle. I Seasonal changes in coat character and their importance in heat regulation. **Australian Journal of Agricultural Research, 5:** 6981.

ADDENDUM

FERRIS, G.F. (1951). The sucking lice. **Memoirs of the Pacific Coastal Entomological Society 1:** 1-320.

HYLAND, J. & NICKEL, W.E. (1968). Technical Report. Bayer Leverkusen Ltd.

MURRAY, M.D. (1961). The ecology of the louse **Polyplax serrata** on the mouse **Mus musculus. Australian Journal of Zoology 9:** 1-13.