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TYDSKRIF VAN DIE SUID-AFRIKAANSE VETERINÊRE VERENIGING

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EDITORIAL

CHANGE OF NAME

At the Sixty-sixth Annual General Meeting of the South African Veterinary Medical Association, it was decided that the Association henceforth be known as the SOUTH AFRICAN VETERINARY ASSOCIATION. Use of the archaic and tautological additional adjective "Medical", which was often a source of confusion to the outsider, is discontinued. Accordingly, this Journal will be known as the JOURNAL OF THE SOUTH AFRICAN VETERINARY ASSOCIATION. Format and cover are retained; the numbering of the volumes will be continued uninterruptedly.

THE A.I. INDUSTRY AND THE PRIVATE PRACTITIONER

Considered realistically, it is to be expected that under pressure of day-to-day demands and with the best intentions certain practices may arise within organisations which, when evaluated critically, may perhaps not be strictly in accord with the highest ethical procedures, or which can give rise to malpractices, or create wrong outside impressions. The potential for friction is increased when a polarisation between vested interests becomes more tangible with increase in organisational intensity. In this respect the A.I. industry on the one hand and private practice on the other form no exception. From time to time over the last years complaints have arisen concerning the activities of veterinarians and inseminators in the A.I. Industry.

Well-defined circumscription of the field of work of the various classes of veterinarians and auxiliary personnel does not always follow automatically and is never easy to determine. Yet it is imperative that the ideal of "Unity of Service", as previously oulined [1], be pursued incessantly; particularly such a numerically small profession charged with such onerous national duties may not squander its energies on internal schisms.

The following were the main points of contention:

1. The issuing of certificates by veterinarians or auxiliaries of A.I. Co-operative Societies, which certificates could be interpreted as certifications of health or of fertility.

REDAKSIONEEL

NAAMSVERANDERING

Op die Ses-en-dertigste Algemene Jaarvergadering van die Suid-Afrikaanse Veterinêr-Mediese Vereniging is besluit dat die Vereniging voortaan bekend sal staan as die SUID-AFRIKAANSE VETERINÊRE VERENIGING. Die gebruik van die argaïese, toutologiese en vir die buitestaander verwarrende bykomstige byvoeglike naamwoord „mediese" verval. In ooreenstemming hiermee sal hierdie tydskrif bekend staan as die TYDSKRIF VAN DIE SUID-AFRIKAANSE VETERINÊRE VERENIGING. Formaat en omslag bly onverander; die jaargang-numerering word sonder wysiging voortgesit.

DIE K.I.-BEDRYF EN DIE PRIVATE PRAKTISYN

Realisties gesien, kan 'n mens verwag dat onder druk van daaglikse eise en sonder enige kwade opset, sekere gebruike binne organisasies mag ontstaan wat, krities beskou, miskien nie aan die hoogste etiese standaarde voldoen nie, of wat aanleiding kan gee tot misbruike, of wat wanindrukke na buite kan skep. Die onminpotensiaal word verhoog as daar algaande met hoër bedryfsintensiteit 'n polarisasie tussen belangegroepe intree. In hierdie opsig is die K.I.-bedryf enersyds en die private praktyk andersyds geen uitsondering nie. Reeds jare kom daar van tyd tot tyd klagtes oor die aktiwiteite van veeartse in die K.I.-bedryf en/of die van insemineerders wat in die bedryf se diens staan.

Duidelike omskrywing van die werksvelde van verskillende groepe veeartse en hulppersoneel is nie altyd vanselfsprekend nie en nooit maklik om te bepaal nie. Daarenteen is dit gebiedend noodsaaklik dat die ideaal van „Eenheid van Diens", soos tevore uiteengesit [1], met alle mag nagestrewe word; veral so 'n numeries relatief klein professie met so 'n omvangryke landstaak durf nie sy kragte verspil deur teen homself verdeel te word nie.

Die volgende was die vernaamste wrywingspunte:

1. Die uitreiking van sertifikate deur K.I.-koöperasie-veeartse, of hulle beamptes, wat as gesondheid- en vrugbaarheidsertifikate geïnterpreteer kon word.

2. Herd examinations by veterinarians in the A.I. industry.

3. Pregnancy and even herd examinations by technicians or inseminators in the A.I. industry.

4. Treatment of obviously "dirty" cows by inseminators.

5. Lack of a responsible herd approach by private practitioners.

As is so often the case, misunderstandings and lack of a sense of co-operativeness had aggravated matters.

At the request of the S.A.V.A. Council, a meeting was held at East London on September 13th, 1971, between representatives of Council, of the Reproduction Group, Council Members of the Eastern Cape Branch of the S.A.V.A. and all the veterinary chiefs of A.I. Co-operatives. Proposals arising from this meeting have since been ratified by Council. The ultimate decisions are given and discussed below:

1. *Semen Evaluation Report.*

No objections can be held against a report which merely reflects the results of semen examination after collection and dilution, and before and after freezing of the semen. The important issue lies therein, whether such a report could be accepted and interpreted by the layman as being a report or certification of fertility. Consequently it has been decided to use the following form of certification, which will henceforth be used by all A.I. Co-operative Societies.

SEE CERTIFICATE PAGE 8

The report merely reflects the origin of the semen, the volumes, the quality prior to freezing and the results of freezing. It is addressed *confidentially* to the owner and can never be used or interpreted as a certification of fertility. No rubber stamps may be used for signing the report; technicians may sign on behalf of the Professional Chief of the Co-operative Society.

2. *Which bulls are to be worked by the A.I. Industry.*

Besides their own bulls, certain bulls in private ownership will be worked by A.I.

2. Kudde-ondersoek deur veeartse in diens van die K.I.-bedryf.

3. Dragtigheidsbepalings en selfs kudde-ondersoeke deur tegnici of insemineerders van die K.I.-bedryf.

4. Behandeling van in-die-oog-lopende „vuil" koeie deur insemineerders.

5. Gebrek aan 'n verantwoordelike kuddebenadering deur private veeartse.

Soos so dikwels die geval is, het misverstande en gebrek aan onderlinge begrip sake vererger.

Op versoek van die Raad van die S.A.V.V., is 'n vergadering van verteenwoordigers van die Raad, die Reproduksiegroep, Bestuurslede van die Tak Oos-Kaap van die S.A.V.V. en al die veearts-superintendente van K.I.-koöperasies op 13 September 1971 te Oos-Londen gehou. Voorstelle wat hieruit gevloei het is sedertdien deur die Raad van die S.A.V.V. bekragtig. Die uiteindelike besluite word hier weergegee en bespreek.

1. *Saadevaluasieverslag.*

Daar is geen fout te vind met 'n verslag wat bloot 'n weergawe is van bevindings ten opsigte van die ondersoek van saad na opvang en verdunning, beide voor en na bevriesing daarvan nie. Die belangrikste aspek gaan daarom, of die verslag deur die leek aanvaar kan word as synde 'n vrugbaarheidsverslag of -sertifikaat. Daar is dus besluit op die volgende vorm van sertifisering wat voortaan deur alle K.I.-koöperasies gebruik sal word:

SIEN SERTIFIKAAT BLADSY 8

Die verslag is bloot 'n waargawe van die oorsprong van die saad, die volumes, die kwaliteit voor bevriesing en van die resultaat van bevriesing. Dit word *vertroulik* aan die eienaar gerig en kan nooit na buite aangewend word, of as vrugbaarheidsverslag vertolk word nie. Geen rubberstempels mag ter ondertekening gebruik word nie; tegnici mag namens professionele koöperasiebestuurders teken.

2. *Watter bulle deur die K.I.-bedryf gewerk sal word.*

Benewens hul eie bulle, word in sekere gevalle bulle in private besit deur die K.I.-koöperasies gewerk. Eerstens gaan

Co-operatives. In the first place this will entail working of particular stud bulls of registered breeders, either young bulls, which the owner may wish to sell but of which he wishes to retain semen for his own use (it was in just such cases that problems arose as the result of owners presenting semen freezing reports as certification of fertility), or older bulls of such excellent breeding qualities that their semen was intended to be kept in case of death. Secondly, the A.I. industry will work bulls in which it is interested for its own use.

Final ampulation and freezing of semen can only be done successfully in a laboratory. In many instances private practitioners undertake the collection and dilution of semen and then send it to the laboratory during the equilibration period.

The A.I. industry does not regard it as part of its function or purpose to work bulls destined for sale or for any purpose other than those outlined above.

Herd examination by veterinarians associated with A.I. Co-operatives.

It became clear that no difficulties need arise where veterinarians of A.I. Co-operative Societies examine herds of members of that Society. The A.I. organisations have a duty to their members and it is particularly with herds of new members that difficulties are experienced in obtaining satisfactory results. As a matter of fact, it is mostly with the problem herds that recourse has to be taken to A.I.; it is these herds, especially, that have to be taken in hand by the Co-operatives' veterinarians for the initial six to nine months. If it is considered that there are about 8 000 farmers practising A.I., it becomes clear that the existing five veterinarians can never cope with routine herd examinations.

Although this aspect has only occasionally given rise to friction, there does exist room for improved co-operation between veterinarians in the A.I. industry and private practitioners. It is an elementary ethical requirement that private practitioners should be informed of visits

dit om besondere stamboekbulle van geregistreerde telers, hetsy jong bulle wat die teler moontlik mag verkoop maar van wie hulle saad vir eie gebruik wil uithou (dus juis sulke gevalle wat probleme geskep het omdat die eienaar die bevriesingsverslag as bewys van vrugbaarheid by verkoping aangebied het), of ouerige bulle met voortreflike teelwaarde, waarvan saad gehou wil word as versekering teen sterfte. Tweedens sal die K.I.-bedryf bulle werk waarin hy belang stel vir eie gebruik.

Finale ampulering en bevriesing van saad kan slegs met sukses in 'n laboratorium uitgevoer word. In baie gevalle onderneem private praktisyns die versameling en verdunning van saad, en stuur dit dan na die laboratorium gedurende die ekwilibrasieperiode.

Die K.I.-bedryf beskou dit nie as sy funksie of doel om bulle te werk wat vir verkoop bestem is, of vir enige ander doel as wat hierbo uiteengesit is nie.

3. *Kudde-ondersoek deur koöperasie-veeartse.*

Dit het duidelik geblyk dat daar geen probleme behoort te ontstaan waar K.I.-koöperasie-veeartse kuddes van lede ondersoek nie. Die K.I.-organisasies het 'n plig teenoor hul lede en dit is veral met nuwe lede se kuddes waar dikwels probleme ondervind word om bevredigende resultate te verkry. Trouens, dis meesal die probleemkudde wat op K.I. aangewys word, en dis veral hierdie kuddes wat vir die eerste ses tot nege maande onder die vleuels van die koöperasie se veeartse geneem word. Bowendien, wanneer in aanmerking geneem word dat daar vandag ongeveer 8 000 boere is wat K.I. toepas, word dit duidelik dat die bestaande vyf koöperasie-veeartse nooit as te nimmer die normale roetine-ondersoeke op al hierdie lede se kuddes kan uitvoer nie.

Alhoewel hierdie aspek slegs in enkele gevalle tot wrywing gelei het, bestaan daar beslis ruimte vir beter samewerking tussen K.I.-veeartse en private praktisyns. Dis 'n elementêre etiese vereiste dat laasgenoemde ingelig behoort te word omtrent besoeke deur K.I.-veeartse aan die kuddes van hul kliënte. Vanselfsprekend wil en moet hulle op hoogte bly

to herds of their clients by A.I. veterinarians. More important still, is that closer co-operation between private practitioners and veterinarians in the A.I. industry will not only improve the image of the profession but will undoubtedly serve the best interests of breeders and the livestock industry. On these grounds, a special appeal is made to all parties concerned for greater mutual respect, correct personal relationships and a spirit of whole-hearted co-operation.

4. *Sterility work undertaken by inseminators.*

Instances have been quoted where inseminators have undertaken pregnancy classification and even some sterility work on large herds, either under instruction or with the knowledge of the A.I. management. It is distinctly expressed that sterility work falls outside the normal activities of licensed inseminators. The A.I. industry divorces itself unconditionally from such practices.

On the other hand, note has been taken of the fact that any competent inseminator with some experience can undertake pregnancy classification successfully. The Industry and Council are well aware that inseminators have from time to time undertaken such work on small herds or on a few cows. It was always done *unofficially* and almost always as a special favour for a friend. Everyone present at the East London Meeting understood this problem and no one expressed particular concern about the few cows that may have been examined.

In this respect attention must be paid to certain developments in the dairy industry with regard to A.I. and the official bull testing programmes. This gigantic project will have been launched, or at its inception, by the time this editorial appears in print. In brief, it is proposed that 30 and eventually 50 young bulls will be taken up by the A.I. industry annually. Three to four hundred cows in experimental herds will be inseminated from each bull and the progeny subjected to testing. For the A.I. industry, pregnancy determinations on these cows will be

van werk in sulke kuddes. Nog belangriker is die feit dat nouer samewerking tussen private praktisyns en koöperasieveeartse nie slegs die beeld van die professie na buite sal sterk nie, maar dat dit ongetwyfeld tot heil van die teler en van die veenywerheid sal strek. Om hierdie redes word dus 'n spesiale beroep op al die betrokkenes gedoen vir groter onderlinge respek, korrekte persoonlike verhoudings en 'n gees van hartlike medewerking.

4. *Steriliteitswerk deur insemineerders.*

Gevalle is aangehaal waar insemineerders, òf in opdrag van, òf met die mede-wete van die K.I.-bestuur, dragtigheidsklassifikasies en selfs sekere steriliteitswerk op groot kuddes uitgevoer het. Dit word duidelik gestel dat steriliteitswerk beslis buite die normale aktiwiteite van gelisensieerde insemineerders val. Die K.I.-bedryf distansieer hom sonder voorbehoud hiervan.

Aan die ander kant is kennis geneem dat enige bevoegde insemineerder met ondervinding dragtigheidsklassifikasies met sukses kan onderneem. Die bedryf en die Raad is ook bewus daarvan dat insemineerders van tyd tot tyd sulke werk in klein kuddes of op 'n paar koeie onderneem het. Dit was altyd nie-amptelik en vrywel altyd as 'n spesiale guns vir 'n vriend gedoen. Almal op die Oos-Londense vergadering teenwoordig het hierdie probleem begryp en niemand was juis bekommerd oor die paar koeie wat ondersoek mag word nie.

In hierdie opsig moet kennis geneem word van sekere verwikkelinge in die suiwelbedryf ten opsigte van K.I. en die amptelike bultoetsprogramme. Hierdie grootse projek sal hopelik ten tye van verskyning van hierdie stuk van stapel gestuur wees of op die punt van verwesenliking daarvan staan. Dit kom kortliks daarop neer dat aanvanklik 30 en uiteindelik 50 jong bulle elke jaar in die K.I.-bedryf opgeneem sal word. Drie- tot vierhonderd koeie in proefkuddes sal met saad van elkeen bevrug word en sodoende sal hul nageslag getoets word.

Van die K.I.-bedryf se oogpunt gesien is dit belangrik dat dragtigheidsbepalings op hierdie proefdiere gedoen moet word. 'n getal van 12 000 tot 14 000 per jaar

necessary: a number of 12 000 to 14 000 per annum. The A.I. industry does not have the requisite number of veterinarians available for this work, nor can it afford to employ so many, hence technicians will have to undertake the work. *It will be done solely within the framework of this scheme.* Council has taken notice of these plans.

5. *Treatment of so-called "dirty" cows by inseminators.*

Recognition of venereal infection in cows is part of the inseminator's training and duties. It is not his task to make aetiological diagnoses, nor to institute the requisite therapy. On the other hand, it usually is not practicable to have such a case treated by the private practitioner. For the latter, as a rule, it is an added unnecessary botheration, especially as in most instances it concerns cases of vibriosis that call for treatment during oestrus only. There can be no objection if the inseminator carries out a particular, relatively simple, therapeutic procedure, prescribed by either the private practitioner or by the Co-operative veterinarian. Obviously, the desired procedure would be one whereby the private practitioner undertakes supervision of the herd and calls the A.I. industry to his aid, *inter alia* for the prevention of spread of infectious venereal diseases. The private practitioner must be willing to undertake this task in a responsible manner, rather than to concentrate on "fireman's practice". Of course, the owner should also be conditioned to the herd approach.

With proper insight and understanding of this exposition, but above all with responsible co-operation and correct attitudes, no further difficulties need be encountered. As a matter of fact, the whole occurrence points to a moral and holds an object lesson for all members of the profession. If problems are to be solved, then all those concerned should spring to action, using Council merely as initiator and final authority. They should not try to leave the baby in Council's lap and then conveniently sit back in hope that Council will clear matters up.

1. Editorial 1971 *Jl S. Afr. vet. med. Ass.* 42 : 205

Aangesien die K.I.-bedryf nie oor die getal veeartse beskik om hierdie werk te doen nie en dit ook nie kan bekostig om die werk deur veeartse te laat doen nie, sal tegnici vir hierdie werk gebruik moet word. *Dit sal egter bloot binne die bestek van hierdie skema geskied.* Die Raad is reeds in kennis gestel van die beoogde planne.

5. *Behandeling van die sogenaamde „vuil" koeie deur insemineerders.*

Dit is deel van die insemineerder se opleiding en plig om die veneries besmette koei te herken. Dis egter nie sy taak om 'n etiologiese diagnose te maak of om ooreenstemmende behandeling toe te pas nie. Aan die ander kant is dit meesal nie lonend vir die eienaar om so 'n geval deur 'n privaat praktisyn te laat behandel nie. Vir laasgenoemde is dit dikwels 'n onnodige rompslomp, veral aangesien dit dikwels om gevalle van vibriose gaan, wat behandeling net gedurende bronstigheid verg. Daar kan dus geen fout gevind word as die insemineerder in opdrag van die veearts (praktisyn of koöperasie-veearts) 'n bepaalde, betreklik eenvoudige behandeling uitvoer nie. Dit is duidelik dat die gewenste toestand een is waar die private praktisyn kudde-toesig hou en die K.I.-bedryf op regmatige wyse inspan, onder andere vir voorkoming van verspreiding van aansteeklike geslagsiektes. Die private praktisyn moet gewillig wees om hierdie taak op verantwoordelike wyse op hom te neem, eerder as hom blind te staar op „brandslaan"-praktyke. Natuurlik moet die vee-eienaar ook op kuddebenadering ingestel word.

Met behoorlike insig en begrip van hierdie uiteensetting, maar bowe-al met verantwoordelike medewerking en goeie gesindheid, behoort op hierdie gebied geen probleme meer ondervind te word nie. Trouens, die hele gebeure hou 'n goeie en navolgenswaardige les vir alle lede van die beroep in. As probleme uit die weg geruim moet word, dan behoort al die betrokke instansies self in te spring. Hulle behoort die Raad as inisieerder en finale bekragtiger te gebruik, maar moet nie probeer om die probleem op die Raad se skouers af te laai en dan maar terugsit, gerieflikerwyse, en hoop en vertrou dat die Raad sal sien kom klaar nie.

1. Redaksioneel 1971 *Tydskr. S.Afr. vet. med. Ver.* 42 : 205

SEMEN FREEZING REPORT

BEVRIESINGSVERSLAG

VERTROULIK CONFIDENTIAL

EIENAAR: ..... OWNER

NAAM VAN BUL: ..... NAME OF BULL

RAS: ..... BREED

BEVRIESINGSDATUM: ..... DATE OF FREEZING

HOEVEELHEID BEVRIES: QUANTITY FROZEN

(dosisse) ..... (doses)

EVALUASIE VOOR BEVRIESING:
EVALUATION PRIOR TO FREEZING:

Swak: ..... Poor

Goed: ..... Good

Uitstekend: ..... Excellent

EVALUASIE DIREK NA BEVRIESING:
EVALUATION DIRECTLY AFTER FREEZING:

Uitstekend: ..... Excellent

Goed: ..... Good

Swak: ..... Poor

OPMERKINGS: ..... REMARKS

.....

.....

.....

Geteken deur of namens Hoof, K.I. Koöp.
Signed by or on behalf of Chief, A.I. Co-op.

Jl S. Afr. vet. Ass. 43 (1): 9—12 1972

REFRESHER COURSES IN PHARMACOLOGY

6. MECHANISMS OF DRUG ACTION

W. L. JENKINS

INTRODUCTION

In the previous articles of this series the more important aspects of drug absorption, distribution, biotransformation and excretion were reviewed. These factors all play vital and fundamental rôles in establishing and maintaining an effective drug concentration in the biophase or in the environment in which a drug will produce its characteristic action. However imperative it is to be aware of these concepts when administering drugs, the primary aim is to elicit a well-defined reaction or to have a particular effect which is made use of in the therapeutic management of a diseased state. These drug actions are of prime concern to the attending clinician. Thus it is also essential to understand the basic concepts of drug action and the rationale of the principles involved in order to appreciate the responses encountered. The mechanisms of drug action, therefore, will be discussed briefly, the major emphasis being placed on drug receptors and their interactions.

BASIC MECHANISMS OF DRUG ACTION

The ultimate mechanism by which a drug exerts its effect on a living organism must involve the consequences of physicochemical interactions between the drug and functionally important molecules within such a biological system. Although the precise modes of action have not been described for many drugs, a number of basic mechanisms have been recognized. Examples of these will be presented primarily to illustrate the magnitude of the problem of exact classification of the molecular mechanisms of drug action.

Effects directly due to physical or chemical properties of the drug

1. Physical action, e.g. protective and absorptive powders, lubricant laxatives and emollients.

2. Refrigerant action, e.g. the use of ethyl chloride as a local anaesthetic.

3. Chemical union, e.g. antacids and some chemical antidotes.

4. Physicochemical action, e.g. the effect of the volatile anaesthetics on brain tissue and the possible stabilization of water clathrates in the neurones by these agents.

5. Acidic or basic properties, e.g. urine acidifiers and alkalizers.

6. Osmotic effects, e.g. saline purgatives, osmotic diuretics and plasma substitutes.

7. Oxidation, e.g. oxidizing agents used as disinfectants and antiseptics.

8. Detergent action, e.g. certain disinfectants and antiseptics.

Biochemical and biophysical mechanisms of drug action

1. Direct enzyme inhibition, e.g. the acetylcholinesterase inhibitors, the inhibition of aconitase by fluorocitrate and the inhibition of parts of the cytochrome oxidase system by cyanide.

2. Analogue inhibition of metabolic pathways (antimetabolites), e g. the sulphonamides and antineoplastic agents such as methotrexate, 5-fluorouracil and 6-mercaptopurine.

3. Uncoupling of oxidative phosporylation, e.g. thyroxine and 2,4-dinitrophenol.

4. Alteration of cell membrane structure and function, e.g. the local anaesthetics and polyene antifungal antibiotics.

5. Alteration of membrane and intracellular transport systems, e.g. insulin, the cardiac glycosides and tetrodotoxin.

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6. Chelation (in which an organic grouping binds with another substance which is usually a metallic ion), e.g. EDTA and calcium, penicillamine and copper, and BAL and arsenic.
7. Displacement of endogenous physiologically active substances from protein-bound sites by drugs, e.g. thyroxine, oestradiol and hydrocortisone may be displaced.

8. Release of endogenous physiologically active substances from storage granules, e.g. amphetamine and ephedrine release noradrenaline, and the sulphonylureas release insulin.

9. Interference with the synthesis and release of endogenous physiologically active substances, e.g. botulinus toxin prevents the release of acetylcholine and bretylium prevents the release of noradrenaline.

10. Depletion of mediators in nerve terminals, e.g. reserpine depletes entire catecholamine depots of adrenergic nerves.

11. Interference with the inactivation of endogenous physiologically active substances, e.g. monoamine oxidase inhibitors.

In some of the instances noted above a drug receptor may be involved. Nevertheless, in these cases the mechanisms of action do fit into the classification as presented.

Receptors and effects mediated by drug-receptor interaction

Many drugs are effective in extremely low concentrations: as the drug does not supply the energy necessary for the effect, it is believed that such drugs exert their action on cells only when combined with sites within the cells or on cell membranes. These sites have been called receptors. The characteristic action of a specific drug depends on its combination with specific receptors. These drug-receptor interactions are usually reversible and appear to be governed by the Law of Mass Action.

The binding forces which play a rôle in drug-receptor combinations include ionic bonds, dipole-induced dipole bonds, hydrogen bonds, van der Waal's forces and covalent bonds.

Drugs that are capable of reacting with specific receptors and which then produce a characteristic response are said to possess both *affinity* and *efficacy* (or *intrinsic activity)* and are termed *"agonists"*. Nevertheless, certain drugs are capable of combining with the same receptors without producing a response, i.e. they possess affinity but lack efficacy. These compounds are termed *"antagonists"* and may be of two types. Firstly, a *"competitive antagonist"* combines reversibly with the same receptor site as the agonist; the blockade can be overcome by increasing the concentration of the agonist. Secondly, a "non-competitive antagonist" inactivates the receptor so that an effective complex with the agonist cannot be formed regardless of the concentration of the agonist. Finally, it is possible for some drugs to interact with the same receptors as an agonist so that only a limited or less than maximal response occurs. These compounds thus possess affinity and an intermediate efficacy and are termed *"partial agonists"*.

In addition to the above, there are also a number of drug interactions at the molecular level which do not initiate a pharmacodynamic effect. These include the binding of drugs to macromolecules such as the plasma proteins, cellular protein fractions, enzymes and transport systems. These sites are known as drug acceptors, sites of loss, or storage sites.

A lack of tissue response to a drug may be due to the presence of *"silent receptors"*. These are receptors to which an agonist may become attached but which are not capable of producing a pharmacological response although the intrinsic activity of the agonist is in order. The presence of *"spare receptors"* may also lead to agonist-receptor interaction without an effect being observed. These spare receptors arise in the situation where only a certain percentage of receptors needs to be occupied in order to produce a maximal response.

The concentration of a drug in the biophase (which is directly depedent on the dose administered) plays a vital rôle in the reaction of the responsive tissues. There are two well-described and fundamental types of dose-effect relationships, namely:

(a) A graded response in which the tissue is capable of a progressively greater response with increasing amounts of the drug. The plotted graph of a graded response is usually a hyperbolic curve.

(b) A quantal or all-or-none response in which the responsive tissue reacts maximally. Increasing amounts of the drug will

then activate more units giving a maximum response in each case. The plotted graph of a quantal response is usually a sigmoid curve.

Attempts have been made to explain the shapes of the dose-effect curves by application of the receptor theory. The classical concept was proposed by Clark in 1937 and is known as the "simple occupation theory". In this instance it was assumed that the drug effect is proportional to the fraction of receptors occupied and that the maximum effect results when all the receptors are occupied. Deficiencies were soon exposed in the simple occupation theory and Ariëns in 1954 presented a "composite occupation theory" which was supplemented by Stephenson in 1956. Ariëns proposed and defined the two unrelated attributes of a drug, namely, affinity and intrinsic activity, which are discussed above. Stephenson suggested that a maximal effect can be produced by the occupation of only a small proportion of available receptors (thus there will be "spare receptors") and that the response is not always linearly proportional to the number of receptors occupied. Furthermore, he proposed that different drugs have different capacities to initiate a response and hence that they occupy different proportions of the receptors when producing equal responses. This property Stephenson called "efficacy" and the concept resembles that of Ariëns' "intrinsic activity" rather closely.

A hypothesis of drug action different from those described above was proposed by Paton in 1961. This concept, known as the "rate theory", suggests that the effect produced by an agonist is proportional to the rate of combination of a drug with its receptor. It also relates drug efficacy to the rate of dissociation of the drug-receptor complex.

There is as yet no finality as regards the above hypotheses and each theory appears to apply in certain instances.

There is always an intimate relationship between the action of a drug and its chemical structure. A series of chemically related drugs known as a congeneric series, in which substituents are added or removed at various positions and in different steric configurations, often produce a spectrum of biological potency or activity. These relationships are known as structure-activity relationships (SAR) and they represent a very useful tool with which to draw inferences regarding the structure and function of receptors. The SAR of a series of drugs are often quite stringent and relatively minor alterations of the drug molecule may result in considerable modification of the pharmacological effects.

It is important to note that a drug may act on more than just one receptor system. Furthermore, well-defined responses produced by a particular substance may be due to interaction with different receptors whose combined actions produce the gross effects observed. It is sometimes possible to separate these component receptors by using suitable agonists. Two excellent examples of this concept are the "muscarinic" and "nicotinic" receptors of the parasympathetic nervous system, and the alpha- and beta-receptors of the sympathetic nervous system.

No receptor site has yet been isolated or visualized and the receptor concept really represents a convenient way of explaining many facets of drug action. In recent years, with the elucidation of the mechanisms of action of certain hormones, some progress has been made towards the identification of receptor mechanisms. There is today a great deal of evidence that a number of hormone-mediated reactions are dependent on the availability of the cyclized form of adenosine monophosphate (cyclic AMP). This cyclic AMP is formed from ATP under the influence of the enzyme, adenyl cyclase. A phosphodiesterase is responsible for the breakdown of cyclic AMP. Thus, when adenyl cyclase is stimulated or the phosphodiesterase inhibited, an increase in the formation of cyclic AMP results. This leads to an increase in the reactions which are dependent on cyclic AMP.

An example of the above is encountered in the action of adrenaline. Adrenaline stimulates adenyl cyclase and the cyclic AMP produced stimulates the activity of a kinase which converts an inactive phosphorylase to an active form known as phosphorylase a. The active phosphorylase catalyses the glucogenolysis reaction. Furthermore, the stimulation of adenyl cyclase appears to be the major mechanism whereby adrenergic stimuli affect lipolysis. It would appear, therefore, that the receptor responsible for these beta-adrenergic effects is either adenyl cyclase itself or is a receptor in close association with this enzyme. On the other hand, there is also much experi-

mental evidence that there are discrete receptors for each group of agonists in tissues where cyclic AMP mediates the response to several types of hormones and drugs. These receptors may possibly converge on a single adenyl cyclase.

In conclusion then, one may simply state the obvious: although many administered drugs probably act on "receptors" to bring about their desired effect, there remains a tremendous paucity of knowledge regarding the precise modes of action of such compounds at the molecular level.

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CIBA-GEIGY (PTY.) LIMITED

NEW R1-MILLION COMPLEX OPENED BY CIBA-GEIGY

The Ciba-Geigy group has world-wide sales of more than R1 000-million, operates in approximately 45 countries and employs about 70 000 people. It invested no less than R90-million in research and development last year. Its activities cover dyestuffs, textile auxiliaries, pharmaceuticals, augricultural chemicals, plastics and additives, pigments, photographic materials and consumer products..

For the past 25 years Ciba and Geigy have been serving the medical and pharmaceutical professions in South Africa as separate companies. The first South African branch of Ciba started operating in Johannesburg in January 1958. It commenced marketing pharmaceuticals and later added dyes and agricultural chemicals to its activities.

Geigy South Africa started marketing dyes, insecticides and industrial chemicals in 1946. In 1949 a company—Pharmakers (Pty.) Ltd.—was formed in order to distribute, amongst others, Geigy's pharmaceutical products and established its headquarters in Cape Town. In December, 1962, J. R. Geigy S.A., Switzerland, acquired Pharmakers which became a subsidiary of J. R. Geigy, Basle. The company transferred its registered office to Johannesburg in September, 1963. In January, 1969, Pharmakers amalgamated with Geigy South Africa and from that date all Geigy pharmaceuticals were marketed through Geigy South Africa.

As a result of the merger of the parent companies in Switzerland in October, 1970, a joint company, Ciba-Geigy (Pty.) Ltd., has operated in South Africa from January 1 last year. The merger has resulted in considerable rationalisation and better usage of plant and facilities.

A new R1-million office and factory complex for Ciba-Geigy South Africa was opened at Spartan, Johannesburg by the Hon. Dr. Nico Diederichs, Minister of Finance, on November 18, 1971. The Swiss Chargé d'Affaires, Mr. F. Rochat, and top Ciba-Geigy executives from Basle, Switzerland, Dr. Samuel Koechlin, and Dr. Otto Niederhauser, Chairman and member of the Management Committee respectively, and Mr. H. V. Muri, regional manager for Southern Africa, were also present.

The new Spartan complex consists of a three-storey office block, containing 3 500 sq. metres of office space, a modern laboratory building, and two factory blocks with packaging, warehousing and production facilities.

The pharmaceuticals and agricultural chemicals divisions are now based at Spartan. The dyestuffs, textile auxiliaries, pigments and additives divisions are at Isando. Administration, finance, personnel and training are at head office, Spartan.

Forming the board of directors of Ciba-Geigy South Africa are Mr. J. G. Dekker (chairman), Mr. D. C. Bodley, Mr. E. R. de Fries, Dr. J. Waldvogel, Mr. J. J. van Heerden (alternate) and Mr. B. S. Greyling. According to Mr. E. R. de Fries, general manager of Ciba-Geigy South Africa, the group now has assets of more than R10-million and a turnover in excess of R12-million a year. "We are considering investing at least a further R2-million over the next four years on a new pharmaceutical compounding and packaging plant and the chemical production of certain active ingredients. There is adequate space for this planned expansion on our 5,45 hectare site at Spartan or at the group's Isando site."

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THE HISTOLOGY OF THE PLACENTOME OF THE EWE BEFORE AND DURING PARTURITION

L. C. VAN WYK*, C. H. VAN NIEKERK** AND P. C. BELONJE**

SUMMARY

A histological study of the placentome of the ewe before and during parturition revealed that shortly before parturition the connective tissue of the proximal areas of the maternal villi and adjacent caruncular tissue becomes hyalinized. This results in narrowing of the arteries and compression of the veins in this area leading to a disturbance in the blood flow in the maternal villi. This may account for the anoxic stress found in the foetus at the end of gestation.

INTRODUCTION

As early as 1891, Spiegelberg[1] suggested that parturition was stimulated by a substance produced in the foetus due to nutritional deficiencies at the end of gestation.

This view has gained support in the last decade: in 1962 Mitchell[2] demonstrated an ever-decreasing oxygen tension in the umbilical vein towards the end of pregnancy, leading to a definite foetal anoxic stress immediately prior to parturition. Furthermore, the foetal adrenal cortex has been implicated in the termination of gestation[3, 4, 5, 6] and, in fact, the foetal plasma cortisol has been shown to rise dramatically near full term. In addition, it has been shown that injections of synthetic cortisone about 10 days before the end of gestation results in abortion within 24 hours[7]. It seems that an anoxic stress condition results in an increased formation of foetal cortisol which in turn may initiate parturition.

This report deals with an investigation into the histological changes in the placentome*** which may be responsible for the foetal anoxia.

MATERIALS AND METHODS

Three primiparous pregnant ewes were used. The first was slaughtered about 20 days prior to calculated date of parturition, the second three days prior to and the third at the beginning of parturition.

Immediately after the animals were killed, the uteri were dissected free, opened and an area in the pregnant horn in the vicinity of the bifurcation of the uterus was fixed in Bouin's solution. After fixation, individual placentomes were prepared for histological sections and then stained with haematoxylin-eosin (HE), Mallory-azan and periodic acid Schiff (PAS).

RESULTS

The placentome 20 days before parturition (Plate 1.) (For general orientation see schematic drawing, Plate 3, Fig. 1).

The base of the maternal area of the placentome consists of a layer of connective tissue (Figs. 3 and 4). From this area the maternal villi extend to the base of the foetal allantochorionic villi. The maternal villi consist of blood vessels in a bed of reticular connective tissue and are covered by elongated, flat epithelial cells (Figs. 1 and 2). On the epithelium there are clusters of multinuclear symplasma cells which have been described as conglomerates of maternal epithelial cells[8] but morphologically they appear more like the trophoblast cells of the chorion as described by Assheton[9] and Wimsatt[10]. Large blood vessels are present in the maternal base area. From these vessels, arterial branches extend into the maternal villi, divide into capillaries, which then drain into the veins of the villi which return the blood

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**Dept. of Animal Physiology, University of Stellenbosch.
***The placentome is considered as the unit of the multiplex ruminant placenta and consists of the maternal aruncle and the foetal cotyledon.

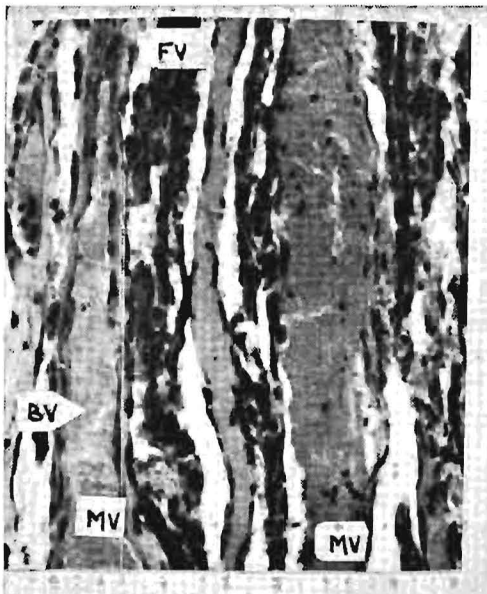

Fig. 1

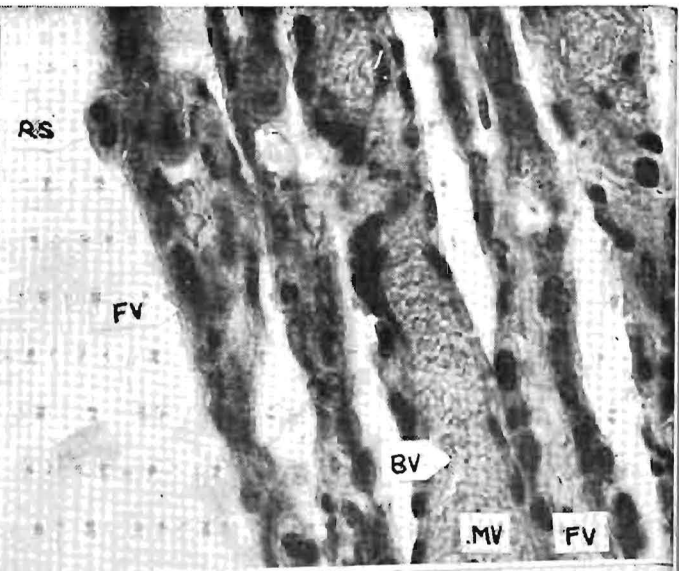

Fig. 2

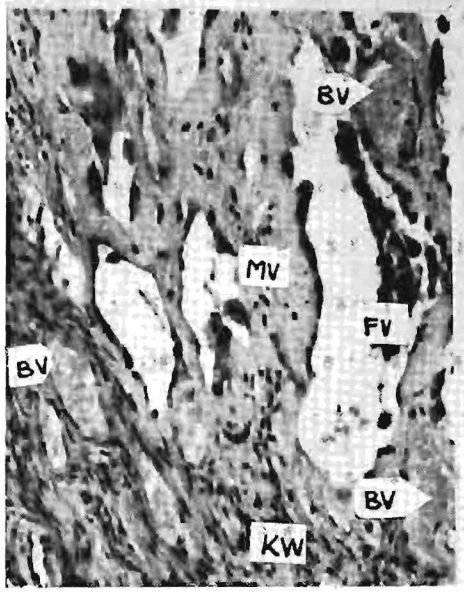

Fig. 3

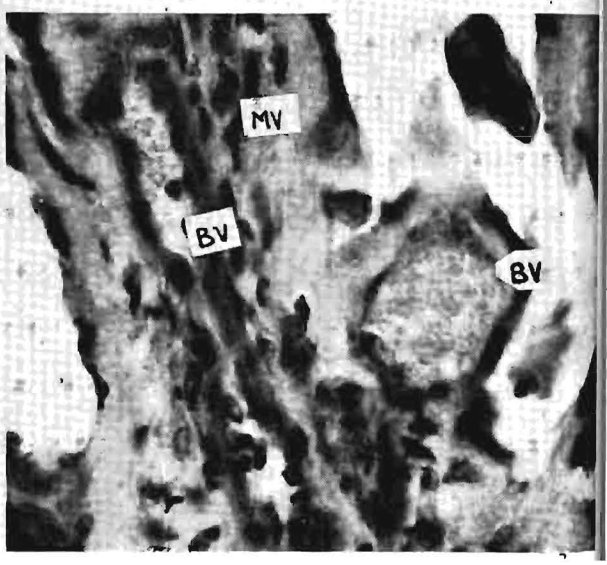

Fig. 4

PLATE 1

Placentome of a pregnant ewe about 20 days before parturition.

Fig. 1: Maternal and foetal villi just distal to the caruncular base.
Fig. 2: Enlargement of Fig. 1 showing prominent maternal villi with large blood vessels.
Fig. 3: The proximal area of the maternal villi (caruncular base) with adjacent caruncular connective tissue and prominent blood vessels.
Fig. 4: Enlargement of Fig. 3 showing the most proximal part of the maternal villi.

KW	=	caruncular connective tissue	BV	=	blood vessels
MV	=	maternal villus	RS	=	giant cells
FV	=	foetal villus			

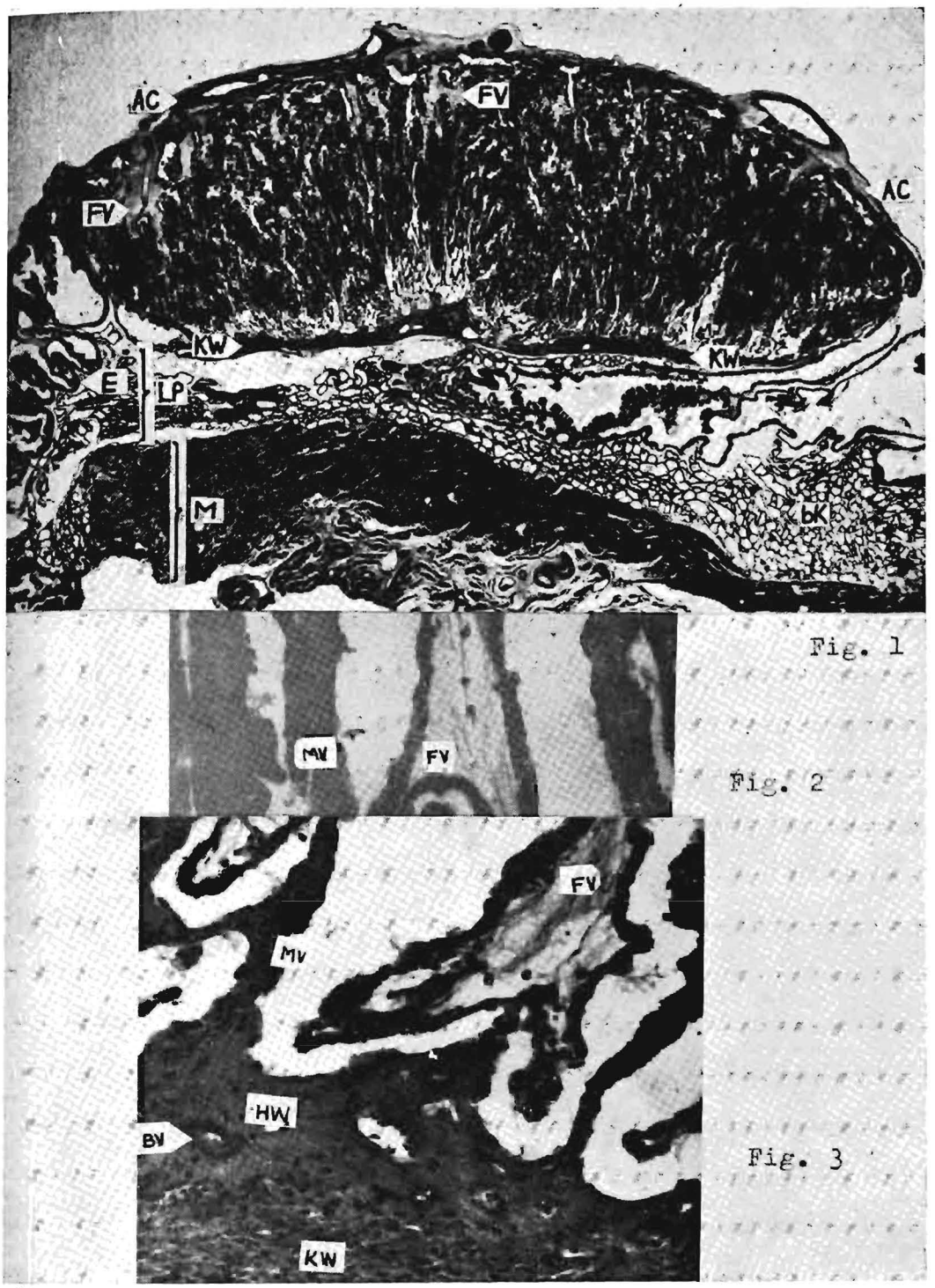

PLATE 2

Placentome of a pregnant ewe about 3 days before parturition.

Fig. 1: Cross section of placentome with intact foetal membranes.
Fig. 2: Maternal and foetal villi in the area just distal to the caruncular base.
Fig. 3: The proximal area of the maternal villi at the caruncular base, with adjacent caruncular tissue.

KW	=	caruncular connective tissue	AC	=	allantochorion
MV	=	maternal villus	LP	=	lamina propria
FV	=	foetal villus	M	=	myometrium
BV	=	blood vessels	bK	=	uterine glands
HW	=	hyalin tissue	E	=	uterine epithelium

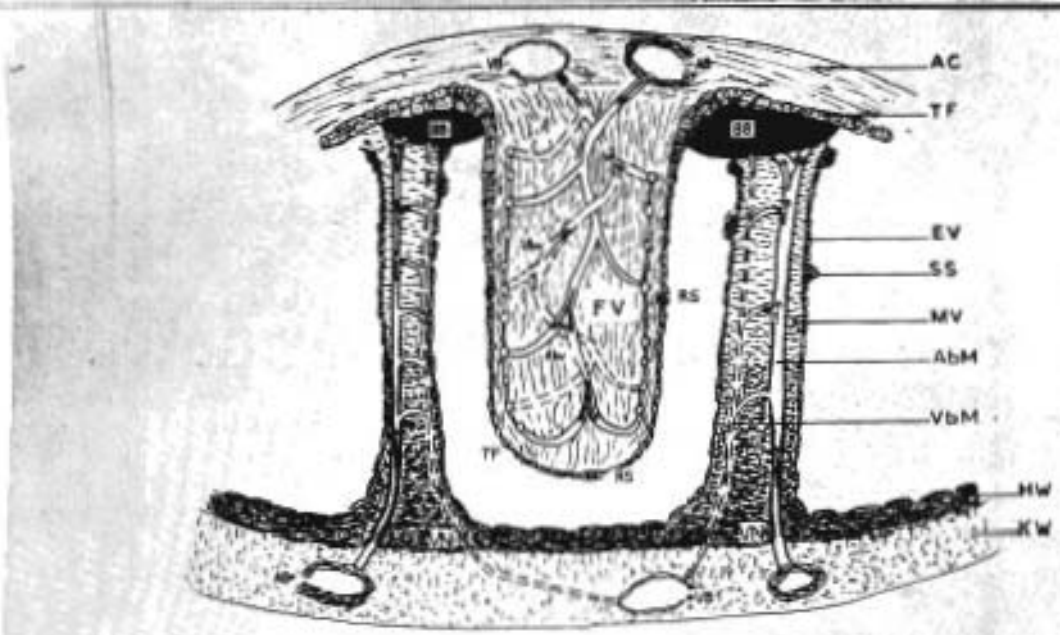

Fig. 1

Fig. 2 Fig. 3

PLATE 3

Placentome of a pregnant ewe at parturition.

Fig. 1: Schematic diagram of a sector of the placentome.
Fig. 2: Cross section of placentome showing prominent blood baths.
Fig. 3: The proximal area of the maternal villi with adjacent caruncular tissue. Stained Mallory-azan.

KW	=	caruncular connective tissue	SS	=	symplasma cells
MV	=	maternal villus	RS	=	giant cells
FV	=	foetal villus	AbM, AB+	=	maternal arterial vessels
BV	=	blood vessels	VbM, +VB	=	maternal venous vessels
HW	=	hyalin tissue	EV	=	epithelium of maternal villus
AC	=	allantochorion	Abv, −AB	=	foetal arterial vessels
BB	=	blood baths	Vbv, VB−	=	foetal venous vessels
TF	=	trophoblast cells			

to the large veins in the maternal base area.

On the other hand, the foetal villi originate from the allantochorionic base, interdigitate with the maternal villi and extend as far as the base of the maternal area. These foetal villi contain blood vessels in embryonal reticular connective tissue and are covered by an epithelium of trophoblast cells in which a large number of binucleate giant cells (RS, fig. 2) can be seen.

The placentome 3 days before parturition (Plate 2).

Certain striking changes have taken place. Both the basal part of the maternal villi as well as a thin area of adjacent maternal base connective tissue show signs of hyalinization (Fig. 3, HW). This is seen in HE sections as a loss of the normal connective tissue structure and the replacement thereof by a structureless, homogenous, acidophilic tissue with few nuclei. Furthermore, this tissue is strongly PAS positive, suggesting the active deposition of mucopolysaccharides. This zone stains dark blue with Mallory-azan compared with the light blue colour of normal connective tissue.

As a result of this hyalinization, the veins in the maternal base area appear compressed. The lumina of the arteries are also smaller as a result of hyalinization of their walls. Moreover, the portions of the maternal villi adjacent to the base area are thinner and stain darker. This is in marked contrast to the more distal parts maternal villi, which are broad as a result of vascular congestion.

On the other hand, the foetal villi show no signs of hyalinization, but their more distal areas appear to have spread out in the area where the maternal villi have contracted (Figs. 2 and 3).

The placentome during parturition (Plate 3)

The hyalinization of the maternal base and adjacent villi is more extensive. On the othed hand the PAS reaction is far less marked, while the Mallory-azan is even darker blue, probably as a result of ageing of the hyalin tissue. The lumina of the blood vessels in this area are even smaller than before, while the distal blood vessels of the maternal villi are markedly distended: extensive blood baths can be seen immediately adjacent to the foetal chorionic base area (Fig. 2).

DISCUSSION

It appears from these findings that shortly before parturition the connective tissue of the proximal portions of the maternal villi and an adjacent area of the caruncle undergoes hyalin degeneration. As a result of this degeneration the arterial walls become thickened and the thin-walled veins become compressed. As a result of these changes, vascular congestion occurs in the blood vessels in the distal parts of the maternal villi as well as the formation of large blood baths just under the allantochorion as described by Wimsatt [10]. No changes were noticed in the foetal villi.

We suggest that these vascular changes in the maternal tissue shortly before parturition lead to the anoxic condition of the foetus as described by Mitchell [2].

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INVOLUTION OF THE *POST PARTUM* UTERUS OF THE EWE

L. C. VAN WYK*, C. H. VAN NIEKERK** AND P. C. BELONJE**

SUMMARY

The involutionary changes in the caruncles of ewes have been studied histologically from a few hours after parturition to 28 days *post partum*. The foetal villi are withdrawn with the placenta. During the first 12 days *post partum* the distal two thirds of the maternal villi liquify in two stages. The remaining hyalinized caruncular tissue detaches as plaques between 16 and 20 days *post partum* to leave an area of naked caruncular connective tissue. The uterine epithelium grows over this connective tissue to complete involution by 28 days *post partum*.

INTRODUCTION

The macroscopic changes during the involution of the *post partum* uterus of the ewe have been described by several authors [1, 2, 3, 4, 5]. The only comprehensive histological study found in the literature was that by Uren in 1935 [6]. As it may be logically assumed that this process of involution has to run to completion before pre-implantation changes can occur, it may well be one of the most important limiting factors in an intensive breeding programme. Hence it was considered necessary to investigate the changes more fully.

MATERIALS AND METHODS

Eleven four- to six-tooth primiparous ewes were slaughtered one each at the following times *post partum:* three hours, 12 hours, 24 hours, 48 hours, four days and then every four days up to and including the 28th day.

Immediately after the animals were killed, the uteri were removed, opened and an area of the pregnant horn in the region of the bifurcation of the uterus was fixed in Bouin's solution. Subsequently individual placentomes were sectioned and stained with haematoxylin-eosin (HE), Mallory-azan and periodic acid Schiff (PAS).

RESULTS

For the purposes of the description which follows, the villous area of the caruncle has been divided into three zones. Area A is the most distal portion, area B the middle third and area C the proximal third under which is found a band of hyalinized connective tissue (plate 2, fig. 1).

1. *Three hours post partum* (Plate 2).

This ewe was killed just after the expulsion of the after-birth.

The foetal villi have been withdrawn together with the foetal membranes and the parallel spaces which they had occupied are easily discernible (Figs. 1, 2 and 3). Nevertheless, a large amount of foetal cells such as trophoblast cells, binucleate giant cells and symplasma cells remain in these spaces (Fig. 3).

The blood baths in area A have ruptured and free red cells are found on the surface of the caruncle (Fig. 1, A). The blood vessels in area B are distended with blood (Fig. 1, B).

In area C (Fig. 1, C) the connective tissue of maternal villi is hyalinized and the blood vessels are constricted. At the base of the villi, there is a distinct band of hyalinized caruncular connective tissue (Figs. 1 and 3, hw) in which the blood vessels are also markedly constricted.

2. *Twelve hours post partum*

The maternal villi begin to collapse; the parallel arrangement, particularly in area A, is no longer discernible and degenerative changes such as pyknosis are seen. The blood vessels in area B are still prominent and distended. Many of the remaining trophoblast cells have coalesced and appear similar to symplasma cells. The zone of hyalinization is prominent.

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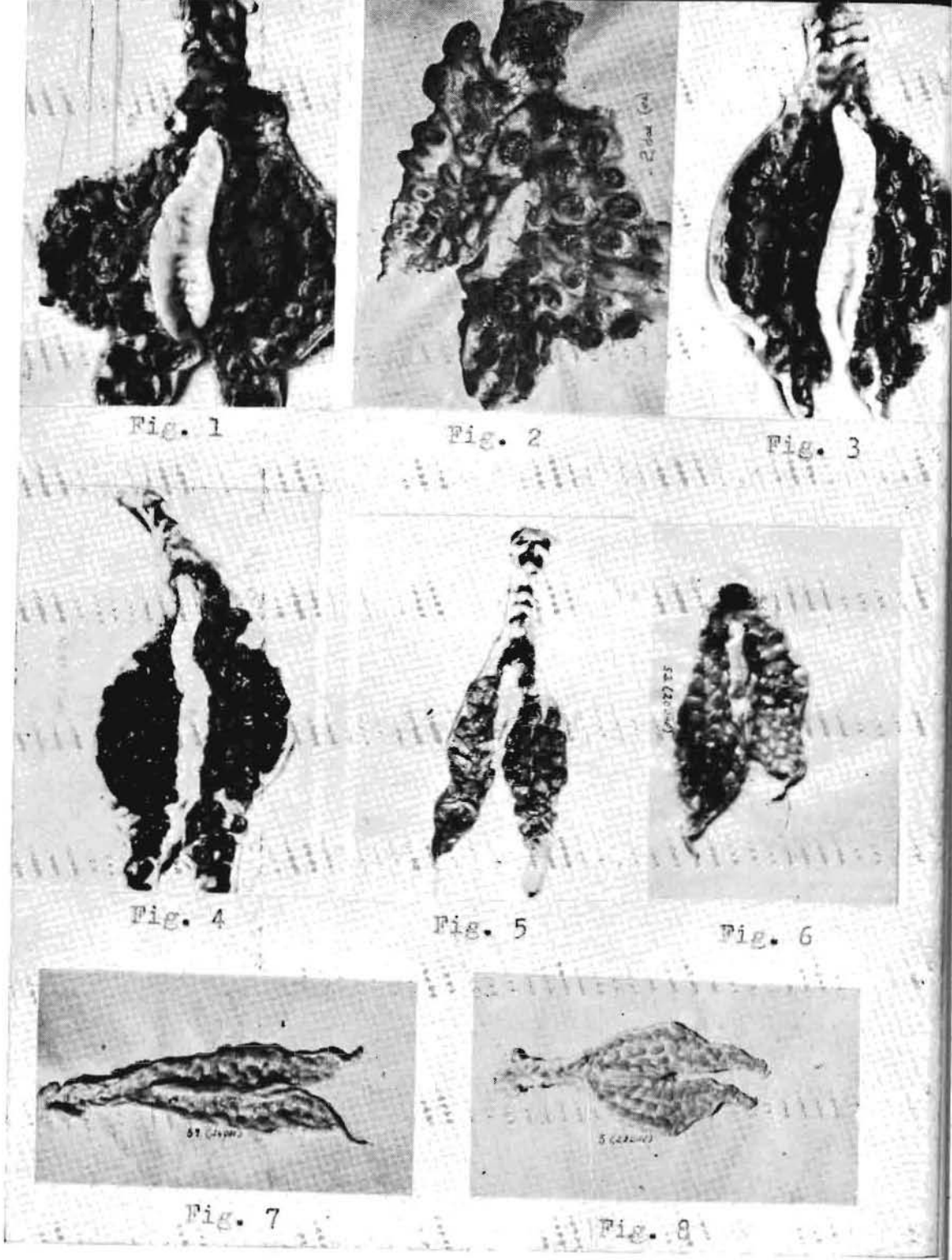

PLATE 1

The opened **post partum** uterus of the ewe.

Fig. 1: 24 hours **post partum**
Fig. 2: 48 hours **post partum**
Fig. 3: 4 days **post partum**
Fig. 4: 8 days **post partum**
Fig. 5: 16 days **post partum**
Fig. 6: 20 days **post partum**
Fig. 7: 24 days **post partum**
Fig. 8: 28 days **post partum**

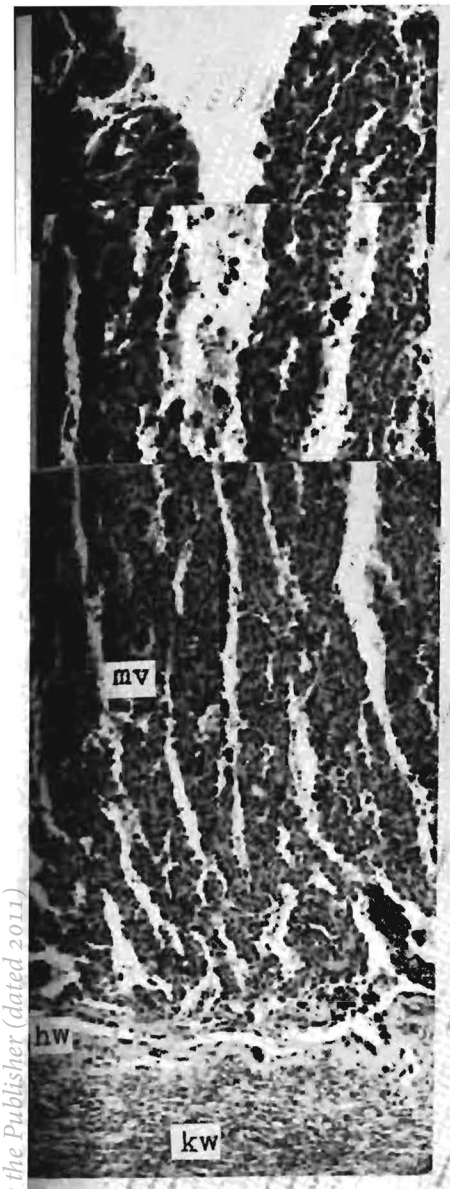

Fig. 1

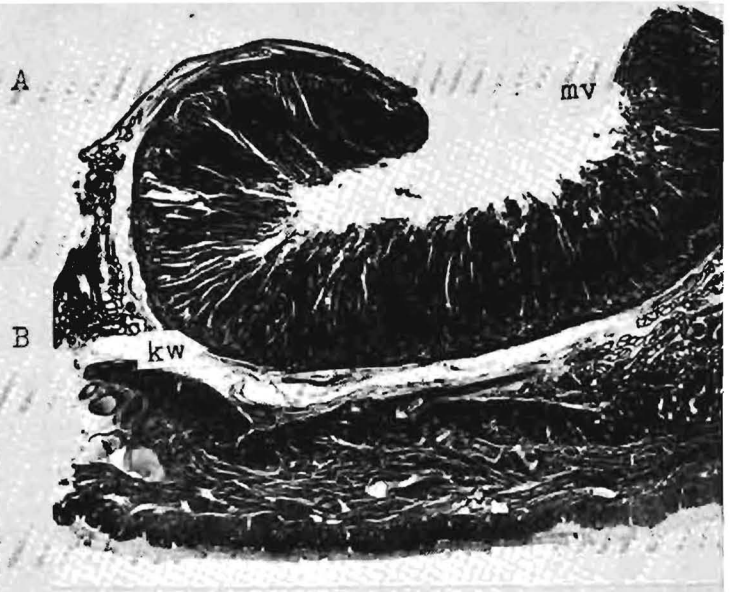

Fig. 2

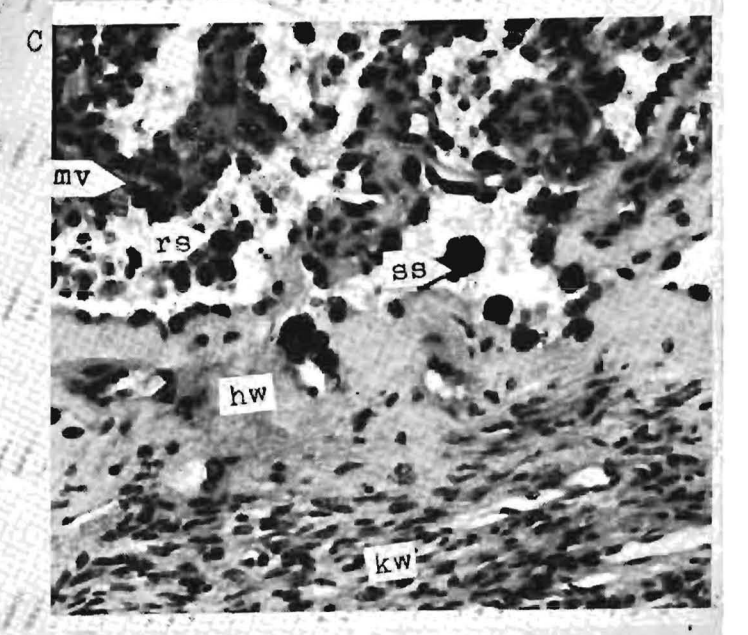

Fig. 3

PLATE 2

The caruncle of the ewe three hours **post partum.**

Fig. 1: Area A. The most distal area of the maternal villi in which the blood baths are found before parturition.
Fig. 1: Area B. The middle area of the maternal villi with congested blood vessels.
Fig. 1: Area C. The proximal area of the maternal villi with adjacent band of hyalinized caruncular connective tissue.
Fig. 2: Cross section of the caruncle.
Fig. 3: Enlargement of the most proximal portion of the maternal villi and adjacent band of hyalinized connective tissue.

mv = maternal villus
kw = caruncular connective tissue
hw = hyalinized connective tissue
rs = binucleate giant cells
ss = symplasma cells

3. *Twenty-four hours post partum* (Macroscopic: Plate 1, fig. 1. Microscopic: Plate 3)

Dark red, autolysed blood from the blood baths is seen macroscopically on and between the caruncles. Microscopically, necrotic changes have occurred in area A (Fig 1A). The tissue has degenerated into an amorphous mass and the cells have undergone pyknosis, karyorrhexis and karyolysis. Furthermore, the blood vessels have degenerated and red cells are found spread throughout the area.

In area B (Figs. 2 and 5) the blood vessels are extremely distended but the endothelial cells are still clearly visible. Between the vessels pyknotic nuclei can be seen.

In area C (Fig. 3) the hyalinized villi retain some of their original structural form and are still covered with an epithelium and symplasma cells; nevertheless, certain of the nuclei are pyknotic. In this area, as well as the adjacent hyalinized band (Fig. 4, hw), the blood vessels are very compressed.

4. *Forty-eight hours post partum* (Macroscopic: Plate 1, fig. 2)

The endometrium appears cleaner than at 24 hours: the degenerated blood from the blood baths has been evacuated through the cervix. Microscopically, the necrotic changes in area A have progressed and the area appears more amorphous.

The cells in area B do not really differ from those in the 24 hour specimen.

In area C, the band of hyalinized tissue appears broader, more prominent and has a more convex appearance but this is probably due to a shrivelling of the entire caruncle. The necrotic changes in the epithelial lining of the basal portions of the villi have advanced to such an extent that the epithelium is not identifiable as such.

5. *Four days post partum* (Macroscopic: Plate 1, fig. 3)

There is a dark brown to black, semi-solid substance on and between the caruncles as a result of autolysis and liquefaction in area A.

Area A has disappeared as a result of liquefaction and cannot be seen, even microscopically.

Area B still contains prominently filled blood vessels with pyknotic endothelial cells.

The cells of the villi in area C have degenerated to such an extent that the villi themselves cannot be identified easily but the hyalin in the villi is still visible. Below that the broad hyalinized layer is still prominent.

6. *Eight days post partum* (Macroscopic: Plate 1, fig. 4. Microscopic: Plate 4).

A black tarry substance is seen on and between the caruncles and extends into the cervix. When studied microscopically, it is evidently the result of autolysis of the blood-filled area B (Figs. 1 and 3). The blood vessels in this area are in an advanced state of degeneration and by the 12th day area B has autolysed completely.

The villi in area C have degenerated into an amorphous mass and pyknotic nuclei are spread throughout (Fig. 2).

The hyalinized band below area C is still broad and prominent but between this and the permanent caruncular connective tissue a layer of young connective tissue has formed (Figs. 2 and 4, jb). This young tissue consists of a vast amount of closely packed fibroblasts between which fine reticular fibres can be seen with silver impregnation techniques.

7. *Sixteen days post partum* (Macroscopic: Plate 1, fig. 5. Microscopic: Plate 5, fig. 1)

The upper part of the caruncle is now a brown necrotic plaque. Some of these plaques have loosened and are lying free in the uterus whereas those which are still attached are very easily removed, leaving a clean glistening translucent caruncular surface (Plate 5, fig. 2).

Microscopically, the young connective tissue layer below the hyalinized band is even more prominent and the reticular fibres are easily seen. The hyalinized band above it is very folded, possibly as a result of contraction of the underlying connective tissue. Spaces appear to have formed between these two layers. It is in this area of disruption that final separation between the plaque and the underlying healthy, young connective tissue occurs (Plate 5, Figs. 1 and 2).

8. *Twenty days post partum* (Macroscopic: Plate 1, fig. 6. Microscopic: Plate 5, fig. 3)

The caruncles are all free of plaques. Some of these loose plaques are still present in the uterus, cervix and vagina. The caruncle now consists of a naked connective tissue surface over which the epithelium is beginning to extend from the sides.

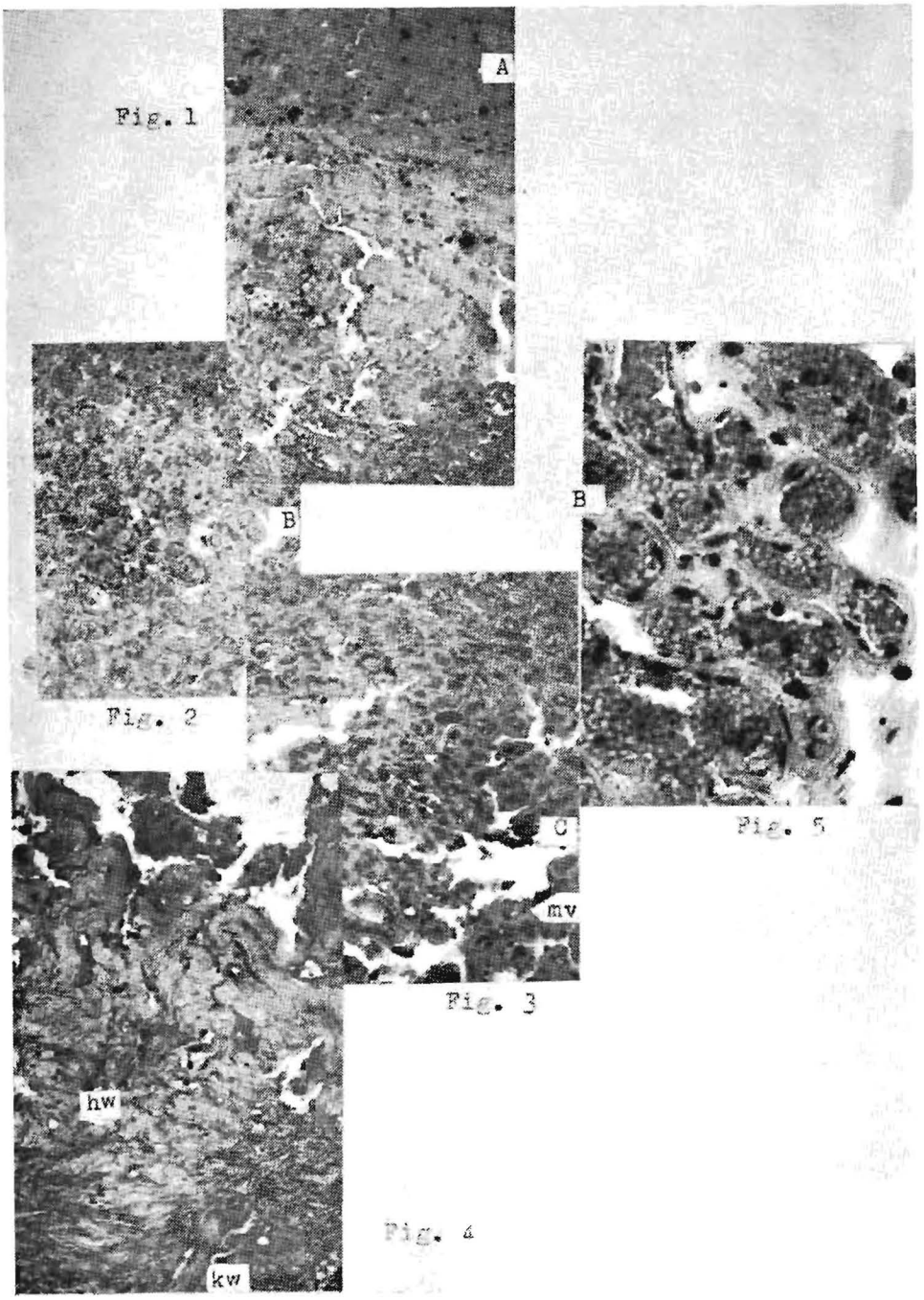

PLATE 3

The caruncle of the ewe 24 hours **post partum.**

Fig. 1: Area A. The most distal area of maternal villi.
Fig. 2: Area B. The middle area of the maternal villi.
Fig. 3: Area C. The most proximal area of the maternal villi.
Fig. 4: The base of the maternal villi and adjacent hyalinized connective tissue of the caruncle.
Fig. 5: Enlargement of area B showing prominently filled capillary blood vessels.
mv = maternal villi hw = hyalinized connective tissue
kw = caruncular connective tissue

9. *Twenty-four days post partum* (Macroscopic: Plate 1, fig. 7)

The uterus is completely clean. The epithelium has covered about 75 per cent of the caruncle.

10. *Twenty-eight days post partum* (Macroscopic: Plate 1, fig. 8. Microscopic: Plate 5, fig. 4)

Uterine involution is complete. The epithelial layer has completely covered the caruncle.

CONCLUSIONS

After parturition the foetal villi are withdrawn together with the foetal membranes, leaving a certain amount of foetal cells in the spaces between the maternal villi.

The process of involution of the caruncle occurs in three distinct stages, viz.:

1. Degenerative changes in the most distal portion (area A) of the maternal villi commence soon after parturition and this area has completely liquified by four days *post partum*.

2. The central portion (area B) of the villi, in which distended blood vessels are found, degenerates more slowly and by the 12th day this area has autolysed and exfoliated completely.

3. The remaining tissue, which includes the proximal hyalinized portions of the maternal villi (area C) as well as the adjacent hyalinized connective tissue, does not liquefy but comes off as a plaque between the 16th and 20th day and is expelled through the cervix.

This band of hyalinized tissue, which Uren [6] considered as a *post partum* involutionary change, actually forms before parturition [7]. It appears to increase in size during the process of involution but the apparent broadening of this band is the result of contraction of the whole caruncle and especially of the hyalin tissue, which is then thrown into folds. About eight days *post partum* a layer of young connective tissue forms between the hyalin band and the permanent caruncular tissue. It is between the young connective tissue and the hyalin layer that eventual disjunction of the hyalin plaques occurs leaving a naked caruncular connective tissue surface. At about 20 days *post partum* the uterine epithelium starts growing over from the sides of the caruncle and by the 28th day *post partum* the epithelium has completely covered the caruncular surface: involution is complete.

The very sparse infiltration by leucocytes into the caruncle during involution indicates the relative insignificance of the rôle played by these cells.

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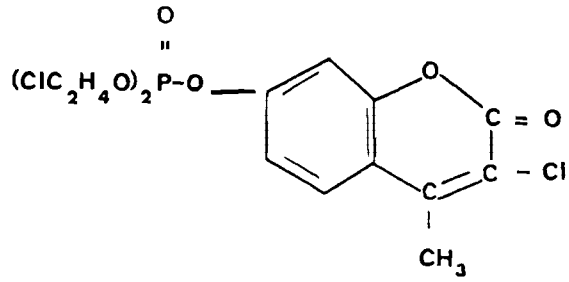
HALOXON



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COOPER ONTDEKKING

Dit is 'n OXON van 'n

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is 'n hoogsdoeltreffende en veilige middel veral teen perde-, skaap-, bok-, en bees-rondewurms met 'n terapeutiese indeks van 4 tot 25 afhangende van die dieresoort.

J. 4790

Jl S. Afr. vet. Ass. 43 (1): 29—33 1972

FURTHER OBSERVATIONS ON THE INVOLUTION OF THE *POST PARTUM* UTERUS OF THE EWE

L. C. VAN WYK*, C. H. VAN NIEKERK** AND P. C. BELONJE**

SUMMARY

A total of nine 4—6 tooth primiparous Mutton Merino ewes were slaughtered at intervals until 28 days *post partum*. From various macroscopic measurements of the ovaries and uterus it was concluded that uterine involution is complete between 20 and 24 days *post partum*. This is somewhat earlier than the 28 days required for the maternal caruncles to be covered completely by epithelium, as determined previously. There was a small but definite decrease in weight of the pituitary gland.

INTRODUCTION

Altough the macroscopic involution of the uterus of the ewe has been described [1, 2, 3, 4, 5], this investigation formed part of a wider experiment which included the histology of the preparturient [6], parturient [6] and postparturient [7] changes in the placentome of this species. This article deals with and adds to the knowledge of certain gross changes in the pituitary gland, the ovaries and the uterus of the ewe during the first 28 days *post partum*.

MATERIALS AND METHODS

Nine 4 to 6 tooth primiparous Mutton Merino ewes were used in the experiment. As they lambed, the ewes were allocated to be slaughtered after 1, 2, 4, 8, 12, 16, 20, 24 or 28 days *post partum*. Care was taken in each case that the lambs suckled only for the first 24 hours, after which they were removed. At slaughter the pituitary gland, ovaries and uterus were removed, dissected free from all extraneous tissues, and the following data were recorded:

1. Weight of the pituitary gland.
2. Diameter of the largest follicle.
3. Total weight of follicular fluid (i.e. the loss in weight after each ovary had been finely chopped and the fluids absorbed on filter paper).
4. Total weight of uterus.
5. Volume of uterus, determined by displacement of water.
6. Length of each uterine horn plus body and cervix, measured from the *ostium uterinum externum*.
7. Diameter of each uterine horn at the bifurcation of the uterus.
8. Diameter of the body of the uterus.

Thereafter the uterus was opened dorsally along its length, the surface was examined and the diameter of largest caruncle measured.

During the recording of the data, each horn was examined separately and pregnant and non-pregnant horns were distinguished. In the case of discrete data, where both horns were pregnant, mean values were utilized.

The data were analysed acording to curvilinear regression methods [8] after logarithmic transformation of the independent variate. The coefficient of the second degree term was examined and found to be either non-significant or of negligible magnitude in all cases. This shows that each set of data could be described effectively by a simple exponential curve.

RESULTS AND DISCUSSION

The observations on the pituitary glands, ovaries and uterus of the ewes slaughtered between 1 and 28 days *post partum* are presented in Table 1. Using the statistical methods mentioned above, an estimated value for each character at parturition and the mean percentage change per day in the

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Table 1: CHANGES IN THE PITUITARY GLANDS, FOLLICLES AND UTERI OF EWES DURING 28 DAYS POST PARTUM

Days post partum				1	2	4	8	12	16	20	24	28
Lambs	Number			2	1	2	2	1	2	1	2	2
	Total birth weight (kg)			8,81	4,81	7,00	6,00	4,90	9,36	4,77	5,70	5,50
Weight of pituitary gland (g)				2,22	1,33	0,77	0,93	1,09	0,99	0,86	0,60	0,81
Diameter of largest follicles (cm)				0,1–0,2	0,1–0,2	0,1–0,2	0,1–0,2	0,2–0,4	0,2–0,4	0,4–0,6	0,4–0,6	0,4–0,6
Weight of follicular fluid (g)				0,06	0,09	0,04	0,07	0,19	0,19	0,18	0,27	0,41
Uterus	Weight (g)			1 206	531	541	326	151	140	101	64	60
	Volume (ml)			1 100	500	520	310	143	135	98	62	56
	Length (cm)		Left horn	50,0	35,0	36,0	41,5	33,0	30,5	22,0*	24,5	23,0
			Right horn	47,0	29,0*	32,0	42,0	28,5*	30,0	25,5	26,0	21,5
			Mean	48,5	—	34,0	41,8	—	30,3	—	25,3	22,3
	Diameter of horns (cm)		Left horn	5,9	5,7	5,3	3,6	3,9	2,4	2,1*	1,8	2,0
			Right horn	6,2	2,7*	4,5	3,7	1,2*	2,4	2,4	1,8	1,8
			Mean	6,1	—	4,9	3,7	—	2,4	—	1,8	1,9
Diam. of body (cm)				15,6	8,9	8,8	7,4	4,7	4,3	4,5	3,6	3,4
Diameter of largest caruncle (cm)			Left horn	2,6	1,8	1,9	1,3	1,2	0,7	0,6*	0,7	0,8
			Right horn	2,9	1,2*	2,1	1,3	0,6*	0,7	0,7	0,7	0,8
			Mean	2,8	—	2,0	1,3	—	0,7	—	0,7	0,8

*=Non-pregnant horn

Table 2: AN ANALYSIS OF THE STATISTICAL EQUATION ($\log y = \log A + Bt + Ct^2$) USED TO DESCRIBE THE CHANGES MEASURED DURING THE **POST PARTUM** PERIOD

y (macroscopic measurements)	A (value at parturition)	% error	B (% change in y per day ± SE)	C (% change in y per day when C is not equal to 0 ± SE)
Weight of pituitary gland ((g)	1,56	26	−2,51 ± 0,04	0,0005 ± 0,0015
Weight of follicular fluid (g)	0,05	35	+3,03 ± 0,05	0,0000 ± 0,0019
Weight of uterus (g)	1 053	19	−7,70 ± 0,03	0,0012 ± 0,0011
Volume of uterus (ml)	971	18	−7,44 ± 0,03	0,0011 ± 0,0011
Length of pregnant horn (cm)	42	10	−0,77 ± 0,02	0,0001 ± 0,0006
Diameter of pregnant horn at bifurcation (cm)	6,47	9	−2,95 ± 0,02	0,0003 ± 0,0005
Diameter of body of uterus (cm)	13,59	13	−4,45 ± 0,02	0,0009 ± 0,0008
Diameter of largest caruncle in pregnant horn (cm)	2,79	13	−5,01 ± 0,02	0,0011 ± 0,0008

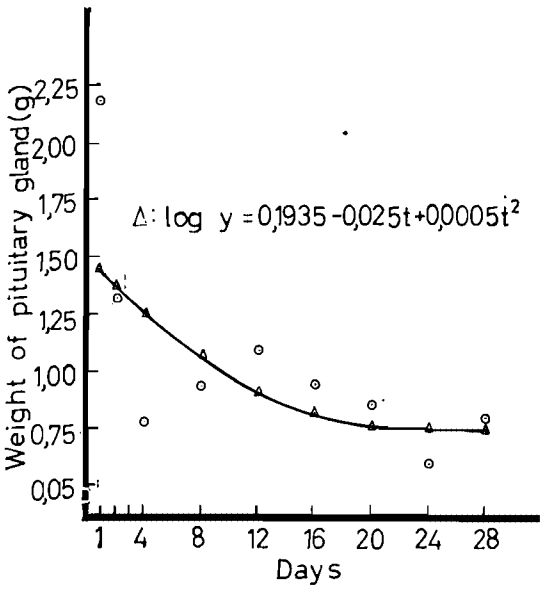

Fig. 1. Graphic representation of reduction of pituitary weight.

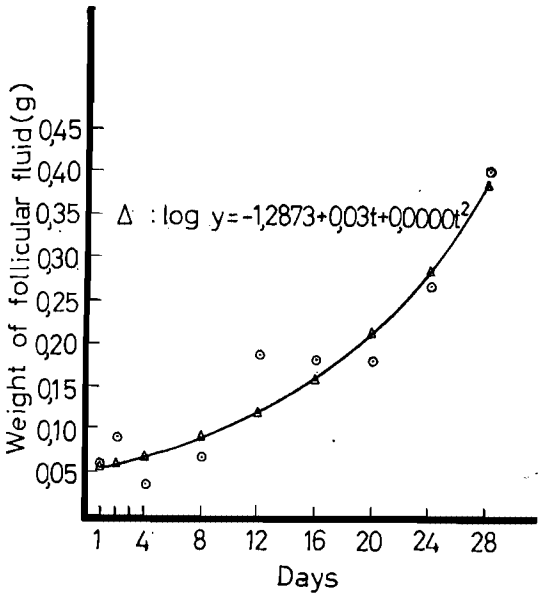

Fig. 2. Graphic representation of increase of follicular fluid.

characters during involution have been estimated and are given in Table 2. These estimated changes, together with the actual observations, are plotted in Fig. 1. The data suggest that in the cases of uterine weight and volume and diameter of the body of the uterus and of the largest caruncle, the simple model does not adequately represent the *post partum* changes which occurred, and that involution in respect of these characters was not a constant percentage per day, but was faster during the early stages after lambing.

There appears to be a small but definite decline *post partum* in the weight of the pituitary gland weight (Tables and Fig. 1). As no hormone levels were analysed, any explanation would be speculative. It is interesting, however, to note the increase in ovarian fluid (Fig. 2), indicating a gradual

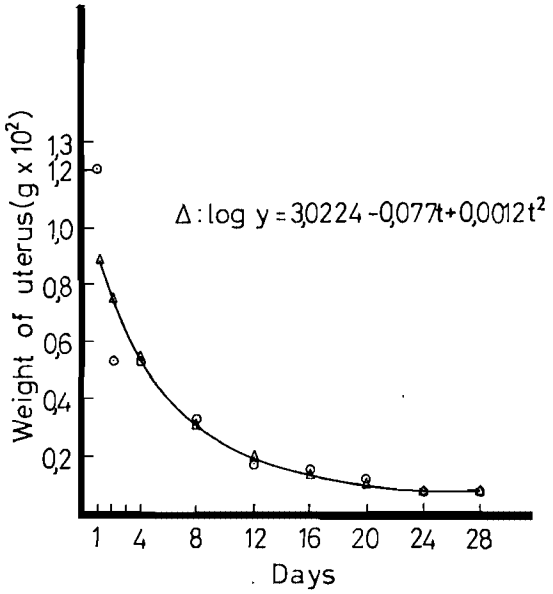

Fig. 3. Graphic representation of decrease of uterine weight.

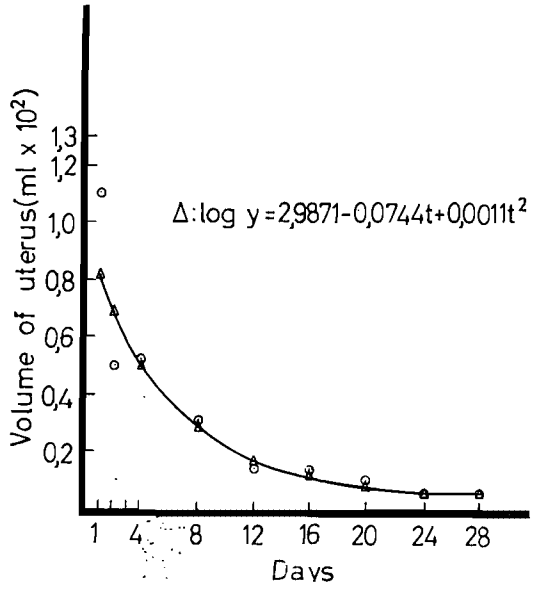

Fig 4. Graphic representation of decrease of uterine volume.

increase in follicular activity during this period.

The rapid reduction of the weight of the uterus (Fig. 3) during the first eight days agrees with previous findings [2, 5, 9]; the greatest decrease occurred during the first twelve days. Furthermore, as can be seen in Table 1, the decline in the volume of the uterus (Fig. 4) approximated very closely the loss of weight of the organ.

Although the recently gravid uterine horns had undergone their maximal reduction in length by the 20th day *post partum* (Fig. 5), the diameter had not yet done so

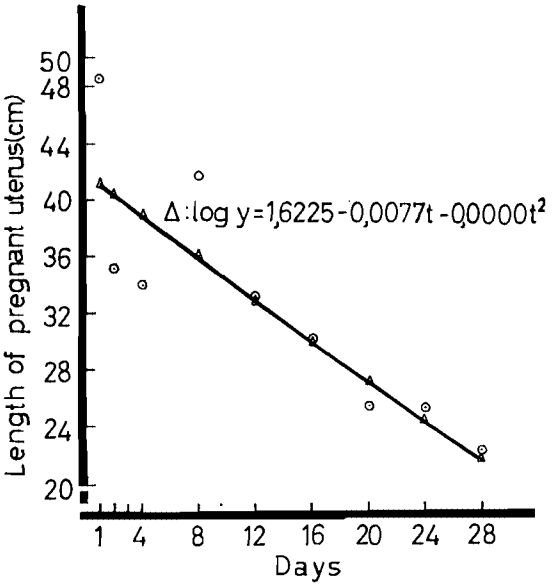

Fig. 5. Graphic representation of decrease of uterine length.

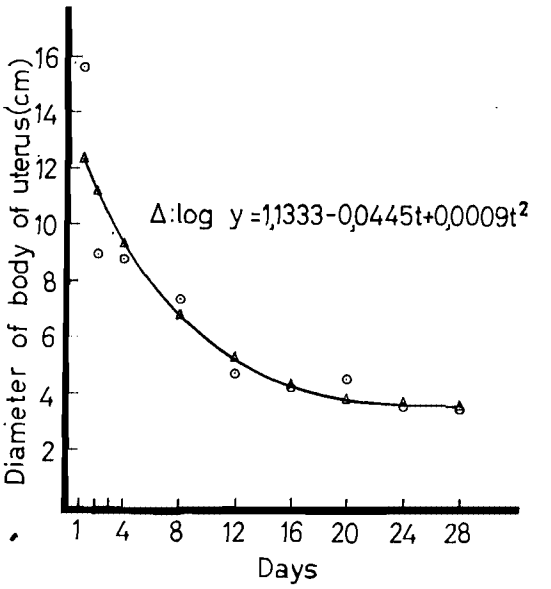

Fig. 7. Graphic representation of decrease in diameter of uterine body.

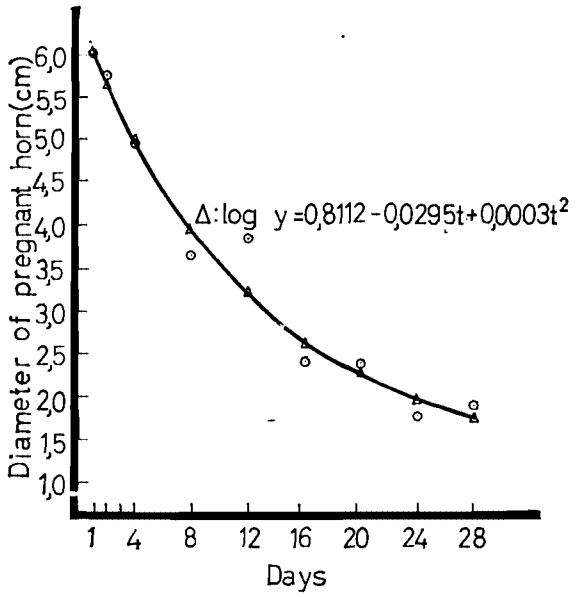

Fig. 6. Graphic representation of decrease in diameter of pregnant horn.

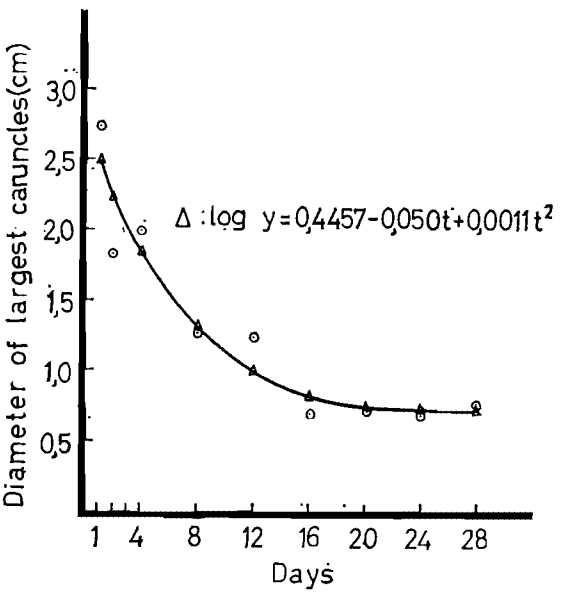

Fig. 8. Graphic representation of decrease in diameter of largest caruncle.

even by the 28th day (Fig. 6). On the other hand the diameters of the largest caruncles were at their resting size by about the 16th day (Fig. 8) while the body of the uterus had reached its quiescent diameter by the 24thday (Fig. 7)[10].

In both this and in the microscopic study [7], no foetal membranes were observed after the afterbirth had come away. It was clear that the foetal villi had been withdrawn together with the afterbirth and the debris found in the uterus was autolysed tissue from the distal portion of the maternal caruncles. By the 24th day the uterus was cleared of all debris.

From a macroscopic point of view, then, the uterus appears to have completed involution by the 20th to the 24th day *post partum*. This finding is in agreement with previous work [1]. On the other hand, as shown histologically [7], the caruncles are covered completely with epithelium only by the 28th day.

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GONADOTROPHIC HORMONE ACTIVITY IN FEMALE ANGORA GOATS EXHIBITING NORMAL AND ABERRANT REPRODUCTIVE ACTIVITY*

P. S. PRETORIUS**

SUMMARY

Luteinizing hormone activity was determined in the pituitary glands of normal pregnant goat does, of females which had aborted during previous pregnancies but carried a live foetus in their present pregnancy, of does which had aborted recently and of animals in which embryos had been resorbed or had died. Pituitary weight, corpus luteum size and ovarian follicular activity of these animals were compared.

Does which had aborted recently displayed lowered pituitary LH activity compared to normal pregnant animals. This lowered LH activity was accompanied by advanced luteal regression. Gestational failure due to embryonic resorbtion did not seem to be due to luteotropic failure. Animals in which the foetus had died but had not been expelled, displayed normal levels of LH activity, although luteal degeneration was observed. Females which had aborted during previous pregnancies seemed capable of completing a gestational period successfully under optimum conditions. Neither weight of the pituitary gland, nor ovarian follicular activity appeared to be related to the outcome of gestation.

The anterior pituitary, through its lowered LH activity, appears to be involved in the syndrome of gestational failure in the Angora goat.

INTRODUCTION

The serious proportions which reproductive losses had assumed in the Angora goat were investigated and outlined in several Karoo districts by van Heerden [1, 2, 3]. These studies revealed that a large percentage of apparently barren does had actually conceived but had aborted or that the embryo, so it seemed, had been resorbed. Early regression of the corpus luteum was described as the cause of abortion [1, 2, 3, 4]. On pituitary cytological evidence van Heerden [3] advanced a theory of an inherent weakness in the pituitary-gonadal axis, characterized by early degeneration of the *corpus luteum graviditatis*.

In later studies van Rensburg [5, 6, 7] attributed foetal death and abortion to hyperadrenocorticism, which exterted its effect through the interrelated hormonal mechanisms of the mother and foetus. The level of adrenal function seemed to cause profound disturbances in the secretion and ratio of pituitary gonadotrophins.

The afore-mentioned investigations revealed an apparent weakness in the endocrine mechanisms of the Angora goat, which culminated in various aberrations of the normal reproductive pattern. Nevertheless, apart from the above-mentioned speculations on the rôle of the pituitary gland in gestational failure, no investigations seemed to have been performed on the pituitary endocrine relationships in this species. Acquiring data on the luteinizing hormone activity in animals with normal, and does exhibiting aberrant reproductive function, was necessary.

MATERIALS AND METHODS

Pregnant Angora goat does, which all had successfully reared a kid during the previous kidding season, were slaughtered six each on day 21, 28, 35, 42, 56, 70, 84, 98, 112, 119, 126 and 140 of gestation. Fourteen pregnant females, which had aborted during previous pregnancies but were carrying a live foetus in their present pregnancy, were also slaughtered; two each on day 28, 42, 98, 112, 126, 133 and 140 of gestation. In two animals, sacrificed at 21 and 42 days of gestation, signs of embryonic resorption were noted, while in another two does dead foetuses were found when slaughtered on day 28 and 84 of gesta-

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tion respectively. Thirteen animals had aborted during their recent pregnancy and were slaughtered immediately after abortion.

The animals were slaughtered at the local abattoir, situated about 200 m away from the feeding pens. Care had been taken to subject the animals to minimum conditions of stress prior to slaughter.

Following death, both ovaries were removed, dissected clean and fixed in Bouin's fluid. After fixation for at least 14 days, each ovary was cut serially into approximately 1 mm slices. The number and diameter of all macroscopical follicles and corpora lutea were recorded. The pituitary gland was removed intact from the cranial cavity within 10 min after death and deep-frozen. Following thawing and removal of extraneous tissue, the anterior lobe was separated from each gland and placed in acetone at 4°C. After 3 days and five changes in acetone it was dried at room temperature and stored in a desiccator, which was kept under refrigeration. The acetone-dried pituitary glands were ground to a fine powder and homogenized in cold 0,9 per cent saline. The homogenate was eventually diluted to the desired concentration required for assay. Pituitary glands of animals in similar gestational stages were pooled, while glands of does which had aborted recently, resorbed their embryos or carried dead foetuses were assayed individually.

Pituitary luteinizing hormone activity was assayed by the ovarian ascorbic acid depletion method described by Parlow [8]. Younger rats and some minor modifications in the pretreatment of the rats were adopted in the standard assay procedure. Pituitary material was administered during a single intraperitoneal injection at a dose level of 0,8 mg pituitary powder in 0,5 ml 0,9 per cent saline. Six immature female Sprague Dawley rats (23 to 24 days) were employed in each group. Ascorbic acid concentration was determined according to the method of Mindlin & Butler [9]. Pituitary luteinizing hormone activity was expressed as per cent ovarian ascorbic acid depletion activity over that of control animals, the latter taken as 100 per cent. The value for each gestational stage represents a mean of three independent assays.

RESULTS

Pituitary weight

To ascertain if deviations from the normal reproductive pattern were reflected in the weight of their pituitary glands, a comparison was drawn between normal pregnant females and does in which pregnancy was terminated either by abortion, resorption or foetal death. These values are summarized in Table 1.

Pituitary glands of animals which carried normal healthy foetuses at the time of slaughter outweighed those from does which had aborted recently; the difference was relatively small and statistically not significant. Although the difference in pituitary weight between normal pregnant animals and does, in which embryos had been resorbed or were found to be dead but not expelled, appeared to be large, it was also statistically non-significant. The considerably higher pituitary weights recorded in animals which had aborted during previous pregnancies but carried a live foetus at the time of slaughter, was most probably a function of their higher body weight compared to those of other pregnant females (44 against 39 kg).

Luteinizing hormone activity

The pituitary luteinizing hormone (LH) activity of does carrying live foetuses was compared to that of does which had lost

Table 1: WEIGHT OF THE PITUITARY GLAND IN ANGORA GOAT DOES WITH NORMAL PREGNANCIES, NORMAL PREGNANCIES BUT ABORTION DURING PREVIOUS PREGNANCIES, RECENTLY ABORTED, RESORBED AND DEAD FOETUSES (Mean ± S.E.)

Type of pragnency	Total gland intact (mg)	Anterior intact (mg)	Posterior (mg)
Normal pregnancy (21 to 140 days)	444,8 ± 40,6	365,7 ± 35,9	79,1 ± 7,0
Normal pregnancy, previously aborted	587,9 ± 27,9	481,9 ± 25,9	106,1 ± 5,8
Recently aborted	427,0 ± 26,4	350,4 ± 23,0	76,6 ± 6,2
Resorbed foetuses	493,2 ± 22,8	397,3 ± 33,3	95,9 ± 19,5
Dead foetuses	379,8 ± 69,9	311,9 ± 62,9	68,0 ± 7,1

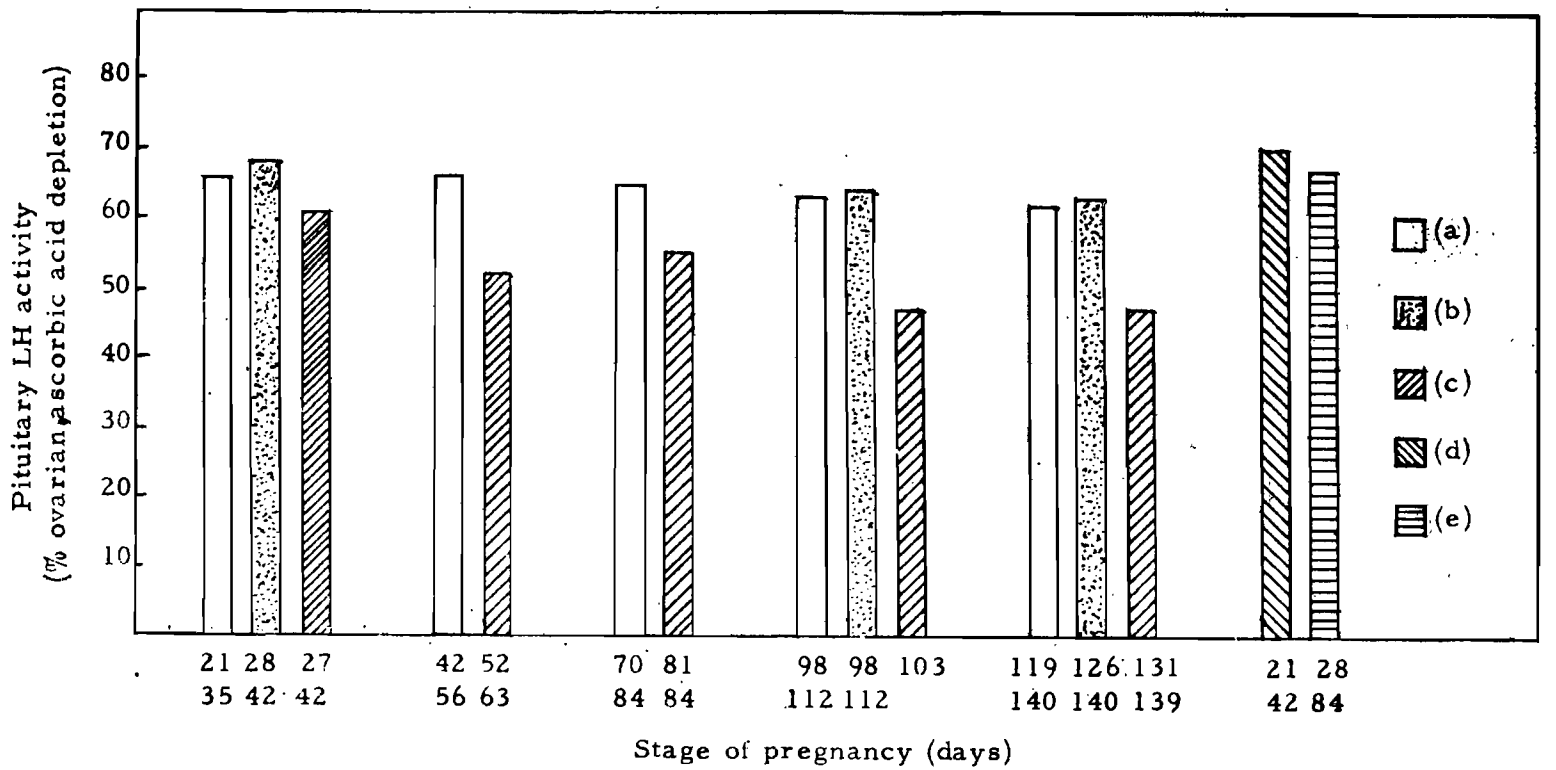

Fig. 1. Luteinising hormone activity of the anterior pituitary gland in Angora goat does with (a) normal pregnancies; (b) normal pregnancies, but abortion during previous pregnancies; (c) recent abortions; (d) resorbed foetuses and (e) dead foetuses.

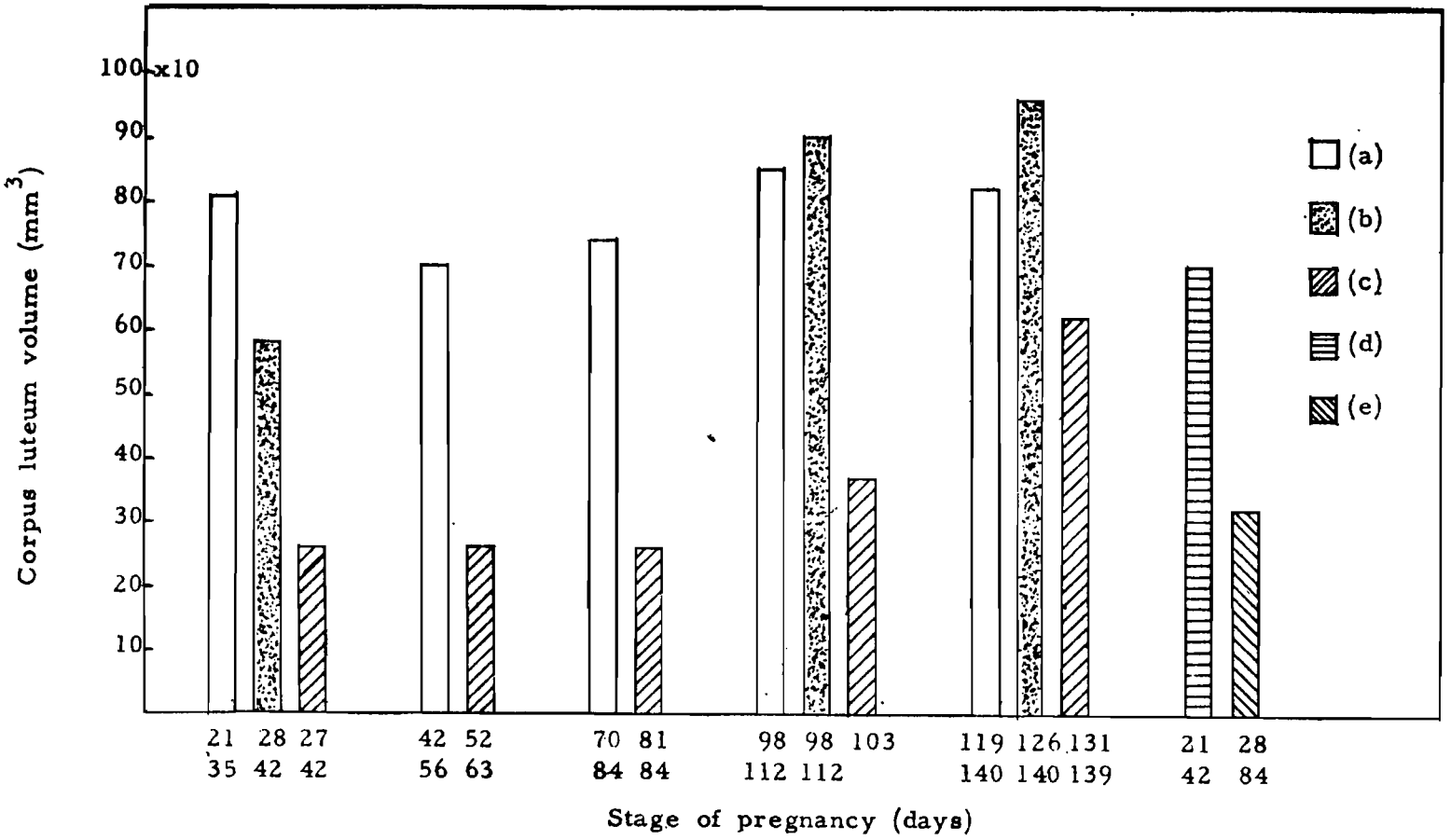

Fig. 2. Corpus luteum volume in Anfiora goat does with (a) normal pregnancies; (b) normal pregnancies but abortion during previous pregnancies; (c) recent abortions; (d) resorbed embryos and (e) dead foetuses.

their foetuses either by abortion, resorption or foetal death. These values are represented graphically in Fig. 1 for combined gestational stages.

Pituitary LH activity displayed no major changes during the gestation period in normal pregnant animals, although a slight declining tendency was noticeable as the period of pregnancy advanced. In does which had aborted recently, LH activity was constantly lower than in the pituitaries of animals carrying a live foetus at the time of slaughter. These differences in LH activity varied from 9 to 25 per cent. Statistically significant differences ($P<0,05$) in LH activity were proven only between normal pregnant does and does that had aborted after 100 days of gestation (Fig. 1).

Does which had aborted during previous pregnancies but carried a live foetus at the time of slaughter, displayed similar values in LH activity as females with normal previous reproductive function. The similarity in pituitary LH activity is quite remarkable (Fig. 1).

LH activity of does in which embryos had been resorbed or the foetuses had died, did not differ significantly from those of normal pregnant animals. In fact, does in which embryos had been resorbed displayed slightly higher LH values. Compared to does which had aborted recently, pituitary LH activity was significantly ($P<0,05$) higher in does which had resorbed their embryo (Fig. 1).

Corpus luteum

Corpus luteum size is graphically illustrated in Fig. 2 for does who differed in gestational outcome. The corpora lutea of normal pregnant animals displayed no major changes in size during pregnancy. Although some variation in corpus luteum size was recorded between gestational stages, it was small and statistically not significant. By contrast, all the corpora lutea of does which aborted recently were considerably smaller in size ($P<0,01$) than those of animals carrying a live foetus. The corpora lutea of these does were about one third the size of those of normal pregnant females and resembled in morphological appearance that of a non-functional corpus albicans (Fig. 2). These corpora lutea were embedded in the ovarian stromal tissue; on cross section no central cavities were observed.

Corpora lutea of does in which the foetuses were dead at the time of slaughter were also smaller in size ($P<0,01$) than those of normal pregnant animals. These corpora lutea had the same morphological appearance as those of does which had actually aborted. The corpora lutea of animals in which the embryos had been resorbed were also somewhat smaller in size than those of normal pregnant females, but the differences were not significant. Central cavities, with a mean diameter of 3,2 mm, were recorded in these corpora lutea.

The values in Fig. 2 also proved that the corpora lutea of animals which had aborted during previous pregnancies, but carried a live foetus in their present pregnancy, did not differ in size from those of pregnant does with normal previous reproductive histories (Fig. 2).

Ovarian follicular activity

In order to ascertain if differences in the reproductive function of pregnant does are reflected in their ovarian activity, follicular activity was compared (Table 2).

Table 2: FOLLICULAR ACTIVITY IN OVARIES OF ANGORA GOATS WITH NORMAL PREGNANCIES, WITH NORMAL PREGNANCIES BUT ABORTION DURING PREVIOUS PREGNANCIES, DOES WHICH HAD RECENTLY ABORTED AND DOES WITH RESORBED OR DEAD FOETUSES

Type of pregnancy	FOLLICULAR ACTIVITY				
	Total follicle number	Mean follicle diameter (mm)	Total follicle volume (mm^3)	Diameter largest follicle (mm)	Diameter 2nd largest follicle (mm)
Normal pregnancy	22,6	1,60	180,7	5,9	4,6
Normal pregnancy but abortion during previous pregnancies	18,3	1,87	179,3	5,8	4,8
Recently aborted	19,0	1,69	150,80	4,9	4,0
Resorbed or dead foetus	23,1	1,80	211,9	6,4	5,1

Neither follicle number nor follicle diameter seemed to be related to the outcome of gestation. Nevertheless, total follicle volume was slightly lower in does which had aborted recently compared to that of normal pregnant animals. This difference was probably due to the smaller diameter of the largest and second largest follicles recorded in the ovaries of does which had aborted recently (Table 2).

Ovarian follicular activity was higher in does in which embryos had been resorbed or which carried dead foetuses. According to Table 2, the bigger number of follicles and their larger size seemed to be responsible for this difference. None of the above-mentioned differences in follicular activity, however, was statistically significant, except in the case of the largest and second largest follicles, which were significantly larger ($P<0,05$) in animals with resorbed or dead foetuses compared to animals which had aborted.

DISCUSSION

The most significant finding of this study is probably the lower LH activity recorded in the pituitary glands of animals which had aborted. Although these differences in some instances were relatively small, they were invariably accompanied by advanced luteal regression. This observation tallies with the earlier reports of van Heerden [3] and van Rensburg & van Rensburg [4], who had also ascribed foetal death and abortion to the premature regression of the corpus luteum of pregnancy.

In the doe with a normal live foetus, corpus luteum size remained unchanged during pregnancy. This seems to indicate that its functional activity is largely maintained during the gestational period. A more functional approach was made by Ghannam [10] and van Rensburg [7] who determined the progesterone content of corpora lutea in pregnant does. According to these studies, progesterone content rises up to the 90th to 110th day of gestation followed by a slow decline. Nevertheless, the essentiality of the corpus luteum for maintaining pregnancy, specially during the early gestational stages, is generally accepted. The importance of the ovaries in maintaining pregnancy during the later stages of gestation varies with species [11]. In the pregnant goat, luteal enucleation during any gestational stage results in foetal abortion [12], [13]. Moreover, the placenta of the goat appears to be exceedingly dependent upon progesterone. Within a few hours following corpus luteum enucleation, blood progesterone levels will drop to the basal level maintained by the adrenals [7]. Raeside & Turner [14] were unable to demonstrate any progesterone in the placenta of the female goat, which indicates that the placenta of the goat produces no or insufficient amounts of progesterone for maintaining pregnancy. A constant supply of adequate amounts of progesterone, secreted by the corpus luteum, thus seemed to be essential for maintaining pregnancy in this species.

Maintenance of the corpus luteum and its secretory activity depends on some "luteotrophic factors"; substances which differed between species [15]. Prolactin appears to be the active substance in most laboratory animals [16], [17], while LH seems to exert luteotrophic properties in the ovine and bovine female [18], [19]. Indirect evidence points to pituitary LH as being luteotrophic in the doe [20]. This assumption is supported by the results of the present investigation. Luteal regression in does which had aborted was invariably accompanied by lowered pituitary LH activity. On the other hand, animals in which the embryos had been resorbed or had died early in pregnancy, displayed apparently "normal" levels of pituitary LH. The corpora lutea of animals in which the embryos had been resorbed appeared morphologically normal and seemed to be functionally active. Whether this was actually the case is not known, as progesterone secretion was not determined. The fact that the physiological mechanisms for maintaining pregnancy appear to be normal in the mother, gave reason to suspect that the cause of this type of gestational failure arose from the embryo's side. The difference in pituitary LH activity between animals which aborted and those in which the foetus had died but had not been expelled, remain difficult to explain, as both types of does displayed advanced luteal regression. The pituitary might have produced sufficient amount of LH, but these were not released, or released in too limited amounts to support luteal function. The limited number of females in which embroynal resorption or foetal death had occurred, precludes valid conclusions at this stage and calls for further study.

The LH activity in animals which had aborted during previous pregnancies but carried a normal healthy foetus in their pre-

sent pregnancy, was equal to that of other normal pregnant animals. This observation presents evidence that under optimum conditions these animals are quite able to complete a gestational period successfully. The similarity in pituitary LH levels between the former and latter group of does is quite remarkable. That the former might be more prone to abort during conditions of stress is readily admitted.

Neither weight of the pituitary gland nor ovarian follicular activity seemed to be related to the outcome of gestation in the Angora goat.

The anterior pituitary gland thus appeared to form a link in the chain of events (factors) which are involved in the syndrome of gestational failure in the Angora goat. Interference with the normal pituitary function might arise from aberrations in adrenal activity as described by van Rensburg [5, 6, 7]. The lowered pituitary LH activity presumably results in failure of the luteotrophic stimulus of the corpus luteum of pregnancy and hence in abortion. The employment of quantitative assays to furnish additional data on both pituitary and blood hormone levels would be of great value to elucidate further the rôle of the pituitary gland in this syndrome of gestational failure.

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Jl S. Afr. vet. Ass. 43 (1): 43—45 1972

DEEP FREEZING OF RAM SEMEN

N. A. VINHA* AND R. I. COUBROUGH**

SUMMARY

The freezing of ram semen diluted in an egg-yolk-milk-glycerol diluent is described. The results of the *in vitro* evaluation after 16 days storage at −196°C are given.

INTRODUCTION

Since the discovery of Polge, Smith & Parkes[1] that glycerol had a protective action on spermatozoa during freezing, long-term semen preservation has become a reality. The constant results obtained with the freezing of bovine semen, however, have not as yet been repeated consistently with ram semen[2]. More recently, following the overall benefits accruing from the payette method of freezing and storage for bovine semen[3], this method has been adapted for use in sheep semen freezing. Salamon[4] compared the freezing of ram semen in ampoules, synthetic straws and as pellets. The samples were diluted in an egg-yolk-citrate-glucose mixture and stored at −196°C for 2—4 weeks. Regardless of the type of container used, he found no significant difference in lambing rate after a double insemination. Using a lactose (11%), egg-yolk (20%) and glycerol (5%) diluent, Sainsbury[5] froze ram semen in straws after allowing an equilibration period of four hours. Evaluating samples stored for one year at −196°C, he found from 60—80% live spermatozoa, indicating that long-term semen storage in straws had little visible effect on spermatozoan survival rate. Aamdal & Andersen[6], testing different extenders, found that lactose diluents gave good results when the semen was frozen in straws. Insemination of ewes with semen stored for one month resulted in a conception rate of 62,5%. With these encouraging results of freezing ram semen in straws, the current trial was carried out using the universal diluent, skim milk, for dilution purposes.

MATERIAL AND METHODS

Semen from four Merino rams was collected, diluted and frozen on two occasions (A & B). The rams were kept under identical conditions which remained constant over the trial period. All rams were mature, and were worked shortly after the normal breeding season. The semen was collected by means of electrical stimulation using the Ruakura ejaculator[7], a double ejaculate being collected each time.

The diluent consisted of skimmed milk (pH 6,7), 10% egg-yolk plus a final concentration of 6% glycerol. Penicillin and streptomycin were added at the rate of 500 IU penicillin and 500 μg of streptomycin per ml of diluent. No pH adjustment was carried out. The dilution rate was based on the work of Gunn, Saunders & Granger[8], resulting in a total of 300 million spermatozoa/ml before cooling and freezing.

The dilution and glycerination techniques employed were those routinely used for bull semen[9, 10, 11]. The straws were filled and sealed at 4°C, and allowed to stand for 17—18 hours at 4°C. The equilibrated samples were frozen to −79°C in dry ice and alcohol according to the temperature degradation scale used by Fraser[12].

+ 4°C to 0°C : a period of 30 minutes
0°C to − 5°C : ½°C / minute
− 5°C to −10°C : 1°C / minute
−10°C to −17°C : 2°C / minute
−17°C to −79°C : 4°C / minute

Once the straws had reached −79°C they were placed directly into liquid nitrogen at −196°C.

The two batches of semen (A & B) were stored at −196°C for 16 and 14 days respectively. Samples from each batch were thawed in a waterbath at 37°C for 30 seconds before being evaluated.

RESULTS

The semen quality of each ejaculate collected on both occasions (A & B) was very

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similar and well within the standards set for dilution and freezing. An example of the semen quality of an ejaculate prior to dilution is given in table 1.

Table 1: TYPICAL SEMEN QUALITY OF SAMPLES DILUTED (Ram 27622)

Ejaculate	Volume (ml)	Motility	Density	% Alive	pH
1st	0,8	5	Thick creamy	90	7,0
2nd	1,4	5	Thick creamy	90	7,0

Straws thawed from each batch (A & B) frozen were very uniform in semen quality. An example of the typical semen picture obtained on evaluation is given in table 2.

Table 2: TYPICAL SEMEN PICTURE OBTAINED AFTER STORAGE AND THAWING (Ram 27622)

	Time stored	% Alive	Linear movement
Batch A	16 days	70%	80%
Batch B	14 days	60%	90%

DISCUSSION AND CONCLUSIONS

Although several workers [5, 6, 13] have found that a lactose diluent was most suitable for the freezing of ram semen, the favourable results obtained in this trial with skim milk augurs well for the use of this universal diluent.

The concentration of glycerol used in ram semen dilution for freezing purposes has varied greatly, ranging from as low as 4% to as high as 15% final concentration [2, 6, 5, 13]. In the trial reported, the 6% final concentration of glycerol appeared to have had no apparent untoward effect on the spermatozoa. This is contrary to the thoughts of Frazer [14], who considered that a glycerol content of above 4% was detrimental to the motility of ram spermatozoa.

An equilibration period of not less than four hours and not more than eight hours was considered ideal for ram semen [5]. Laboratory routine dictated an equilibration time of between 17—18 hours during this trial, again without apparent adverse effect on either the percentage live spermatozoa or on the linear movement of the thawed semen.

Aamdal & Anderson [6] thawed the samples in their trial at 75°C for 12 seconds and at 35°C for 30 seconds. They found that thawing at the higher temperature gave considerably better results. Although the semen in our trials was only thawed at one temperature, namely 37°C for 30 seconds, our results were considered good, and certainly do not compare with the poor results obtained by Aamdal & Andersen [6] at the lower temperature.

From the *in vitro* results in this trial, the freezing of ram semen diluted in egg-yolk-skim-milk-glycerol appears to warrant further investigation and suggests that the general avoidance of milk as a diluent for ram semen may not be justified. The *in vitro* evaluation of semen stored for up to 16 days at −196°C shows that ram semen tolerates milk dilution and freezing well. Since milk diluents are generally available and cheap, they may be considered universal diluents; their use in further ram semen freezing trials with subsequent insemination seems warranted.

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BOOK REVIEW — BOEKRESENSIE

THE PIG AS A LABORATORY ANIMAL

L. E. Mount and D. L. Ingram

Academic Press, London and New York 1971

pp VIII+175, Figs 41, Tabs 4, Publ. price £3.00

The authors of this book are to be congratulated on achieving their stated aim of providing "a practical guide to those wishing to work with pigs as laboratory animals" in such compact form. Not only are the important biological characteristics of the species lucidly discussed, but the practical application of this information to the housing, handling, feeding and supply of experimental pigs is also set out with commendable brevity. Completeness, however, has not been sacrificed to brevity, and reference will also be found to such mundane but essential topics such as weighing methods, transport of pigs and effluent disposal.

There is an interesting chapter on behaviour, both natural and laboratory-conditioned, and stress is laid on prior conditioning of animals to the conditions of the experiment if unreliable and confusing results are not to be obtained. Further chapters on anaesthesia, surgery and the uses of the pig as an experimental animal provide more detailed information on the methodology available. The final chapter (by G. A. Embleton) discusses the symptomatology and control of the common pig ailments in an authoritative fashion and includes a brief but valuable section on preventive medicine.

The book's value is greatly enhanced by an extensive bibliography of nearly 400 references to the international literature. A surprising omission here is any reference to halothane-induced malignant hyperpyrexia in some Landrace pigs. The bibliography is followed by an author index and a somewhat incomplete subject index.

This is a useful book which most admirably supplements the well-kown "Swine in Biomedical Research". While its chief value will obviously be to non-veterinary research workers it is also confidently recommended to practising colleagues desiring a brief but informative text on the species.

R. K. L.

Jl S. Afr. vet. Ass. 43 (1): 47—56 1972

BLEEK, SAGTE, WATERIGE (BSW-) VARKVLEIS*

R. T. NAUDÉ**

SUMMARY

Pale, soft, exudative (PSE) pork is the result of accelerated *post-mortem* glycolysis in the musculature whilst the carcass is still warm. The muscular damage does not occur in the live stage: it is aggravated by the degree of stress experienced by the animal directly before or during slaughter, as well as immediately thereafter, leading to very active muscular contraction.

Selection for more rapid and effective growth and for better muscling with a minimum of fat has probably resulted in animals that are more susceptible to stress and therefore react more drastically to environmental stimuli. Stunning by captive bolt results in highly accelerated lactic acid production and almost invariably leads to PSE pork. Electrical stunning has the least deleterious effect on pork quality. The incidence of PSE pork, evaluated according to pH_1 value***, at three pork factories and one municipal abattoir varied from 5 to 28 percent. Quietness of handling directly before slaughter and efficiency and speed of stunning are all factors which decrease the rate of *post-mortem* glycolysis.

Under ordinary factory conditions it is neither practical nor economical to effect a cooling of the carcass of more than 3 to 4°C within the usual time lapse of 45 minutes. The pork industry is thus faced with the challenge of decelerating *post-mortem* glycolysis, either by better *ante-mortem* handling, or by breeding pigs less susceptible to stress.

OPSOMMING

Bleek, sagte, waterige (BSW-) varkvleis is die gevolg van versnelde nadoodse glikolise in die spiere terwyl die karkas nog warm is. Die spierbeskadiging word nie by die lewendige dier aangetref nie: dit word in die hand gewerk deur die mate van stress wat op die dier direk voor, of tydens slagting en ook nadoods uitgeoefen word en wat tot baie aktiewe spiersametrekkings lei.

Seleksie vir sneller en meer doeltreffende groei en vir gespierde varke met 'n minimum hoeveelheid vet het waarskynlik meegebring dat varke meer stress-gevoelig is en dus drastieser op omgewingstimuli reageer. Bedwelming met die penpistool het besonder versnelde nadoodse melksuurvorming in die spiere en byna sonder uitsondering BSW-vleis tot gevolg. Elektriese verdowing het die minste nadelige invloed op vleiskwaliteit. Die voorkoms van BSW-vleis in elektriesbedwelmde spekvarke, geoordeel volgens die pH_1-waarde***, wat by drie spekfabrieke en een munisipale abattoir bepaal is, lê tussen 5 en 28 persent. Rustigheid van direkte voordoodse hantering, en vaardigheid en spoed van bedwelming is faktore wat die snelheid van nadoodse glikolise vertraag.

Onder fabrieksomstandighede is karkasafkoeling met meer as 3 tot 4°C binne die gebruiklike 45 minute na slagting onprakties en onekonomies. Vir die varkbedryf is daar dus 'n uitdaging om nadoodse glikolise te vertraag, hetsy deur gunstiger voordoodse hantering, hetsy deur teling van minder stress-gevoelige varke.

INLEIDING

Die variasie in prosesseerbaarheid van varkvleis is reeds sedert 1771 'n kwaliteitseienskap waaroor vervaardigers begaan was[1]. Die swak waterbindende vermoë van die vleis van sekere varkkarkasse wat na Engeland uitgevoer is, het reeds sedert vroeg in die

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***pH_1: Die pH-waarde soos bepaal 45 minute na dood.
The pH value as measured 45 minutes post mortem.

twintigste eeu [2] 'n bron van kommer vir die varkbedryf van Denemarke geword. Vervaardigers van Wiltshire-speksye, ingemaakte en gerookte hamme, wors en verpakte vars varkvleis, stel almal baie hoë eise aan die vermoë van die spiere in vleissnitte om die 75 persent water, wat dit in die spierselle bevat, aan die strukturele proteïene tydens prosessering gebonde te hou. Die verskynsel van bleek, sagte en waterige (BSW-) spiere is vir die eerste keer in 1953 deur Ludvigsen [3, 4] by Deense Landrasvarke as „spierdegenerasie" beskryf. Hy het tot die slotsom gekom dat die oorsprong van BSW-vleis nie by die verwerking geleë is nie, maar dat dit tot die dier voor, tydens en direk nadoods teruggevoer moet word. In Nederland word besondere aandag verleen aan die vrektes wat onder gesonde varke voorkom, veral tydens vervoer na slagplase of na ander vorms van stress, soos uiterste of vinnig variërende omgewingstemperature, uitputtende oefening of bakleiery voor slagting [5]. Duitse navorsers het dit „akute hartversaking" genoem en Selye 'n „aanpassingsiekte" [6]. Ludvigsen [3, 4] het dan ook afdoende bewys gelewer dat dié „spierdegenerasie", wat nie by die lewendige dier voorkom nie, maar dikwels reeds binne tien minute nadoods in die spiere van die karkas waargeneem word, slegs sekondêr is tot die gebrek van die aanpassingsmeganismes van die dier om bevredigend tydens 'n stresstoestand te reageer. Oormatige prikkeling van die byniermurg veroorsaak 'n sodanige adrenalienafskeiding en 'n gevolglik versnelde glikolise, dat die glukokortikoïede-afskeiding van die bynierskors nie in staat is om homeostase te handhaaf nie [7-9]. Tipiese „spierdistrofie" wat by die lewendige dier waargeneem en gekenmerk word deur 'n oormatige bindweefsel- en lipiedneerlegging kan dikwels deur vitamien-E of seleniumaanvulling van die rantsoen oorkom word. Varke met BSW-spiere reageer egter gladnie op laasgenoemde voedingsbehandeling nie [10].

Gedurende die tydperk 1926 tot 1963 is die varke in Deense nageslagtoetsstasies geteel en geselekteer om die toetsperiode, wat van 20 tot 90 kg lewende gewig strek, met agt dae te verminder en terselfdertyd 20% meer spier en 20% minder vet in die karkasse te produseer. Die verhoogde proteïen-neerlegging in die karkas was verantwoordelik vir 77% van die verhoogde doeltreffendheid van voerverbruik, wat ook as gevolg van hierdie seleksie teweeggebring is [7].

Seleksie van diere vir snelle spiergroei het 'n verhoogde groeihormoon-afskeiding van die hipofise as gevolg, vermoedelik ten koste van die bynierkortikotrofiese hormoonafskeiding van genoemde klier [7]. Vanweë die feit dat varke se vermoë om stress te weerstaan baie kan verskil, word hulle dus as „stressgevoelig" of „stress-weerstandbiedend" geklassifiseer [6, 7]. Onder dieselfde omgewingstoestande reageer eersgenoemde varke fisiologies baie hewiger as laasgenoemde. Dit het 'n anoksiese toestand in die spiere tot gevolg. Indien hierdie toestand van stress direk voor of tydens die dood van die dier voor kom, veroorsaak dit abnormale spierspanning. Die bedwelmings- en slagproses kan ook tot 'n mindere of meerdere mate sodanige spierkontraksie stimuleer. Aktiewe sametrekking van die spiere kort vóór en tydens die dood van die dier veroorsaak dat normale nadoodse glikolise teen 'n baie verhoogde tempo plaasvind. Die gevolg hiervan is dat die vorming van melksuur, wat in 'n daling van spier-pH vanaf 7,0 tot 5,4 weerspieël word en normalerwys binne 12 uur plaasvind [10], reeds binne 30 tot 90, en soms binne 10 minute na bedwelming voltrek is en die spiere dan reeds in *rigor mortis* verkeer. Hierdie toestand word dan ook „suurrigor" genoem, in teenstelling met die normale rigor wat 12 uur na dood intree. 'n Toestand van „alkaliese rigor" kan egter ontstaan nadat spieruitputting vir 'n paar dae voordoods plaasgevind het en die glikogeen sodanig opgebruik is dat die pH slegs tot ongeveer 6,8 kan daal en *rigor mortis* dan intree en donkerkleurige vleis tot gevolg het [9]. In die praktyk duur dit by slagplase en spekfabrieke ongeveer 30 tot 60 minute alvorens die karkasse in die verkoelkamers gestoot word. Die spiertemperatuur is dan nog bokant 35°C en, indien die pH snel gedaal het, sal 'n kombinasie van 'n hoë melksuur en hoë temperatuur in die spiere voor kom, wat die proteïene sodanig sal denatureer dat die normale helderrooi kleur en redelik droë, ferm oppervlakte na die kenmerkende BSW-toestand verander. Die pH van spiere wat voor en tydens bedwelming meer ontspanne is, daal tot slegs 6,4 voordat die karkasse afgekoel word en bogenoemde eiwit-ontaarding vind dan nie plaas nie [11].

EIENSKAPPE VAN BSW-SPIER

Bleek

Teenoor die relatief donkerrooi kleur van bees- en skaapvleis, het varkvleis 'n ligter, dog helderligroos kleur (Fig. 1). BSW-spier het egter 'n baie ligte, dowwe grys kleur. Die verskil in die kleur van normale en hierdie afwykende spiere word nie aan 'n verskil in

mioglobienkonsentrasie toegeskryf nie[6, 11]. Hierdie kleurverskille is egter die gevolg van koagulasie van die sarkoplasma-proteïene onder toestande van hoë suur en temperatuur, wat die spierpigment vermom. Genoemde proteïene word ook op die miofibrillêre proteïene, aktomiosien, gepresipiteer, wat 'n groter ligweerkaatsing tot gevolg het[12]. Die gekombineerde effek van hierdie twee proteïenafwykings gee dan aan die spiere die onnatuurlike bleek kleur.

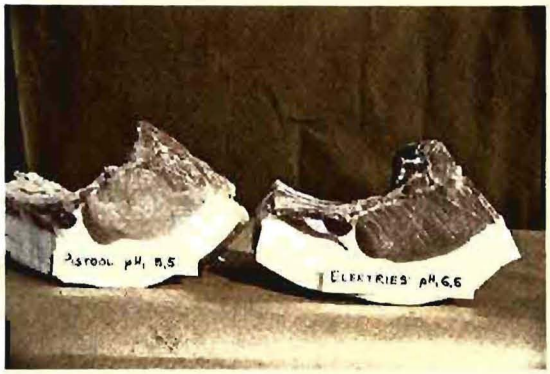

Fig. 1: Die voorkoms van BSW-vleis (links) vergelyk met normale vleis (regs).

Sag

'n Verdere gevolg van versnelde nadoodse glikolise is dat die kollageen van die spierselomhulsel gedeeltelik gehidroliseer word, gevolglik beland baie van hierdie gevormde gelatien saam met die sarkoplasmavog in die tussenselruimtes en 'n ernstige edeem kan daar waargeneem word[1, 10, 13, 14]. Hierdie aansameling van intersellulêre vloeistof laat die spiere hul normale fermheid verloor en 'n papperige voorkoms aanneem.

Waterig

Die waterbindende vermoë van vleis is vir die vleisfabrikant seker die heel belangrikste kwaliteitseienskap. BSW-spiere het swak waterbindende eienskappe vanweë presipitasie van die sarkoplasma/eiewitte op dié posisies van die aktomiosienbinding waar die los watermolekule gebind word. Twaalf uur na slag is die pH van normale sowel as BSW-spiere ongeveer 5,4, wat ongeveer gelyk is aan die iso-elektriese punt van miofibrillêre proteïene. Die denaturering en gelokaliseerde presipitering van die proteïene van BSW-spiere, en in 'n mate die versnelde intrede van *rigor mortis*, is dus die faktore wat waterbindingsvermoë nadelig beïnvloed. Water wat nie meer aan die aktien- en miosienfilamente gebind is nie, word in die sarkoplasma vrygestel en as gevolg van die hidrolise van die omhullende kollageen word baie van die selvloeistof na die tussenselruimtes verplaas. Sodra die vleis gesny word, word dan groot hoeveelhede van hierdie selsap, wat baie opgeloste selplasma bevat, vrygestel[6-9].

Biochemies

Stress direk voor dood of tydens slagting veroorsaak 'n sametrekking in die spier. Vir spiersametrekking is ATP nodig; onder aërobiose vind hersintese plaas. Onder anaërobiese toestande, soos wanneer spiere anoksies raak as gevolg van vasokonstriksie tydens stress of nadat die dier uitgebloei het, vind 'n minder doeltreffende anaërobiese glikolise deur middel van die sarkoplasma-ensieme plaas en daal die ATP-vlak van die spiere terwyl melksuur aansamel. Hierdie proses word baie versnel by 'n temperatuur van 35°C en hoër. Die aktiwiteit van glikolitiese ensieme, soos fosforilase[15], is verhoog in spiere wat BSW-geneig is. Die vinnige daling in die ATP-konsentrasie van BSW-spiere veroorsaak dat *rigor mortis* reeds kort na dood (binne 2 uur) intree in vergelyking met normale varke waar rigor eers 4 tot 6 uur nadoods in tree[10]. 'n Lae ATP-vlak en 'n hoë melksuurkonsentrasie word dus in BSW-spiere, waarvan die temperatuur nog nie benede 35°C gedaal het nie, aangetref. As gevolg van die verhoogde aktiwiteit van ATP styg die nadoodse temperatuur van spiere, soms tot 45°C. Die graad waartoe en die snelheid waarmee *rigor mortis* intree, bepaal in hoeverre die aktien- en mosienfilamente oorvleuel wanneer aktomiosien gevorm word. Hoe meer die oorvleueling, hoe taaier sal die vleis wees. Stolling van die sametrekkende en sarkoplasmatiese proteïene by 'n hoë temperatuur veroorsaak ook 'n verlaging in die oplosbaarheid van genoemde eiewitte[9, 10, 14].

Mikroskopies

Die lengte van die sarkomere en I-bande variëer as gevolg van die mate van oorvleueling van die aktien- en miosienfilamente tydens spierkontraksie. Gedurende *rigor mortis* vind hierdie proses onomkeerbaar plaas, sodat die filamente 'n permanente binding vorm en nie meer ontsluit word en weg van mekaar skuif nie[8-10]. By BSW-spiere is die gestreepte voorkoms baie onduidelik as gevolg van die proteïen-denaturasie wat plaasgevind het. Omrede die aktiewe spierkontraksie wat tydens *rigor mortis* bestaan, is sekere spiervesels

sterk saamgetrek, wat aan die kort sarkomere en I-bande gesien kan word, terwyl spiervesels daarnaas lang sarkomere en I-bande vertoon en gegolfd lê, omdat die nabygeleë vesels meer saamgetrek is.

Spiere bevat hoosfaaklik twee spierveseltipes: die wit vesels het 'n groot deursnee en 'n anaërobiese metabolisme wat melksuurophoping bevorder, die rooi vesels se metabolisme is aërobies, oksideer melksuur geredelik en het 'n klein deursnee. Sommige spiere bevat oorwegend óf rooi óf wit spiervesels en word dan ook dienooreenkomstig geklassifiseer. Spiere soos byvoorbeeld die *M. semitendinosus* of *M. semimembranosus* het wit en rooi elemente [13]. Dit is veral die wit spiere of wit gedeeltes van tweekleurige spiere waarin melksuur maklik ophoop, ernstige proteïendenaturasie voorkom en al die tipiese verskynsels van BSW waargeneem word wanneer varke voor en tydens slagting aan stress onderhewig is. Die *M. longissimus dorsi* en die *M. semimembranosus* is die twee spiere wat die meeste aangetas word.

Fisiologies en Geneties

Die fisiologiese reaksies van die lewendige dier speel 'n baie belangrike rol in die bepaling van die kwaliteit van die vleis. In Nederland het Sybesma & van Logtenstijn [16] gevind dat die opgewonde toestand van sekere varke veroorsaak dat die bloedsomloop gestrem word, 'n suurstoftekort in die spiere ontstaan en terselfdertyd 'n hipertermie in die spiere ontwikkel as gevolg van die aktiewe afbraak van ATP. Die gevolglike lae ATP-vlak tydens slagting versnel dan die totstandkoming van *rigor mortis*. As gevolg van die verswakte bloedsomloop versnel die hartslag en asemhaling in 'n poging om die suurstoftekort aan te vul.

Sekere varkrasse is meer geneigd tot hierdie biochemiese en fisiologiese afwykings en toon 'n hoër voorkoms van BSW-vleis as die minder gevoelige rasse. Die Poland China-varke van Amerika en die Pietrain-varke van België is gevoelig vir stress-toestande en dus vir die ontwikkeling van BSW-vleis. 'n Besonder snelle nadoodse daling in spier-pH kom by hierdie varke voor, terwyl Groot Wit-varke besonder stressbestand is en die pH van hul vleis stadig daal. Deense en Nederlandse Landrasvarke beklee 'n posisie tussen hierdie twee uiterstes en daar is reeds tussen 35 en 40% van die varke by Deense toetsstasies wat dié verskynsel begin toon [7, 10]. Die Varkontwikkelingsvereniging van Engeland, PIDA, het onlangs 'n toename van 1 tot 4½% in die voorkoms van BSW-vleis in nageslagstoetse van hul varke gerapporteer [6].

Na 'n baie deeglike opname in die Verenigde Koninkryk het Bendall en sy medewerkers gevind dat 6,6% van die varke van nageslagstoetsstasies BSW-spiere het en slegs 1,8% van die kommersiële varke wat by dieselfde slaglokale geslag is. In dieselfde opname is vasgestel dat BSW-spiere by 10,5% van die Landras- en by 3,9% van die Groot Wit-varke voorkom. Alhoewel spesifieke rasverskille dus voorkom, is dit baie duidelik dat seleksie binne rasse vir groeisnelheid en gespierdheid ook meegewerk het om varke te teel wat meer stress-gevoelig en tot die nadoodse ontstaan van BSW-spiere geneig is. Die spiere van sulke diere bevat dan ook relatief meer van die wit spiervesels, waarin melksuur maklik ophoop met versnelde nadoodse glikolise en gevolglike ontstaan van BSW-vleis [13].

Stress en Omgewing

Lawrie [8] beskryf die metaboliese stresstoestande wat die spier beïnvloed baie duidelik en volledig. 'n Ewewigstoestand vir die ongeskondenheid van lewende weefsels is afhanklik van die dinamiese balans tussen kataboliese en anaboliese prosesse. Dit is dus duidelik dat die onderskeie metaboliese ewewigstoestande in 'n komplekse organisme fisiologies gekoördineer moet wees indien die organisme in sy geheel nadelige invloede moet kan weerstaan. Wanbalanse, wat as gevolg van hierdie nadelige invloede ontstaan, word na verwys as „stress". 'n Groot verskeidenheid van stress-effekte („stressors") kan die organisme beïnvloed, soos byvoorbeeld oefening, temperatuur, humiditeit, lugdruk, suurstofdruk, voeding, patologie, nadelige kunsmatige stowwe of invloede, verdowingsmiddels, toksiene, ioniserende bestraling, elektriese skok en sielkundige effekte (temperament, vrees, lig, geluid). Die hantering van 'n stress-toestand of die handhawing van homeostase deur die dier is hoofsaaklik afhanklik van die komplekse reaksies van die hipotalamus-hipofisebynier-reeks, dit wil sê die „algemene aanpassingsindroom". Indien die intensiteit van stress sodanig is dat die normale balans versteur en nie weer herstel kan word nie, het dit Selye se „aanpassingsiekte" of die dood tot gevolg.

In die praktyk word varke aan 'n verskeidenheid stress-toestande blootgestel. Dié

wat die ernstigste gevolge vir die nywerheid inhou, is toestande wat die dier kort voor slagting beïnvloed [1, 7, 8, 10, 16]. Varke word dikwels vervoer wanneer uiterste omgewingstemperature heers. Dit is veral hitte wat nadelig is vir varke wat in 'n voertuig saamgedrom is. Bakleiery kan verdere hipertermie by die diere veroorsaak en so ernstig wees dat aansienlike vrektes kan voorkom [5]. As gevolg van skok, vrees of bakleiery ontstaan vasokonstriksie, met reeds voordoodse opeenhoping van die melksuur in die spiere en 'n meer aktiewe anaërobiese metabolisme, sodat die spiere meer onderhewig is aan anoksiese toestande [10]. Diere wat in 'n hipertermiese, anoksiese toestand verkeer en dan natgespuit word, ondervind ook ernstige skok as gevolg van skielike temperatuurverskille en kan gevolglik maklik vrek. Skok, vrees en inspanning direk voordoods en sparteling direk nadoods veroorsaak versnelde glikolise en pH-daling in die spiere. Stress-gevoelige varke reageer meer ernstig onder sulke omstandighede, maar selfs minder gevoelige varke se spiere ondergaan nadoods 'n vinnige pH-daling indien hul skok opdoen vóór, en spartel na bedwelming. Ruwe hantering in die aanhoudingshokke en aanjaag na bedwelmingshokke, soms selfs met behulp van ligte elektriese skokke, het in alle gevalle 'n oplaaiende stress-effek. In Ierland [18] en Engeland [17] is bevind dat die bedwelmingsproses as sodanig ook 'n potensiëel nadelige effek op spierkwaliteit uitoefen. Vrees as gevolg van suurstofgebrek by varke wat die koolsuurgastonnel binnegaan veroorsaak meer sparteling as by varke wat elektries verdoof word [17]. Die ernstigste sparteling na bedwelming word egter aangetref wanneer varke met die penpistool in die brein geskiet word: gevolglik toon byna alle varke wat so bedwelm word die tipiese BSW-verskynsels in die spiere [11]. Onnodige vertraging van die afslag-, ontweiding en speksyvoorbereidingsprosesse kan ook vinnige suurvorming onder hoë temperatuurtoestande in die spiere bewerkstellig [10].

Ekonomies en Tegnologies

Finansiële verliese kan reeds ten opsigte van vrekte onder die lewendige diere voorkom as gevolg van een of meer van die reeds bespreekte faktore. Baie opvallend en dramaties is egter die afwykende spier- of vleiseienskappe wat by varkkarkasse voorkom wat versnelde *rigor mortis* en glikolise ondergaan terwyl die temperatuur van die karkas nog bokant 35°C lê. By normale hamme het al die spiere min of meer dieselfde kleurintensiteit. By BSW-hamme kom 'n kenmerkende tweekleurige effek voor wat die gevolg is van die verskil in nadoodse glikolise in die verskillende spiere waarvan die proteïene nie tot dieselfde mate gedenatureer is nie [10]. Die tegnologiese probleme wat met BSW-vleis ondervind word bring egter die ernstigste finansiële verliese vir die vervaardiger mee. Gewigsverliese van spiere van selfs 6 tot 10% as gevolg van verlies aan vrygestelde sarkoplasmavog kom voor [10]. Tydens die verpakking van verbruikersporsies transudeer BSW-spiere meer as normale spiere, tydens inpekeling vind tot 5% meer gewigsverlies plaas, tydens gaarmaak word 20% meer vogverlies ondervind en tydens inmaak vorm 4 tot 8% meer gelatien buite om die hamme of ander snitte in die blikke. Hierdie nadelige eienskappe van BSW-spiere is reeds die afgelope 15 jaar baie deeglik eksperimenteel onder gekontroleerde toestande en koöperatief in die bedryf in baie lande van die wêreld gedemonstreer [1, 6, 9, 10, 14]. Briskey [10] het na aanleiding van hierdie kwaliteitsafwyking reeds in 1964 bereken dat dit vir 'n groot spekfabriek in Amerika, wat ongeveer 8 000 varke per dag, of twee miljoen per jaar, slag, 'n jaarlikse verlies van bykans twee miljoen dollar meebring.

Voorkoms

Om die voorkoms van BSW-vleis vas te stel, word verskeie parameters soos kleur, temperatuur, rigorontwikkeling en die pH_1-waarde van spiere bepaal, waarvan laasgenoemde, wat ongeveer 45 minute nadoods bepaal word, die mees betroubare en algemene maatstaf is. Indien 'n pH_1-waarde van 6,0 en laer waargeneem word, terwyl die spiertemperatuur by spekvarke gewoonlik nog hoër as 35°C is, kan met 'n redelike mate van sekerheid voorspel word dat die vleis BSW-eienskappe tydens verwerking sal openbaar.

Sulke opnames het aan die lig gebring dat die voorkoms van BSW-karkasse in die V.S.A. 18%, in Denemarke by hul toetsstasies tot 35%. in Ierland tussen 6 en 22% en in Engeland tussen 2 en 10% onder verskillende toestande beloop [10, 17, 18].

NAVORSING IN SUID-AFRIKA

Opnames

In Tabel 1 word die syfers van verskeie opnames in Suid-Afrika aangedui [19, 20].

Tabel 1: pH_1-WAARDES VAN SPEKVARKE BY VYF SENTRA

Sentrum	Getal	$\bar{X}$ pH_1	%pH ⩽6,0	Min p.m.*	Spier**	Dwelm-tegniek***
Fabriek A	580	6,43	8,6	45	L.D.	E
Fabriek B	1 019	6,51	5,0	30	L.D.	E
Fabriek C	626	6,24	28,6	45	S.M.	E
Slagplaas D	285	6,41	17,5	45	S.M.	E
Abattoir E	32	5,50	100,0	45	L.D.	P
Abattoir E	(32)	5,64	96,3	45	S.M.	P
Abattoir E****	29	5,75	87,2	45	L.D.	P
Abattoir E	(29)	5,83	79,3	45	S.M.	P

*Min. p.m. — minute **post mortem**
L.D. — **M. longissimus dorsi
S.M. — **M. semimembranosus
***E — Elektriese bedwelming (90—110 Volt)
***P — Penpistool-bedwelming
**** — Speen- en vleisvarke

Die getalle tussen hakies dui aan dat dieselfde karkasse betrokke was as in die onmiddellik bostaande ry.

A, B en C is drie groot spekfabrieke in verskillende dele van die land en D en E is twee munisipale abattoirs in beheerde gebiede. Klingbiel en Naudé[19] het in hul publikasie die prosedure wat by fabrieke A en B gevolg word, noukeurig beskryf. Die fabrieksprosedure by hierdie twee fabrieke het baie nou ooreengekom.

Die laer pH_1-waarde van die varke by fabriek A is verklaar deur die feit dat die waarnemings vyftien minute later nadoods geneem is en die pH dus verder kon gedaal het. Hierdie aanname is bevestig deur pH-bepalings op verskillende tye na dood te neem. 'n Daling van 0,15 pH-eenhede is gedurende 15 minute waargeneem, wat 'n verhoging van ongeveer 10 persentasie-eenhede in die melksuurkonsentrasie van die spier verteenwoordig[21]. Dit kan dus met redelike sekerheid aanvaar word dat die pH-lesings en die persentasie karkasse wat pH_1-waardes van 6,0 en laer by die twee fabrieke gehad het, ongeveer dieselfde is, naamlik pH_1 6,43 en 8% respektiewelik, wat baie goed ooreenstem met die syfers van Bendall en sy medewerkers[17] in Engeland. Die *M. longissimus dorsi* by die tiende rib is as 'n indikatorspier vir die ontwikkeling van 'n BSW-toestand gebruik, aangesien dit een van die mees vatbare spiefe in die karkas is[6, 8, 15]. Dit is relatief maklik om pH-lesings 45 minute na dood van hierdie spier te neem, nadat die werwelkolom by spekkarkasse verwyder is. Indien die vloei van karkasse egter stadig verloop, of lesings geneem word by abattoirs waar karkasse nie halveer word nie, is pH-waarnemings gedoen by die ontblote gedeelte van die *M. semimembranosus*. Hierdie spier ondergaan ook baie vinnige nadoodse glikolise[5, 8, 9], alhoewel effens stadiger as in die geval van die *M. longissimus dorsi*. Dit word duidelik weerspieël in die pH_1-waarnemings by slagplaas E (Tabel 1), wat op hierdie twee spiere in dieselfde karkasse gedoen is. By eersgenoemde is die verskil tussen die twee spiere 0,14 pH-eenhede en by laasgenoemde is dit 0,08.

Die opvallende verskil tussen fabrieke is eerstens die veel hoër voorkoms van BSW-karkasse (persentasie met pH_1 gelyk aan of laer as 6,0) naamlik 28,6% by fabriek C en 17,5% by abattoir D, teenoor slegs 8,6% by fabriek A, ten spyte van die feit dat C en D se syfers ten opsigte van die relatief minder sensitiewe *M. semimembranosus* gemeet is. Die verspreiding van pH_1-waardes en die verskil in die vorm van die frekwensiedistribusie van genoemde waardes van fabrieke A en C word ook duidelik in figuur 2 geïllustreer.

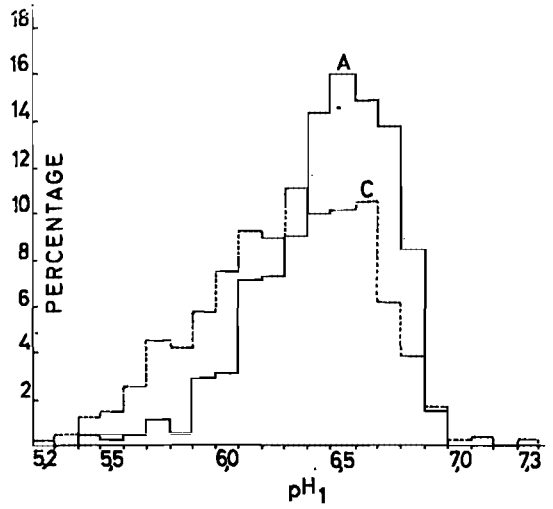

Fig. 2: Die verspreiding van pH_1-waardes van spiere in speksye by twee fabrieke (A——; C- - - -).

By lokale A, B, C, en D is alle varke elektries bedwelm. By abattoir E, waar alle varke met die penpistool bedwelm is, het 100% van die spekvarke en 87,2% van die jonger varke se oogspiere snel nadoodse glikolise ondergaan. 'n Daling in pH van 7,0 by die lewendige dier tot 5,5 45 minute nadoods verteenwoordig 'n toename in die melksuurkonsentrasie van die spiere van 20 tot 120 μ mol/g spier [18, 21]. Hierdie hoë voorkoms van BSW-spiere by abattoir E kan byna uitsluitlik gewyt word aan die sparteling van varke wat deur penpistool-bedwelming veroorsaak word [6]. Dit is ook by abattoir E gevind dat jong varke minder sensitief is as spekvarke ten opsigte van die gevolge van spierkontraksies. Carroll [22] is van mening dat die jong diere nog 'n groter verhouding rooi teenoor wit spiervesels het en dus minder geneig is om melksuuropeenhoping in die spiere te ondergaan as ouer diere.

Die baie hoë voorkoms (28,6%) van BSW-spiere by fabriek C is vervolgens ondersoek. In Tabel 2 word die resultate van een van die vernaamste oorsake aangedui, naamlik die vertraagde ontweiding by hierdie fabriek.

Tydens die bepaling van pH_1-waarnemings (45 minute *post mortem*) was 'n groot persentasie karkasse nog nie ontwei nie en baie karkasse het tot 150 minute gehang alvorens die ingewande verwyder is. Terwyl 22,87%

Tabel 2: DIE INVLOED VAN ONTWEIDING OP pH_1 BY FABRIEK C

Behandeling	n	$\bar{x}$ pH_1	%$pH_1 \leq 6,0$
Karkasse nie ontwei	116	6,15	41,38
Karkasse ontwei en onbekend	510	6,28	22,87
Totaal	626	6,25	28,59

van die vroeg ontweide karkasse pH_1-waardes van 6,0 en laer getoon het, was dit die geval by 41,38% van varke wat 45 minute na dood nog nie ontwei was nie. Hierdie gegewens stem baie goed ooreen met dié van Bendall [15] en van Wismer-Pedersen [1].

Tabel 3: DAAGLIKSE pH_1-WAARDES (FABRIEK C)

Datum	Getal	$\bar{x}$ pH_1	$pH_1 \leq 6,0$%	
			Getal	%
29/7	123	6,26	41	33,3
4/8	115	6,22	38	33,0
5/8	49	6,18	18	36,7
11/8*	91	6,40*	15	16,5*
18/8	103	6,27	23	22,3
19/8	145	6,21	44	30,3
	626	6,24	179	28,6

*Fabrieksprosedure ten opsigte van vloei van karkasse verloop besonder gunstig.

In Tabel 3 word die daaglikse wisseling in die voorkoms van pH_1-waardes aangedui. Voor verwerking van die syfers is aangeteken dat op die 11de Augustus by fabriek C die vloei van karkasse vanaf bedwelming tot verkoeling baie vlotter verloop het as wat dit die vorige drie opnamedae die geval was. By verwerking van die data het dit dan ook geblyk dat baie minder lae pH_1-waardes op die betrokke dag gevind is, naamlik 16,5% in vergelyking met meer as 30% die vorige drie dae.

Dit is ook by fabriek C en abattoir D opgemerk dat varke baie ruwer hanteer word tydens aflaai en aanjaag na die bedwelmingshok (dikwels met 'n elektriese skokker). Be-

dwelming met die elektriese verdowingsapparaat is baie onhandiger en dikwels nie regoor die brein gedoen nie, en moes gevolglik langer toegedien word, wat dan meer sparteling tydens dood veroorsaak het. Lister [7] maak in sy oorsig ook melding van die rol wat die vaardigheid van bedwelmingstoepassing speel ten opsigte van spierkontraksies.

Klingbiel en Naudé [19] het gevind dat geslag van die dier, karkasgewig en rugvetdikte by die tiende rib weinig of geen rol speel in die voorkoms van lae pH_1-waardes nie. Dit is in ooreenstemming met die werk van oorsese navorsers [10, 17, 23]. Hulle het ook rigor-ontwikkeling in die *M. semimembranosus* bepaal en gevind dat 'n hoër mate van spier-rigor gepaard gaan met 'n laer pH_1. Van die hoë rigor-spiere het 24,0% 'n pH_1 van 6,0 en laer gehad en by die lae rigor-groep was dit slegs 4,5. Nederlandse werkers [24] gebruik dan ook die rigor-meter in hul fabrieke om kwaliteitsklassifikasie van karkasse voor verkoeling te doen soos wat Deense fabrikante die pH-meter [10] gebruik. Die oorsprong van die varke het 'n daadwerklike invloed op die lae pH_1-lesings gehad, aangesien sekere boere 'n aansienlike groter hoeveelheid potensiële BSW-varke laat slag het as andere.

Gekontroleerde waarnemings

Naudé en medewerkers [20] het gevind dat die pH_1-waardes hoër en die spiertemperatuur laer by klein speen- as by groter spekvarkkarkasse 45 minute na dood is. In die *M. longissimus dorsi* het die spiertemperatuur 45 minute na bedwelming by speenvarke reeds so laag as 30,6°C gedaal, teenoor die hoë waarde van 39,6°C wat by spekvarke waargeneem is (Tabel 4).

Tabel 4: SPIEREIENSKAPPE VAN PISTOOL-BEDWELMDE VARKE*

	Speenvarke	Spekvarke
Getal	22	32
Ouderdom (md)	2,0	6,0
Karkasgewig (kg)	15,6	68,1
M. Longissimus dorsi		
Temperatuur (45 min)	30,6°C	39,6
pH (45 min) — pH_1	5,8	5,5
pH (24 uur) — pH_f	5,6	5,3
M. Semimembranosus		
Temperatuur (45 min)	34,5°C	40,2
pH (45 min) — pH_1	5,8	5,6
pH (24 uur) — pH_f	5,7	5,4

*Landras en Landras × Groot Wit.

Alhoewel die pH van die pistoolbedwelmde speenvarke se spiere dus reeds tot onder 6,0 binne 45 minute gedaal het, was die temperatuur reeds onder 35°C en sou die tipiese BSW-eienskappe waarskynlik nie ontwikkel het nie. Die groter spekkarkasse het gladnie afgekoel nie, die pH het meer gedaal en proteïen-denaturering het dus ongetwyfeld plaasgevind.

In 'n volgende eksperiment [20] is spekvarkkarkasse direk na ontweiding (25 minute *post mortem)* gehalveer. Een sy het in die slaglokaal by omgewingstemperatuur bly hang en die teenoorgestelde sy is vir 20 minute by −20°C verkoel. Hierdie penpistoolbedwelmde varke het pH_1-waardes van laer as 6,0 gehad, die verkoelde en onverkoelde sye se spiertemperature was nog hoër as 36,5°C, en het nie van mekaar verskil nie. Soos wat Lister [7] en Carroll [13] dan ook tereg gemeld het, kan kommersiële verkoeling nie spekvarkkarkasse vinnig genoeg laat afkoel om spierproteïen-beskadiging onder lae pH_1-toestande te voorkom nie.

In Tabel 5 word die resultate van 'n gekontroleerde eksperiment weergegee.

Die drie bedwelmingstegnieke wat kommersiëel toegepas word is met mekaar vergelyk ten opsigte van die pH_1-waardes by spekvarke. Vorige waarnemings ten opsigte van die penpistool is weereens bevestig [18, 20]. Koolsuurgastoediening het heelwat meer sparteling as elektriese verdowing tot gevolg gehad, wat ook weerspieël word in die laer pH_1-waardes van albei spiere van eersgenoemde varke. Alhoewel die toediening van koolsuurgas nie onder sulke ideale toestande as by fabrieke toegepas is nie, en dus meer sparteling tot gevolg kon gehad nie, blyk dit tog asof elektriese verdowing in ooreenstemming met oorsese resultate [17] minder spieraktiwiteit veroorsaak as koolsuurgasverdowing. By die penpistoolgroep was weereens 'n baie hoë persentasie van die oogspiere BSW (80,0%) teenoor 20% by die koolsuurgasgroep en 0,0% by die elektriesbedwelmde groep. As gevolg van koolsuurgas-toediening vir 50 tot 60 sekondes was die bloed-pH, wat direk nadoods bepaal is, heelwat laer as in die ander twee groepe. Gedurende hierdie tyd sirkuleer die bloed voordat die are afgesny word, terwyl by penpistool- en elek-

Tabel 5: DIE INVLOED VAN BEDWELMINGSTEGNIEK OP pH BY LANDRAS SPEKVARKE

Bedwelming	n	M. longissimus dorsi			M. semimembranosus			Bloed*** pH
		pH_1	$pH_1 \leqslant 6,0$	pH	pH_1	$pH_1 \leqslant 6,0$	pH_f	
			%			%		
Penpistool	10	5,79	80,0	5,50	6,10	60,0	5,49	7,82
Koolsuurgas*	10	6,31	20,0	5,57	6,47	0,0	5,59	7,43
Elektries**	10	6,48	0,0	5,57	6,48	0,0	5,56	7,73

*65% CO_2 **90 Volt ***direk na dood

triese bedwelming die vark byna dadelik na bedwelming uitgebloei word.

In spiere met lae pH_1-waardes het Dreyer en Horn [25] proteïen-denaturasie histologies duidelik gedemonstreer en 'n groter hoeveelheid tussenselvloeistof gevind. Hulle het ook by stressgevoelige varke „reuse-spiervesels" gevind, wat moontlik van die anaërobiese tipe kon gewees het, aangesien baie korter sarkomere gedemonstreer kon word. Hierdie vesels moet dus meer kontraksie as die ander vesels in die spier ondergaan het, wat kan dui op 'n versnelde nadoodse glikolise. 'n Heel verskillende kleurreaksie is ook by die „reusevesels" as by die ander rooi en wit vesels waargeneem. Hierdie vesels kan moontlik as 'n verdere parameter van BSW-spiere gebruik word.

GEVOLGTREKKINGS

Na aanleiding van die bevindinge van oorsese werkers kan dit aanvaar word dat BSW-vleis vir die vleisprosesseerder en -verpakker die nadeel van groot finansiële verliese inhou. Resultate dui daarop dat by Landrasvarke meer gevalle van BSW voorkom as by Groot Wit-varke. Seleksie vir maerder, meer gespierde varke, wat vinniger en meer doeltreffend groei, gaan hoogs waarskynlik gepaard met seleksie vir spierensiem-sisteme wat 'n vinnige nadoodse glikolise in die hand werk. Die invloed van omgewingstoestande, soos hantering van diere vóór, tydens en na dood, kon ook duidelik geïllustreer word. Die gevolgtrekking van Lister [7] en Carroll [13], dat kommersiële verkoelingstegnieke karkasse nie vinnig genoeg kan afkoel om BSW te voorkom nie, is bevestig. Daar moet gevolglik gepoog word om meer gematigde pH-veranderings nadoods te bewerkstellig, hetsy deur die daarstelling van omgewingstoestande wat spanning by varke vóór, tydens en na slagting sal verlig, of deur die seleksie en teling van meer stress-bestande varke. Hierdie toestand van BSW-vleis, wat reeds by pluimvee en varke aangetref word, is ook reeds by beeste in Frankryk [26] en by buffels in Suid-Afrika [27] waargeneem.

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PALE, SOFT, EXUDATIVE PORK, PORCINE STRESS SYNDROME AND MALIGNANT HYPERPYREXIA—AN IDENTITY ?

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SUMMARY

Pale, soft exudative pork (PSE pork) is a stress-related syndrome, resulting in undesirable meat quality. Clinical and biochemical features of the porcine stress syndrome (PSS) seemed similar to those described in the syndrome of anaesthetic-induced malignant hyperpyrexia manifested by swine. An investigation is described which seeks to establish if swine susceptible to malignant hyperpyrexia would manifest PSE pork. Changes occurring *post mortem* in the muscle of swine susceptible to malignant hyperpyrexia and killed by exsanguination were studied. These demonstrated that the muscle of swine susceptible to malignant hyperpyrexia shows the same gain in expressible water with a similar pattern of pH fall to that associated with PSE pork.

INTRODUCTION

The condition now called pale soft exudative pork (PSE pork) has long been recognized [1] and is of economic importance in the pork industry. Characterized by rapid *post mortem* glycolysis, accumulation of lactic acid and rapid fall in muscle pH which results in undesirable meat quality, PSE pork has been shown to be a stress related syndrome [2-4].

The porcine stress syndrome (PSS) is also of economic importance: it causes death of swine in transport, is characterized by progressive dyspnoea, increasing body temperature, death within minutes of onset of symptoms and immediate rigor mortis [2]. This acute death syndrome in stress-susceptible swine bears an extremely close clinical similarity to the syndrome of malignant hyperpyrexia which follows exposure of susceptible swine to halothane anaesthesia and succinylcholine [5-7], and even severe exercise [8].

Such association prompts the hypothesis that PSS, PSE pork and malignant hyperpyrexia in swine are indeed manifestations of the same myopathy. It was of interest, therefore, to establish whether malignant hyperpyrexic swine, which, at initiation of the syndrome, manifest extremely rapid glycolysis, lactacidosis and rapid pH fall immediately *ante mortem* [9], would develop PSE pork *post mortem*.

MATERIAL AND METHODS

The *post mortem* changes in muscle in three groups of swine were studied:

Group 1 normal swine (controls) (7 pigs)

Group 2 swine susceptible to malignant hyperpyrexia (MHS) (3 pigs)

Group 3 Swine, susceptible to malignant hyperpyrexia, in which the syndrome was established *ante mortem* (MHAM) (3 pigs)

The inclusion of Group 3 was motivated by a desire to simulate the possible initiation in susceptible swine of the syndrome *ante mortem* by the exercise, struggle and stress of abattoir slaughter. The swine studied were Landrace and Landrace×Large White crossbreds from the Western Cape region.

Susceptibility to malignant hyperpyrexia was tested by screening all animals with a test challenge of halothane anaesthesia [5] one week or more before each experiment.

All pigs were killed by exsanguination from the aorta following induction of general anaesthesia. Those in Groups 1 and 2 were anaesthetized with thiopentone sodium, orotracheal intubation with nitrous oxide and oxygen administered by an intermittent positive pressure ventilation technique. Those in Group 3 were anaesthetized with halothane, thus effectively initiating the syndrome of malignant hyperpyrexia *ante mortem*. Whereas most reported investigations into PSE pork were undertaken on pigs slaughtered in abattoirs by stunning and exsanguination, our in-

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vestigation was undertaken on animals being used in surgical research as blood donors. Hence our use of general anaesthesia. We regarded the subsequent death of the animal by exsanguination as simulating that of abattoir slaughtered pigs.

Immediately following the death of the animal, it was turned prone. A pH electrode (Metrohm Herisau compensator E 388) and thermistor probe (Ellab Type TE 3) were inserted directly into the belly of one longissimus dorsi muscle at the level of L 2—3, while the opposite longissimus dorsi was exposed, and from it serial sections were taken for analysis.

The following observations were made at 30 mins., 1 hour, 1½ hours, 2½ hours, 3½ hours, 5½ hours and 24 hours *post mortem*:—

1. Intramuscular pH.
2. Muscle temperature.
3. Estimation of expressible muscle water [3].
4. Measurement of muscle lactate (Boehringer Mannheim Test Combination—Biochemica, Cat. No. 15972). This measurement was only performed up to 5½ hours *post mortem*. Between 5½ and 24 hours the carcass was refrigerated to 2—4°C.

Though other workers had graded meat on colour, we found the assessment of various grades of "pink" subjective and abandoned the use of this observation.

RESULTS

Expressible water

The changes recorded in expressible water *post mortem* are presented diagrammatically in Figure 1, with the actual amounts and the statistical significance of the relevant differences in Table 1. The groups of animals displayed 3 grades of increasing expressible water content *post mortem* in the order—

Control (1) < MHS (2) < MHAM (3)

Whereas the differences in expressible water content between the MHAM and the control group are significant over the whole time range, that between the MHS group and controls achieve significance after 5 hours. Though the means of the MHS and MHAM groups differ, statistical significance is only

Table 1: CHANGES IN EXPRESSIBLE WATER

Water content expressed as mg/g of muscle

	HOURS POST MORTEM						
GROUP	½	1	1½	2½	3½	5½	24
CONTROL (1)	374	368	359	354	387	368	455
MHS (2)	400	405	389	393	429	453	533
MHAM (3)	482	506	496	540	516	557	575
	Statistical Significance of Differences						
CONTROL—MHS	NS*	NS	NS	NS	NS	P<0,02	P<0,001
CONTROL—MHAM	P<0,01	P<0,002	P<0,01	P<0,01	P<0,05	P<0,001	P<0,001
MHS—MHAM	NS	NS	NS	P<0,05	NS	N5	NS

*NS = not significant

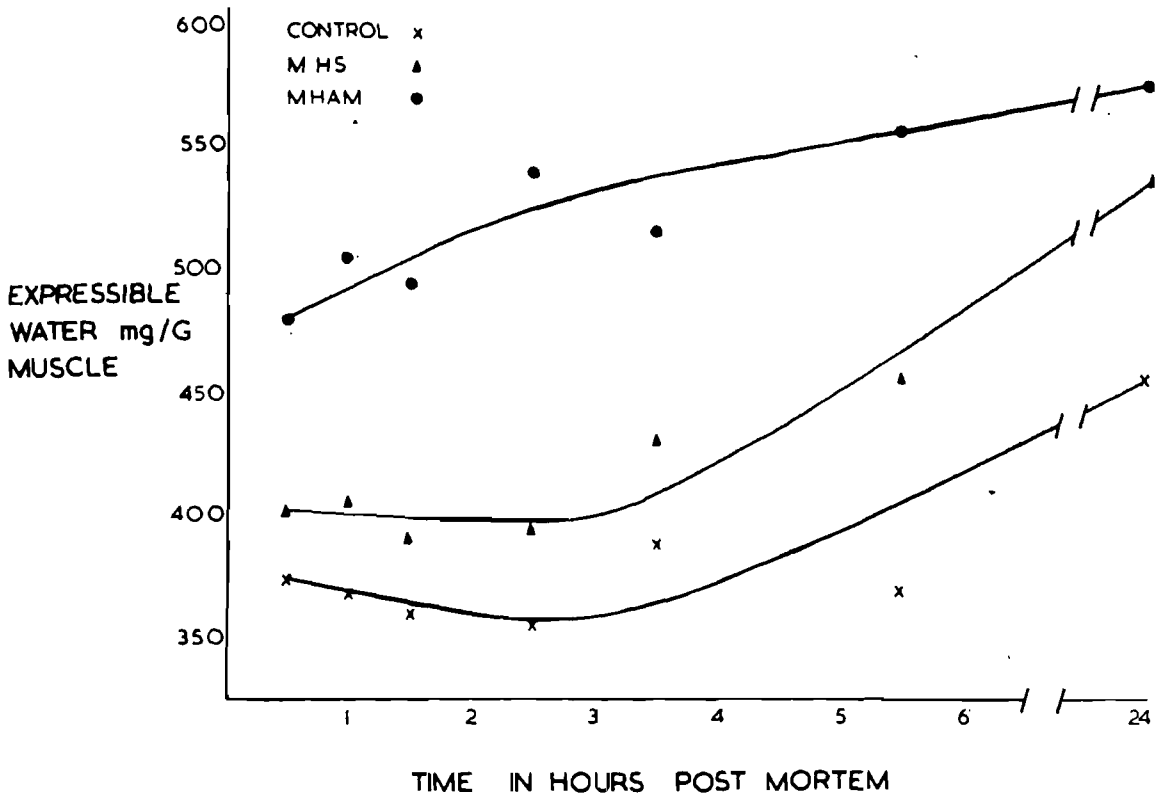

FIGURE 1

achieved at one point. Differences were such, however, that it is felt that statistical significance would have been achieved had more of the MHS animals been available. The expressible water content of both the MHS and MHAM groups at 24 hours is well within the range observed in PSE pork [3, 10].

pH

Changes observed in intramuscular pH are presented diagrammatically in Figure 2 with the actual figures and statistical significance of the relevant differences in Table 2. Though the ultimate pH reached is the same, the rate of fall is different in the three groups of animals in the order—

MHAM (3) > MHS (2) > Control (1)

That of the MHAM group is the most rapid, virtually reaching its lowest limit by the time the first observations were made 30 minutes *post mortem*. That of the MHS group starts at a level near that of the control group but falls rapidly to reach that of the MHAM group by 2½ hours *post mortem*. The control group only reached similar levels at 5½ hours *post mortem*.

The water-holding property of pork has been shown to depend on the rate of pH fall *post mortem*, a reflection of the rate of *post mortem* glycolysis [1-3, 10]. Taking the pH values recorded at 30 minutes, 60 minutes and 90 minutes after exsanguination as reflecting the rate of pH fall, we were able to show a strong inverse correlation between these values and the expressible water content of pork measured at both 5 hours and 24 hours *post mortem*, i.e. the more rapid the pH fall, the higher the expressible water content. The correlation coefficients with their relevant probability values are presented in Table 3.

Lactate

Corresponding with the patterns of pH fall, three grades in the rise of muscle lactate were observed in these groups of animals, in the order, for rate and quantity:

Table 2: CHANGES IN pH

GROUP	HOURS POST MORTEM						
	½	1	1½	2½	3½	5½	24
CONTROL (1)	6,70	6,40	6,23	6,02	5,91	5,82	5,70
MHS (2)	6,42	6,05	5,79	5,61	5,63	5,56	5,58
MHAM (3)	5,78	5,59	5,55	5,53	5,50	5,59	5,77
	Statistical Significance of Differences						
CONTROL—MHS	NS	$P<0,02$	$P<0,02$	$P<0,05$	NS	NS	NS
CONTROL—MHAM	$P<0,01$	$P<0,001$	$P<0,002$	$P<0,01$	$P<0,05$	NS	NS
MHS—MHAM	NS	NS	NS	NS	NS	NS	NS

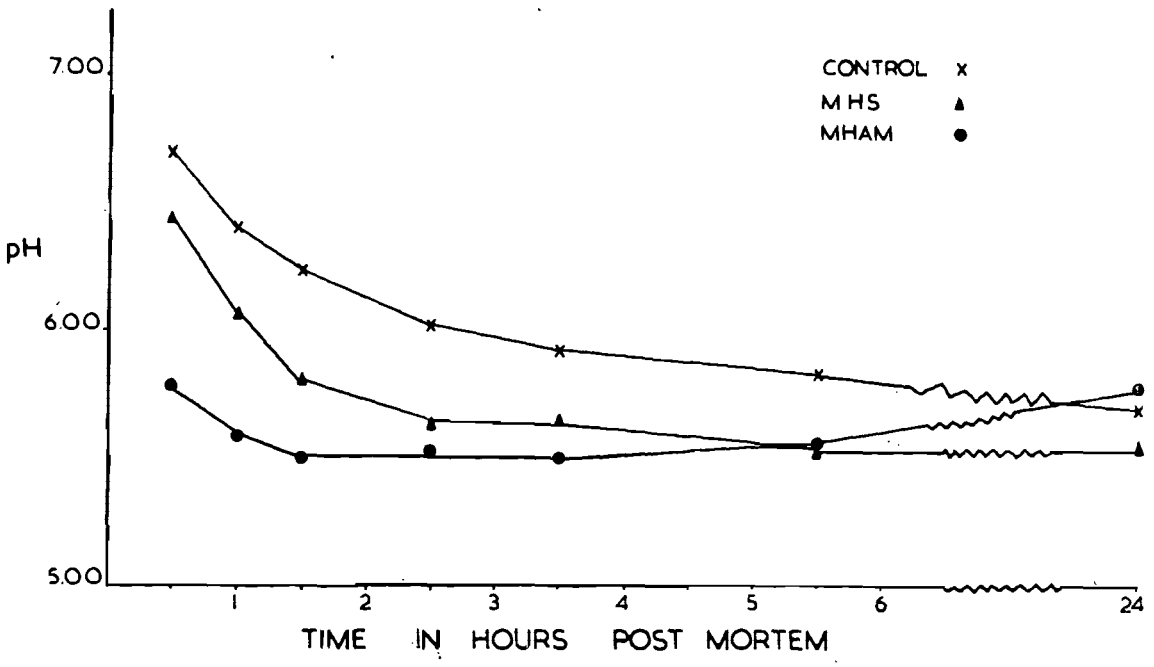

FIGURE 2

Table 3: CORRELATION COEFFICIENTS

EXPRESSIBLE WATER/pH

	pH AT TIME POST-MORTEM (P.M.)		
	30 minutes	60 minutes	90 minutes
Expressible water at 5 hours p.m.	r = –0,89 P < 0,001	r = –0,87 P < 0,001	r = –0,83 P < 0,001
Expressible water at 24 hours p.m.	r = –0,67 P < 0,05	r = –0,75 P < 0,01	r = –0,74 P < 0,01

DISCUSSION

The criteria by which PSE pork is judged are primarily colour and content of expressible water [2, 3]. It has been shown that PSE pork is associated with circumstances which result in rapid *post mortem* glycolysis, myolactosis and rapid fall in pH [10, 11]. Such conditions appear to exist in association with the porcine stress syndrone (PSS). Response of stress-susceptible swine to environmental [12], anoxic [13] and exercise stress [8] appears to resemble closely the clinical [5] and biochemical changes [9] observed in the syndrome of malignant hyperpyrexia. Our observation of the *post mortem* changes in muscle of malignant hyperpyrexial swine shows that it manifests the same reduction in water-holding with a similar pattern of rapid pH fall as that associated with PSE pork. In those swine, in which the syndrome was actually initiated before death, the changes were most rapid and severe, the pH reaching its minimum very shortly after death. When the syndrome was not initiated *ante mortem*, the pattern of glycolysis, pH fall and myolactosis was still much faster than that of controls, with a similar loss in water-holding property. Though the patterns of *post-mortem* pH fall displayed by swine susceptible to malignant hyper-

Table 4: CHANGES IN LACTATE

Lactate expressed in μmol/g muscle

GROUP	HOURS POST MORTEM					
	½	1	1½	2½	3½	5
CONTROL	31	40	43	48	58	70
MHS	43	49	50	62	79	100
MHAM	87	99	108	125	120	123
	Statistical Significance of Differences					
CONTROL—MHS	NS	NS	NS	NS	NS	P<0,05
CONTROL—MHAM	P<0,01	P<0,002	P<0,04	P<0,001	P<0,001	P<0,001
MHS—MHAM	NS	NS	NS	NS	NS	NS

MHAM (3) > MHS (2) > Control (1)

These values are presented diagrammatically in Figure 3 with the actual values and statistical significance of the relevant differences in Table 4. The differences between the control group and the MHAM group were highly significant over the whole range. That apparent between the MHS and MHAM group just failed to achieve significance due to the small size of the two groups of animals. Starting at the same level as that of the control group, the MHS pigs show a higher rate of lactate production, the difference from the controls achieving statistical significance at 5½ hours *post mortem*.

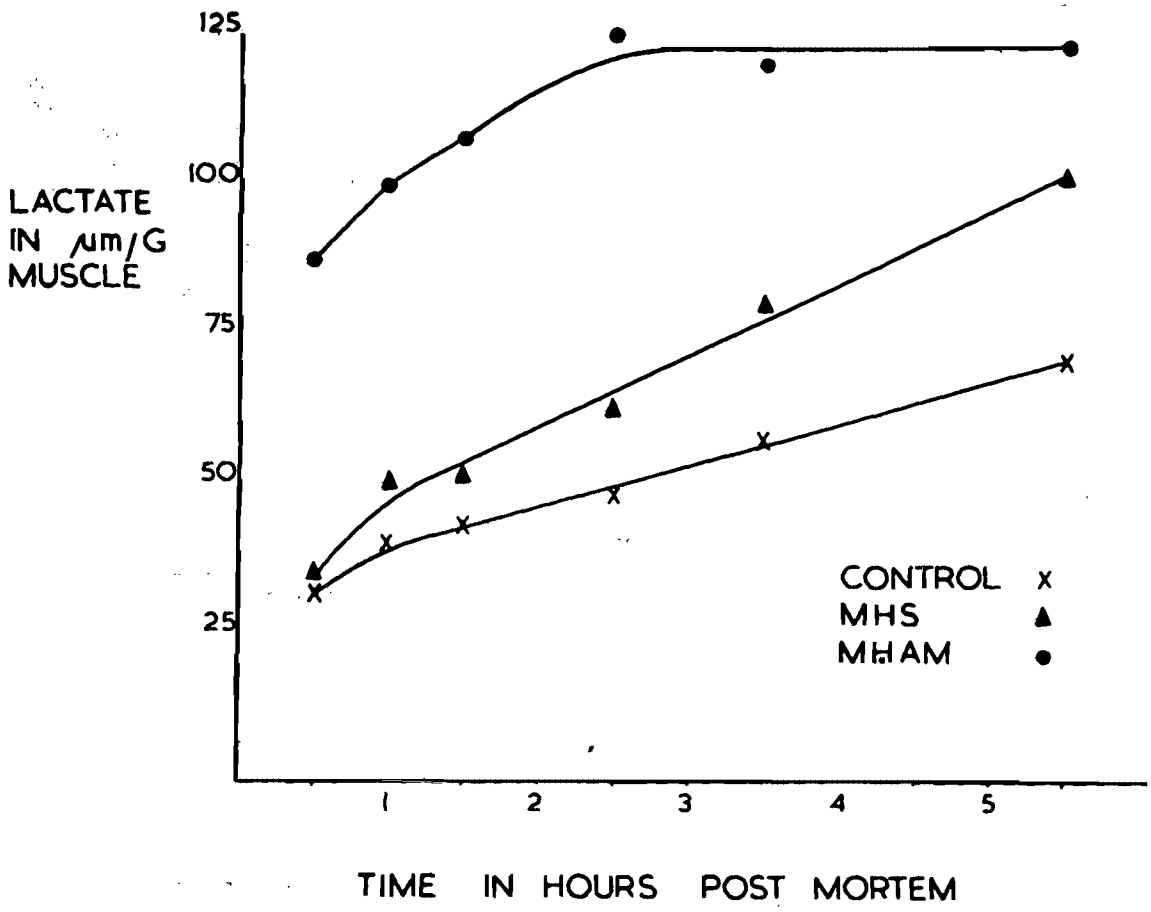

FIGURE 3

pyrexia were similar to those described in PSS by Wismer-Pedersen & Briskey[10] and Toppel[2], we did not observe as low levels, nor the even lower levels described by Lawrie *et al*[11]. This may possibly be the result of our use of anaesthesia before exsanguination, with a resultant tranquil death, whereas stress and struggling usually preceded the stunning and exsanguination at the abattoir as observed in these studies.

Malignant hyperpyrexia swine have been shown to have higher levels of serum CPK[14]. This same enzyme is markedly raised in association wit PSE pork[15]. Our demonstration of the PSE pattern of expressible water in the carcasses of malignant hyperpyrexia-susceptible swine is further evidence that PSS, PSE pork and susceptibility to malignant hyperpyrexia may well be an identity—all expressions of the same myopathy.

Should such an hypothesis be valid, current concepts of the aetiology of malignant hyperpyrexia[16-19] could help towards an understanding of PSE pork and PSS and conversely so.

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REPRESENTATIVE RUMEN SAMPLING*

T. L. TALJAARD*

SUMMARY

The use of a large (80 mm internal diameter) rubber rumen cannula for obtaining truly representative samples of ruminal ingesta from sheep fed long lucerne hay is described. Samples of ingesta removed through the large cannula by bailing or ladling were shown to be similar and more representative than those removed by a small (15 mm) diameter tube through a conventional ruminal cannula (25 mm internal diameter) in respect of dry matter content and numbers of total culturable rumen bacteria. Six parameters were used to demonstrate that the function of the rumen fitted with the large cannula remains physiological for a year at least.

INTRODUCTION

To study the microbial population in the rumen, its substrates and products of fermentation—so vital to the proper understanding of the physiology of ruminant nutrition—adequate sampling is essential. One cannot assume that the commonly used method of sampling by means of a ruminal cannula will provide a true reflection of either the microbial population or the products of fermentation *in vivo*.

ATTACHED AND FREE BACTERIA

Baker [1] found that a large proportion of the bacteria was attached to solid ingesta, while the remainder was freely suspended in the ruminal fluid. There is very little information about the distribution of different species of bacteria between solid and liquid components of the ingesta. Brinkman [2] found that 84 per cent of the glucolytic bacteria in the ruminal ingesta of sheep fed teff hay and dosed with urea and glucose, were attached to the solid components when Gram-positive cocci predominated, and 28 to 69 per cent when Gram-negative curved and straight rods preponderated. Thus from 28 to 84 per cent of the glucose metabolizing activity would be removed by straining the ingesta.

In ruminal ingesta taken by tubes of 15 mm diameter from similarly fed sheep, Schwartz, Schoeman & Färber [3] found the rate of carbohydrate breakdown by the unstrained sample to approximate most closely the rate found *in vivo* and to be as much as three times faster than in the fluid obtained after straining the sample through two layers of cheese cloth. On the other hand, urea was broken down at a similar rate to that *in vivo* by either unstrained or strained samples, thus indicating that the ureolytic bacteria were not attached preferentially to the solid portion of the ingesta. Nevertheless, the rate and the extent of incorporation of the ammonia nitrogen produced from the urea were much lower in the strained sample. Straining, therefore, had changed the potentialities of the ruminal ingesta for converting non-protein nitrogen compounds into bacterial protein.

SAMPLING METHODS

Tube and hand-scooped samples

Gilchrist (unpublished data) compared a sample of ingesta removed through a ruminal cannula with a 15 mm diameter tube and a hand-scooped sample removed from the same sheep by rumenotomy. The sheep had also been fed teff hay and dosed with urea and glucose. On the average, there were 2,2 times as much wet solids in the hand-scooped sample as in the tube sample. In line with this finding, the numbers of the total culturable cellulolytic and glucolytic bacteria were 2,5, 2,6 and 2,3 times greater respectively in the hand-scooped than in the tube sample.

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In view of the similarity of the increment in each of these factors, the greater numbers of bacteria must have been attached to the larger amount of solids in the hand-scooped sample. The use of a small diameter tube for removing ingesta results in a certain amount of straining off of the solid particles, especially if a plug of solid matter should partially occlude the entrance to the tube.

The following results of Schröder (unpublished) on the biuretolytic activity of ruminal ingesta, emphasize the importance of obtaining a representative sample. Comparisons were made between the amounts of biuret decomposed in 24 hours per 100 g ingesta obtained from strained and unstrained samples removed by tube (15 mm diam.) through a rumen cannula, and a hand-scooped sample removed by rumenotomy. The amount of biuret broken down by ingesta from the hand-scooped sample was about four times greater than that from the unstrained sample and about ten times greater than that from the strained tube sample.

Tube and forceps samples

Other enzyme activities have also been shown to be affected by the method of sampling. Hecker [4] determined the proteolytic and deaminase activities and gas production of strained tube samples and of whole rumen contents removed by forceps from a cow. The proteolytic activity was significantly higher in the whole contents than in the strained samples, while the deaminase activity was more than twice as high and the gas production was three times as much.

Regional rumen samples

The different regions in the rumen vary considerably. Smith, Sweeny, Rooney, King & Moore [5] found that the top ingesta from cows were characteristically higher than the bottom ingesta in total nitrogen, ammonia, water- and alcohol-soluble sugars, volatile fatty acids and crude fibre, but lower in ether-extractable compounds, pH, titratable alkalinity to pH 5, and percentage digestion of cellulose added *in vitro*.

Reid, Bailey & Glenday [6] took grabbed regional samples of ingesta from cows after grazing and compared these samples with a drum sample obtained by bailing out the ingesta into a drum and mixing them well. No single region provided samples that consistently matched the drum samples either in dry matter or in volatile fatty acid content. A direct relationship was found between the differences in volatile fatty acid concentration and the degree of rafting. The drum sample could not be matched by averaging the concentration of volatile fatty acids in two or more regions. Drum sampling was associated with low standard errors and coefficients of variation.

Representative rumen samples

In order to take a sample of ruminal contents which is representative of solid and liquid components, as well as of regional concentrations of end-products, it is necessary either to bail out the ruminal contents or to mix these *in situ* by hand passed through a large ruminal cannula. In cattle, mixing *in situ* can never be complete, because of the very large volume, and bailing may be difficult also on this account. Even mixing in a drum could be incomplete. In sheep, with smaller rumen volumes, bailing is easier. It is possible to mix the contents thoroughly and rapidly before sampling and to replace them as rapidly after sampling. This minimizes the discomfort to the animal and the disturbance of the ruminal flora. If bailing is not entirely acceptable on physiological grounds, then the ruminal contents can be mixed almost equally well *in situ* by hand, after which samples can be scooped out with a ladle. This method is termed ladling.

EXPERIMENTAL RESULTS

SAMPLING METHODS

Tube (15 mm diam.), ladled and bailed samples of ruminal ingesta were removed from each of five sheep fed for 2,5 hours on lucerne hay *ad libitum*. In Table 1 the content of dry solids of tube and ladled samples is expressed as a percentage of that found in the bailed sample from the same sheep. The resemblance of ladled to bailed samples was much greater (87 to 101 per cent) than that of tube to bailed samples (18 to 43 per cent).

Table 1: AMOUNT OF DRY SOLIDS IN LADLED AND TUBE SAMPLES EXPRESSED AS PERCENTAGE OF THE AMOUNT IN BAILED SAMPLES

Sheep	Ladled Sample	Tube Sample
A16	89,2	35,1
A24	96,1	43,4
A26	87,3	17,8
A27	101,0	42,2
A35	100,0	36,7

In view of this, it could be expected that the numbers of total culturable bacteria in the ladled sample would also be found to approximate more closely those in the bailed sample, than would those in the tube sample (Table 2). Not only the unstrained, ladled samples of ingesta, but also the fluid strained from these samples, contained greater numbers of bacteria than the tube samples or the fluid strained from them.

Table 2: NUMBERS OF TOTAL CULTURABLE BACTERIA IN LADLED AND TUBE SAMPLES EXPRESSED AS PERCENTAGE OF THE NUMBERS IN BAILED SAMPLES

	Ladled samples		Tube samples	
	Fluid strained from sample	Unstrained sample	Fluid strained from sample	Unstrained sample
Means for 5 sheep	81,2	124,6	39,0	57,4
S.D.	±35,0	±38,6	±14,5	±42,9

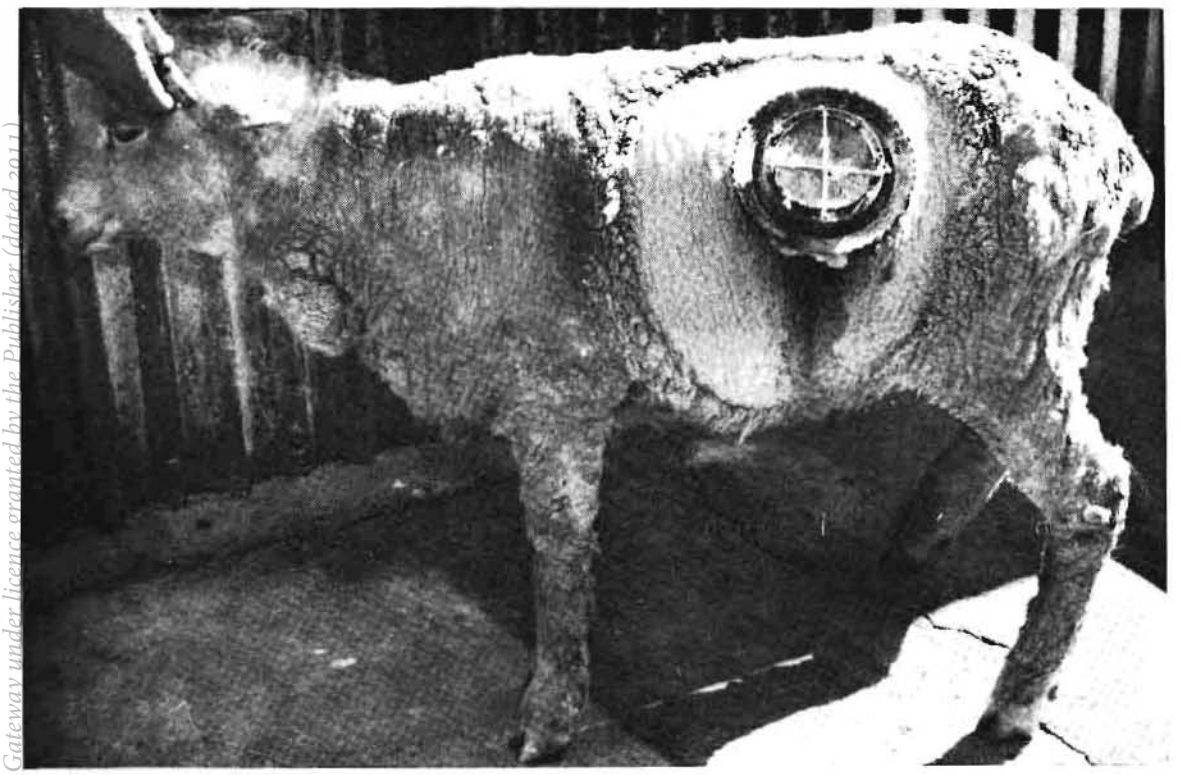

Fig. 1. Sheep one year after insertion of the large (80 mm int. diam.) rumen cannula showing negligible leakage.

A truly representative sample of ruminal ingesta could only be obtained from a sheep provided with a large rubber rumen cannula of 80 mm internal diameter, by mixing the ingesta *in situ*, and then sampling with a ladle.

INSERTION OF LARGE RUMEN CANNULA

The operation for the insertion of the large cannula was carried out in two stages, one week apart. In the first stage, the rumen was attached to the abdominal wall and skin over either a large or a small area, so that the rumen protruded through a slit in the skin. Where attachment was over a large area, a large cannula with an internal diameter which was 30 mm less than that of desired cannula, was inserted through a slit in the rumen in the second stage, and the desired cannula was inserted two weeks later. Where the attachment was over a small area, only a small 25 mm diameter Jarrett-type cannula was inserted in the second stage. The small fistula thus made into the rumen was gradually enlarged at intervals of at least two weeks by inserting a series of four cannulae of increasing diameters, terminating with that of the desired large cannula. Of the two methods, the latter produced the snuggest fit with negligible leakage even up to a year after insertion, as shown in Fig. 1. It only took longer.

The large cannula is now available commercially in the Republic of South Africa (Messrs. Hudson Rubber Co. (Pty.) Ltd., 119 Mitchell Street, Pretoria). Details of the large cannula and the surgery required for its insertion in the rumen of sheep will be published elsewhere.

EFFECTS ON RUMEN FUNCTION

The question arose as to whether the function of a rumen containing such a large cannula would remain physiological. To test this, six parameters were used to compare one group (A) of five sheep, before and after fitting each with the large cannula, with another group (B) of five sheep, before and after fitting each with the small conventional size cannula of 25 mm internal diameter. In Table 3 the findings are summarized for the two groups before and after fistulation when fed lucerne hay *ad libitum*. Although both groups consumed more food after fistulation, this was less marked in the group with the large cannula. After fistulation the weight gain was slightly less for both groups, most probably because the animals were putting on fat at this stage. The digestibility of dry matter was slightly higher after fistulation

Table 3: THE EFFECT OF FISTULATION ON RUMEN FUNCTION

Parameter of rumen function	Fistulation of Group A with large cannulae		Fistulation of Group B with small cannulae	
	Before	After	Before	After
Av. daily food intake (kg)	1,58 (±0,15)	1,68 (±0,06)	1,52 (±0,05)	1,90 (±0,17)
Av. wt gain in 3 weeks (kg)	2,0 (±0,4)	1,7 (±0,6)	2,6 (±1,0)	2,2 (±0,6)
Dry matter digestibility (percentage)	61,04 (±1,44)	66,66 (±1,63)	58,80 (±4,17)	65,89 (±1,68)
Frequency of rumen contractions (per 5 min)		3,72 (±0,22)		3,82 (±1,28)
Strength of rumen contractions (mm water pressure)		66 (±16)		81 (±20)
Rate of passage of solid ingesta (hours)		32,70 (±4,36)		32,00 (±2,81)

in both groups. It can also be seen that the frequency of the rumen contractions and rate of passage of solid particles of ingesta, determined by means of a radioactive marker, were very similar for Group A fitted with the large cannulae and for Group B with the small cannulae. The strength of the contractions was slightly less for Group A than for Group B. This was most probably due to loss of gas through the large cannulae. Further details concerning the effects of large cannulae on ruminal physiology will also be published elsewhere.

The condition of both small and big sheep, fitted with cannulae 12 months previously, indicates that this type of preparation can be maintained successfully. Maintenance offered no problems and demanded no specialized supervision.

CONCLUSION

The insertion of large rubber rumen cannulae of 80 mm internal diameter in sheep is essential to obtain adequate sampling of ruminal ingesta, whether by bailing or ladling. The operation is relatively simple, although for best results the time-consuming procedure of gradual enlargement of the fistula is recommended. The effects on the functions of the rumen are minimal and maintenance—thus far up to one year—is easy and the results of fistulation satisfactory.

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ANTIBIOTIC RESISTANCE AND R-FACTORS IN *ESCHERICHIA COLI* FROM CALVES, MEAT AND MILK

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SUMMARY

Two hundred and twenty-four strains of *E. coli* from apparently normal calves, from diseased calves and pigs, and from raw meat and milk were examined for antibiotic resistance and possession of R-factors. All of 84 strains from a calf unit in which antibiotics were fed routinely at sub-therapeutic levels, were resistant (95% to two or more drugs) whilst 22% were R+. Of 48 strains from a calf unit where antibiotics were only used therapeutically, 93,5% were resistant (86% to two or more drugs) and 63% were R+. Amongst 12 strains from a calf unit where no direct exposure to antibiotics had occurred, 75% were resistant (58% to two or more drugs) and 16,6% were R+. Of 27 strains from ranch calves, 7,3% were resistant (to two drugs only) and one strain was R+. Of 12 "pathogenic" strains, 5 (41%) were resistant (to three or more drugs) and 4 (33,3%) were R+. Amongst 41 strains from meat and milk intended for use as food, 36,5% were resistant (mostly to only one drug) while only one strain possessed R factors. Fifty-four (24%) of all strains were resistant to chloramphenicol and this resistance was transmissible to sensitive *E. coli* and *S. typhimurium* in 25 and 28 instances respectively.

INTRODUCTION

Since Japanese workers first reported on infectious drug resistance in *Shigella* in 1956, this phenomenon has been extensively studied and reviewed [1]. R-factors (resistance transfer factors combined with resistance determinants) may infect all genera of the family Enterobactereae [1]. Lyophilized cultures from the pre-antibiotic era possess R-factors, as do cultures of organisms from antibiotic-virgin areas where the only possible exposure might be to antibiotics naturally produced in the soil [1]. This indicates that use of antibiotics is not responsible for emergence of R-factors, but it is equally well established that the unique characteristics of R+ organisms, i.e. spreading and conferring multiple drug resistance, are facilitated by the positive selection pressure of drugs in the environment [1]. Today R+ bacteria are present in most parts of the world; the increase of drug resistance runs parallel to the increasing use of antibiotics in agriculture and in human and veterinary medicine [1].

Various R-factors may be identified, and they may confer resistance on their host bacteria to streptomycin, sulfonamides, chloramphenicol, tetracyclines, neomycin, kanamycin, spectinomycin, viomycin, gentamycin, penicillin and its derivatives such as ampicillin. Some doubt still exists regarding furazolidone [1].

Man may become infected with R+ bacteria derived from both human and animal sources [1]. Whilst it is generally accepted that the use and abuse of antibiotics in man constitutes the greatest cause of the spread and maintenance of R-factors in the Enterobacteriaceae of human origin, R+ cells from animal sources play a part, and R+ Salmonellae have been transmitted to man [1]. Apparently *E. coli* of animal origin do not readily colonize the human digestive tract but may nevertheless transfer R-factors to man's resident intestinal flora [1]. It is accepted that transfer *in vivo* is subject to inhibition and suppression, brought about by fatty acids, lethal phages and bacteriocins [1]. Transfer *in vivo* as well as *in vitro* is, however, subject to restrictive factors such as donor incompetence, donor-recipient incompatabi-

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lity and strain specific or other inhibitors[1]. It is also known that frequency of transfer from R+ cells in a stable bacterial population is much lower than from newly infected R+ cells. Such R+ bacteria of animals with common pathogenicity for man may thus infect him or transfer R-factors to human commensals or pathogens by way of non-pathogens[1].

Some authors contend that R-factors do not pose a threat to human or animal health[2]: they base this view on work which shows that possession of R-factors is coupled to a decrease in virulence of the pathogen concerned. Jarolmen & Kemp[2] reported marked reduction in the virulence of *S. cholerae-suis* var. *kunzendorf* by acquisition of R-factors, the majority of such R+ clones being "rough" in culture and better recipients than their "smooth" parents, but this situation is not constant. The virulence of multiple drug resistant *Shigella* is not lower than that of drug sensitive cells of the same strain[1]: serious epidemics of infantile diarrhoea have been caused by R+ *E. coli*[1] and the virulence of *S. typhi-murium* is not reduced by the possession of R-factors[1]. Watanabe warns against generalisations based on the findings of Jarolmen *et al*[2] and points out that highly virulent "smooth" strains of pathogenic R+ bacteria have been isolated from natural sources[1].

The contention that the uncontrolled use of antibiotics in animals is a hazard to human and animal health is an important basis for the Swann Committee's report[3] which, *inter alia*, defines "feed antibiotics" as drugs that are of proven economic value, have no therapeutic application in human or veterinary medicine, will not impair the efficacy of therapeutic drugs through development of resistant strains of organisms, and recommends that only "feed anibiotics" should be used and supplied without prescription and then only at levels below 100 ppm.

A similar, rather conservative policy is at present implemented in South Africa, where Maré and Coetzee[5] have isolated R+ strains of both *S. typhi-murium* and a *Citrobacter* from a child and subsequently demonstrated that 19 per cent of drug resistant *E. coli* from human sources are R+[6]. Later, Maré found that none of the 10 per cent of drug-resistant organisms isolated from drug-free human and free-living wild animal populations possessed R-factors. There have been no published reports concerning R-factors in organisms from domestic animals in South Africa.

Food of animal origin may be an important source of human infection with R+ bacteria. In Ireland, R+ *E. coli* was detected in sausages[8]; in Britain 39 per cent and 80 per cent of coliforms from beef and pork carcasses were resistant to one or more antibiotics, while 18 of 20 chloramphenicol-resistant and 40 per cent of other drug-resistant strains possessed R-factors[9].

This report deals with an *ad hoc* survey of the antibiotic resistance pattern and the presence of R-factors in *Escherichia coli* isolated from healthy and diseased calves and from meat and milk intended for use as food for man.

MATERIAL

1 *Calf faeces*

Rectal swabs were taken at random from apparently normal healthy calves, varying in age from a few days to 16 weeks, in three intensive calf rearing units and from herds on two ranches.

In Calf Unit A (CU—A) all were routinely fed subtherapeutic amounts of tetracycline (Tc), streptomycin (Sm), chloramphenicol (Cp), ampicillin (Ap) and neomycin (Nm), either alone or in varying combinations, in their daily ration. (See under "Results" for fuller details.

In CU—B, calves were treated with therapeutic doses of antimicrobial drugs only as required; they received no such drugs at other times.

In CU—C, no antimicrobials were fed and no need had arisen for antibiotic therapy in the months preceding sampling. The calves, however, were held in close proximity to adult cows, some of which had been treated for mastitis.

On Ranch I, calves ran with their dams and no antibiotic exposure was known to have taken place. The ranch, however, was close to intensively reared livestock.

On Ranch II, calves ran with their dams of native stock. The ranch was situated in a remote part of the Transvaal and exposure to antibiotics was considered most unlikely.

2. *Pathogenic E. coli*

Strains of *E. coli* isolated from diseased calves and pigs, and considered responsible for the pathological changes, were obtained from the Department of Bacteriology of the Veterinary Research Institute, Onderstepoort.

3 *Meat*

Samples were obtained from minced meat sent to the State Health Department for chemical analyses.

4 *Milk*

Presumed *E. coli*-positive MacConkey-broth cultures from milk supplied to Pretoria were obtained from the Municipal Health Department.

METHODS

Rectal swabs were streaked directly on to McConkey-agar plates in a fine grid pattern. Discs ("Oxoid") impregnated with 25 μg of streptomycin, 50 μg of tetracycline, 50 μg of chloramphenicol, 25 μg of ampicillin or 30 μg of neomycin were placed on the agar and the plates incubated at 37° C for 24 hours. Colonies of lactose-fermenting microbes that grew right up to the discs were picked off for identification as *E. coli* on the basis of acid and gas production in MacConkey-broth and indol production in peptone water, both incubated at 44 ± 0,25°C for 48 hours. Colonies growing within the zone of inhibition but separated from the edge of the disc by a distance exceeding 2 mm were considered only partially resistant and were not recorded.

Cultures of *E. coli* from diseased calves and from contaminated meat and milk were similarly examined. For the purpose of differention in subsequent transfer tests, all antibiotic-resistant (r) isolates were tested for ability to grow in MacConkey-agar containing 50 μg nalidixic acid/ml.

Possession of R-factors was assessed by the ability of the r-*E. coli* isolates to transfer resistance *in vitro* to completely antibiotic-sensitive recipients: *Salmonella typhimurium* RD 42*, *E. coli* E-27* and the lactose-negative nalidixic acid-resistant *E. coli* K-12**. The method was that described by Maré and Coetzee[6]. Combinations of donor and recipients were incubated, first transferred to enrichment medium where the salmonellae were to be recovered, or otherwise directly streaked on to MacConkey-agar in a fine grid pattern. Before incubation at 37°C for 24 h, high level antibiotic discs were superimposed on the agar as before.

RESULTS

The results are summarized in Table 1.

Table 1: ANTIBIOTIC RESISTANT AND R+ **E. COLI** FROM VARIOUS SOURCES

Source of Isolates	No. Exam.	Antibiotic Resistance multiplicity						Possession of R-factors	
		1	2	3	4	5	Total (%)	Transfers to Sens. Recipients	Total
1. Calves:	84	4	61	9	8	3	84	RD42 : 8	
Unit A							(100)	K12 : 11	
								E27 : 5	19
Unit B	84	3	8	4	13	17	45	RD42 : 1	
							(93,5)	K12 : 29	30
Unit C	12	2	4	2	1	0	9	RD42 : 0	
							(75)	K12 : 2	2
Ranch I	15	0	1	0	0	0	1	RD42 : 0	
							(6,6)	K12 : 1	1
Ranch II	12	0	1	0	0	0	1	RD42 : 0	
							(8)	K12 : 0	0
2. "Path."	12	0	0	3	2	0	5	RD42 : 0	
E. coli							(41)	K12 : 4	4
3. Meat	10	5	2	0	0	0	7(70)	RD42 : 0	
								K12 : 0	0
4. Milk	31	6	1	1	0	0	8(26)	RD42 : 1	
								K12 : 0	1

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1a. *Calf Unit A*

All the *E. coli* were resistant to the antibiotics at high levels, and their resistance patterns (rp) may be summarized as follows:

Antibiotic(s)	*Number*
Sm only	3
Sm and Tc	60
Sm, Tc and Cm	9
Sm, Tc, Cm and Ap	7
Sm. Tc, Cm, Ap and Nm	3
Tc only	1
Tc and Cm	1
Tc, Cm, Ap and Nm	1

Eight (9,5%) strains transferred some or all of their rp to RD42, eleven to K-12 and five to E-27. All transferred resistance (tr) involved Sm and Tc except one instance, where Ap was included.

1b. *Calf Unit B*

Over 93% of the strains were resistant to the antibiotics tested, and their rp is summarized in the following way:

Antibiotic(s)	*Number*
Sm only	3
Sm and Tc	7
Tc only	1
Sm, Tc and Cm	2
Sm, Tc and Ap	2
Sm, Tc, Cm and Ap	13
Sm, Tc, Cm, Ap and Nm	17

One isolate transferred some of its rp to RD42 and 29 to K-12. The transferred resistonce varied from single to quadruple.

1c. *Calf Unit C*

Seventy-five per cent of the isolates were resistant, and their rp is as follows:

Antibiotic(s)	*Number*
Sm only	1
Sm and Tc	2
Sm, Tc, Cm and Ap	1
Sm, Tc and Ap	2
Tc only	1
Sm and Cm	2

None of the strains transferred their rp to RD42, but one strain resistant to Tc and Ap and the other to Tc, Cm and Ap, transferred their rp to K-12.

1d. *Calves on Ranch I*

A single isolate was resistant to Sm and Cm and transferred this rp to K-12 but not to RD42

1e. *Calves on Ranch II*

A single isolate was resistant to Sm and Cm and transferred this rp to K-12 but not to RD42.

2. *Pathogenic E. coli*

Of the five r-strains, three were resistant to Sm, Tc and Nm, while two were resistant to Sm, Tc and Ap. Resistance could not be transferred to RD42 but was transferred to K-12.

3. *Meat Isolates*

Five strains were resistant to Tc and two were resistant to Sm and Tc. None of this resistance was transferable to K-12 or RD42.

4. *Milk Isolates*

Of the eight r-strains, four and two respectively were resistant to Sm and to Tc only, one was resistant to both Sm and Tc, while another strain was resistant to Sm, Tc and Nm. The isolate resistant to both Sm and Tc transferred its rp to 42 but not to K-12.

Chloramphenicol resistance was determined in *E. coli* from the following sources, and was transferred as indicated below:

Source	*No r-isolates*	*Transfer (to . . .)*
Calf Unit A	15 (17,9%)	1 (K-12)
„ „ B	32 (66,6%)	10 (K-12) and 28 (RD42)
„ „ C	3 (25%)	2 (K12)
Ranch I	0	0
Ranch II	1 (9,3%)	1 (L-12)
Pathogenics	3 (25%)	3 (K-12)
Meat	0	0
Milk	0	0

DISCUSSION AND CONCLUSIONS

It is authoritatively stated that the transfer factors and resistance determinants which, when combined, make up the R-factor, possessed by the Enterobacteriaceae, are widespread in nature[1]. The results of this survey confirm this statement by revealing that *E. coli* isolated from meat, milk and from diseased as well as from apparently normal calves in South Africa possess transmissible drug resistance factors.

The results clearly show the difference between the incidence of R+ *E. coli* in calves exposed directly or indirectly to antibiotics and in those raised in remote undeveloped areas where exposure is unlikely to have taken place. This emphasizes that although R-factors may be present in a population, they generally remain unimportant and of low transferability until such time as exposure to the drugs results in a positive selection pressure and active spread of the R-factor[1]. The results confirm that antibiotics should be

used with the utmost discretion and only when necessary, and that the greatest care in the hygenic production of meat of milk is esesntial, if their contamination with intestinal micro-organisms is to be reduced or eliminated.

The survey indicates marked variations in the resistance patterns of *E. coli* strains and shows that this pattern is not necessarily transferred intact to recipient organisms. Spontaneous segregation of resistance markers, however, is well known [1]. Resistance to Cm and Nm is unstable and rather easily lost, whereas resistance to Sm, Ap and Tc is closely linked and quite stable [1].

There are also marked variations in the ability of strains of *E. coli* to transfer drug resistance to recipient test organisms. This may be explained by known forms of incompatability and transfer restriction between donors and recipients, a discussion of which is beyond the scope of this paper.

Chloramphenicol is frequently the drug of choice in the treatment of outbreaks of enteric disease in calves: the rather high incidence of *in vitro* resistance as well as the frequency of transmission of such resistance gives cause for concern. While, as a rule, every effort is made to keep carcass meat free of intestinal organisms, it is common practice for some peoples in African countries to use the gastro-intestinal tract of slaughtered ruminants as food. Such offal is eventually cooked but the distribution, handling and preparation of material which is so highly contaminated with a variety of intestinal commensals and possibly pathogens must inevitably lead to contamination of premises, utensils and ready-to-eat food. Under such conditions the possession of R-factors by these bacteria must inevitably present an even greater hazard to human health.

Until there is generally acceptable proof that R-factors do not represent a threat to human and animal health, a conservative approach to the availability and use of antimicrobial substances in South Africa is fully justified.

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IMMUNIZATION OF SHEEP AGAINST PASTEURELLOSIS*

C. M. CAMERON**

SUMMARY

A brief review of the different forms of pasteurellosis is given with particular reference to the incidence of the disease in South Africa.

Experiments with a polyvalent alum-precipitated vaccine containing *Pasteurella multocida* types A and D as well as four serotypes of *P. haemolytica* were done on mice and sheep. The vaccine produced a sound immunity to challenge with the homologous strain of *P. multocida* and also stimulated a marked increase in the haemagglutination titres and passive protection values of the serum of immunized sheep.

The antibody response to the *P. haemolytica* strains was poorer but possibly sufficient to contribute to an increase in resistance to infection.

The application of the vaccine under practical conditions is discussed.

INTRODUCTION

Aetiology

The term pasteurellosis covers a wide variety of disease syndromes in an extensive host range which includes all classes of domestic and many wild animals, ranging from buffalo and rabbits to poultry. In the past, investigators went to great lengths in an attempt to establish a satisfactory scheme for classification of the isolates. *Pasteurella* organisms show a certain degree of host specificity and species were named according to the animal from which they were isolated, e.g. *Pasteurella oviseptica* or *P. avicida*. This approach was abandoned however, because the differences between the various types were insufficient to warrant separate species and the all-embracing name of *P. multocida* was eventually adopted for all the *Pasteurella* spp. causing a septicaemia.

P. multocida can be divided conveniently into four main types by haemagglutination techniques and into numerous sub-groups by agglutination tests. The diseases caused by the different types, their distribution and host range has been well reviewed and documented by Carter[1]. As shown in Table 1, types B and E are responsible for classical haemorrhagic septicaemia while types A and D are involved in pneumonic infections in numerous species including sheep.

P. haemolytica is an extremely common pathogen of sheep. In fact, *P. haemolytica* has been encountered more often than *P. multocida*, either in conjunction with *P. multocida* but frequently as the only microbe that can be isolated from pathological material. Apart from its rôle in pneumonia, it is the major cause of mastitis (blue udder) and is commonly found in cases of encephalitis. Recently, van Tonder[2] isolated *P. haemolytica* from cases of ovine epididymitis but the aetiological significance of this finding has not yet been established.

P. haemolytica can also be divided into serotypes by a haemagglutination procedure: thus far Biberstein, Gills & Knight[3] have defined twelve types. Table 2 shows the incidence of serotypes isolated from pneumonic lungs of sheep in South Africa.

One of the best known forms of pasteurellosis is shipping fever, commonly encountered in North America. In this syndrome a virus plays a prominent rôle but, in South Africa, no viruses have yet been found to be associated with any form of pasteurellosis. A thorough search is indicated and may yet result in a positive finding.

Incidence in South Africa

Pasteurellosis, as a rule, is a sequel to stress and is most frequently encountered

*Paper read at the Biennial Scientific Congress of the South African Veterinary Association, East London, 13—17 September 1971. Tables 2, 3 and 4 and Fig. 1 have been reproduced by kind consent of the Editor of the **Onderstepoort Journal of Veterinary Research.**

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Table 1: PASTEURELLAE INVOLVED IN PASTEURELLOSIS OF CATTLE, SHEEP AND OTHER RUMINANTS [1]

Carter type	Roberts type	Disease syndrome
Pasteurella multocida		
A	II	Wide host range. Fowl cholera.
B	I	Haemorrhagic septicaemia (Asia).
D	V	Wide host range
E	—	Haemorrhagic septicaemia (East Africa).
Pasteurella haemolytica		
Biberstein serotypes 1 to 12		Pneumonia, encephalitis, mastitis (*blue udder*) and epididymitis(?).

Table 2: SEROTYPES OF **P. HAEMOLYTICA** ISOLATED FROM SHEEP WITH PNEUMONIA

Type	Number of strains typed
1	7
2 (11)	6
3	1
4	8
5	9
6	13
7	7
8	6
9	4
10	1
12	1
Not typable	6
Total	69

when animals are subjected to change of environment, sudden cold and particularly to malnutrition. Consequently, in South Africa, the disease in sheep is most prevalent in areas which are subject to regular and severe droughts, such as the Northern and Western Transvaal, North-Western Cape and South West Africa. Sporadic outbreaks, however, have also been recorded elsewhere in the country under unfavourable conditions. *Pasteurella* infections are also a common terminal complication in sheep suffering from pulmonary adenomatosis (jaagsiekte).

Symptomatology

There are very few overt symptoms except for a rise in temperature, inappetence and dyspnoea. The course of the disease is usually acute or peracute and sheep are usually found dead.

Pathology

The most prominent autopsy finding is extensive pathological change in the lung. The lesions vary greatly in extent and degree, the commonest being an acute bronchopneumonia with disseminated areas of consolidation. The variegated appearance of the lung has given rise to the common name of stock owners of "bontlong" (mottled lung). When *P. multocida* is the aetiological agent, a fibrinous pleuritis is a common feature and sometimes disseminated abscesses may be seen.

In protracted illness, lung lesions may be virtually non-existent, while severe anaemia, icterus and liver degeneration are the main features. This syndrome has been reproduced experimentally and recorded as bacterial hepatitis [4].

EXPERIMENTAL IMMUNIZATION

To control the losses suffered from the various forms of pasteurellosis, a polyvalent formalinized vaccine was prepared and its potency assayed in mice and sheep. The vaccine contained *P. multocida* types A and D and *P. haemolytica* types 1, 4, 6, and 7. Full details of the experimental procedures and results have been published elsewhere [5].

Initial experiments in mice showed that a concentration of 0,16 per cent packed cells per strain would confer a solid immunity. Formalin inactivation was found to be superior to merthiolate, phenol or heat inactivation. Mechanical disruption of the bacteria did not enhance their immunizing potency while an alum precipitated vaccine proved to be superior to oil adjuvant vaccine.

More thorough evaluation of the vaccine was done by administering two injections of 0,2 ml of vaccine subcutaneously to mice at an interval of three weeks. Groups of immunized and control animals were challenged with tenfold dilutions of a standardized suspension of *P. multocida*. The LD_{50} in the im-

munized and control mice was calculated and the difference taken as the degree of protection afforded. It was thus found that the vaccine gave a protection of 6,6 logs to challenge with *P. multocida* Type A and 4,7 logs to Type D.

A standard of 3 logs protection has been set for routine vaccine production. No batch of vaccine has as yet been below this standard.

Similar experiments, aimed at determining the protection afforded to challenge with *P. haemolytica,* failed because of the low pathogenicity of this organism for laboratory animals.

The immunizing potency of the vaccine for sheep was tested by administering two doses of 5 ml each subcutaneously with an interval of four weeks.

In the first experiment the immunized sheep and appropriate controls were challenged two weeks after the second dose of vaccine by the intravenous injection of approximately 2×10^9 virulent *P. multocida* type A organisms. As shown in Table 3, the protection afforded by the vaccine was excellent. Furthermore. serum taken from the immunized sheep just before challenge had a marked passive protection potency for mice. A correlation between protective immunity in sheep and passive immunity appears to be present, as well as with haemagglutination (HA) titre, if considered on a group basis [6].

Table 3: HAEMAGGLUTINATION (H.A.) TITRES AND PASSIVE PROTECTION VALUES OF SERA FROM IMMUNIZED SHEEP CHALLENGED WITH **P. MULTOCIDA** STRAIN A14G (TYPE A)

	Sheep No.	HA Titres at time of challenge	Result of challenge	Logs protection obtained in mice with pooled sera collected before challenge
Immunized group	24 148	40	Survived	
	24 550	20	,,	4,6
	21 395	40	,,	
	24 998	20	,,	
	24 232	20	,,	
	24 547	20	Died	n.t.
Control group	24 810	10	Died	
	24 877	10	,,	0,0
	24 164	10	,,	
	24 921	10	,,	

n.t. = not tested

The second experiment was similar to the first, except that the sheep were challenged with *P. multocida* type D. The immunity was not so good as that obtained with type A as a challenge (Table 4) but was regarded as adequate, bearing in mind that the sheep were exposed to a level of infection greatly in excess of that which they would normally be expected to encounter in nature. It is also noteworthy that the sera from the unvaccinated control sheep had a considerable protective potency for mice (1,9 logs), while the pre-challenge sera from the immunized sheep which subsequently survived was only slightly higher (2,2 logs). A comparatively slight increase in passive protective potency may therefore be sufficient to afford a functional immunity.

Table 4: HAEMAGGLUTINATION TITRES AND PASSIVE PROTECTION VALUES OF SERA FROM IMMUNIZED SHEEP CHALLENGED WITH **P. MULTOCIDA** STRAIN DI (TYPE D)

	Sheep No.	HA Titres at time of challenge	Result of challenge	Logs protection obtained in mice with pooled sera collected before challenge
Immunized group	24 488	40	Survived	
	22 751	20	,,	
	24 481	20	,,	2,2
	23 649	40	,,	
	24 216	80	,,	
	25 329	80	,,	
	24 562	20	Died	
	24 194	20	,,	
	24 997	20	,,	1,9
	23 396	80	,,	
	24 529	320	,,	
Control group	24 539	10	Survived	n.t.
	24 493	10	Died	
	24 842	10	,,	
	26 775	10	,,	1,9
	26 781	10	,,	
	24 226	10	,,	

n.t. = not tested

In order to determine the duration of the antibody response in sheep, polyvalent vaccine was administered to a group of animals and the antibody titres were followed by means of a haemagglutination (*HA*) test, as well as by assaying the passive protective potency of their sera for mice.

The figure shows the antibody response to *P. multocida* type A. After two initial injections of vaccine, there was a marked antibody response which, however, returned to pre-immunization levels within five months. Neither the titre nor the duration could be significantly increased by giving three initial injections. A booster injection given at 5 months resulted in an increase in both HA titre and protective potency but again the response was of limited duration.

Similar assays using *P. multocida* type D gave similar if less pronounced results.

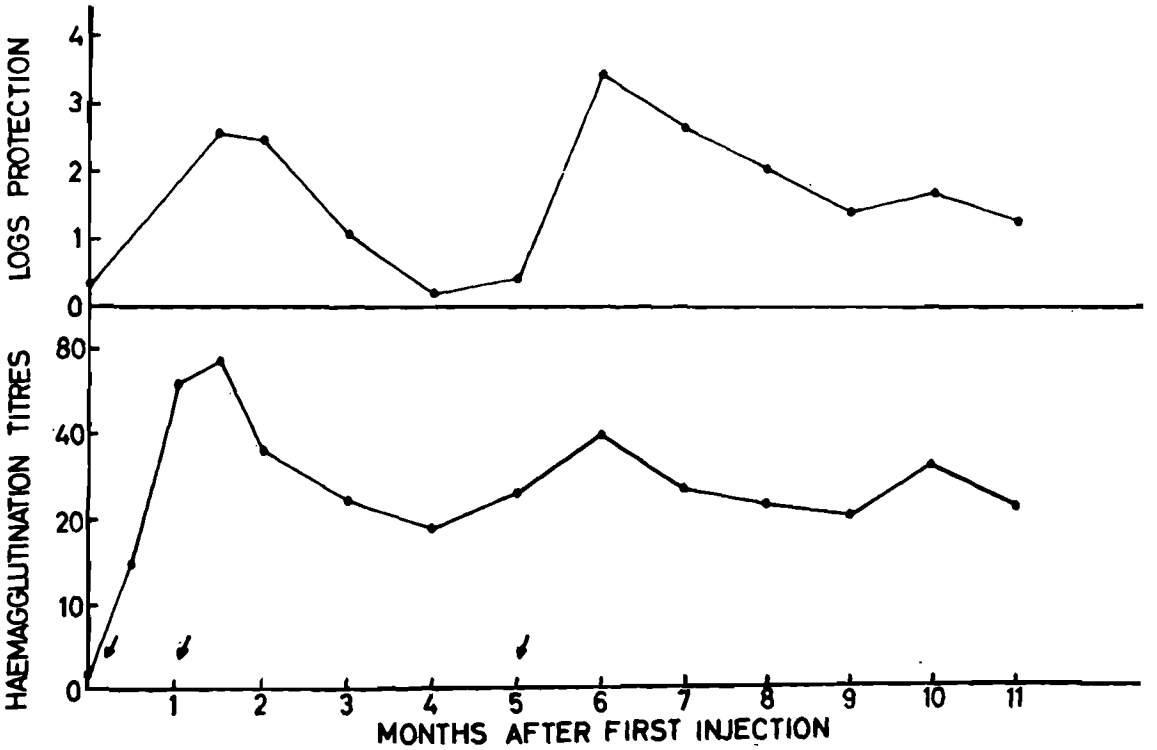
The response to *P. haemolytica* could only be followed by the *HA* test because of the failure to conduct reliable biological assays in mice. The titres obtained, in general, were not as high as those found against the *P. multocida* types and the results were also more erratic.

CONCLUSION

In conclusion it may be stated that the polyvalent vaccine currently produced affords a good protection against *Pasteurella* infections by the homologous serotypes but the brevity of the antibody response may be a major shortcoming. Consequently, immunization should be repeated preferably at least every six months if animals are to be maintained at a high level of immunity. A more practical approach, however, would be to administer the vaccine according to a schedule which would afford maximal immunity at the stage when the animals are most likely to contract the infection, namely before anticipated stress periods.

Occasionally pasteurellosis occurs in young animals before there is time to immunize them actively. One then has to resort to immunization of the ewe. Under such

FIG. 1 MEAN HAEMAGGLUTINATION TITRES AND PASSIVE PROTECTION VALUES OF SHEEP SERA TO P. MULTOCIDA STRAIN A 14G (TYPE A) AFTER ADMINISTRATION OF POLYVALENT VACCINE.



circumstances an injection of vaccine should be given at six and at two weeks before lambing. Administration of *Pasteurella* vaccine to pregnant ewes should be done with great caution. Although abortions are not the rule, they have been reported, particularly in goats. It is therefore advisable to test the vaccine on a small number of animals first, and then on a larger number before the whole herd is done. This method of immunization should give satisfactory results but as yet there are no directly relevant experimental data available.

There is little information regarding the effectiveness of *Pasteurella* vaccine in protecting sheep against udder infections. Incidental reports from the field suggest that the vaccine has considerable value and in some instances has given very good results. It may be used with a fair degree of confidence in outbreaks or on farms where the diagnosis has been bacteriologically confirmed. Should the vaccine fail to give a favourable response, it may be due to the fact that the disease is caused by a serotype which is not represented in the vaccine. In such cases the offending strain should be typed and an autogenous vaccine prepared.

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THE INCIDENCE OF *BRUCELLA OVIS* INFECTION IN SOUTH AFRICAN RAMS: A SEROLOGICAL SURVEY

R. W. WORTHINGTON*, E. M. VAN TONDER** AND MARIA S. G. MÜLDERS*

SUMMARY

Complement fixation (CF) tests using *Brucella ovis* and *Brucella abortus* antigens were done on sera from 9 966 rams. The incidence of *B. ovis* infection in non-vaccinated rams was 5 per cent. Vaccination with Rev I appeared to reduce the incidence of *B. ovis* infection. *B. abortus* or *B. melitensis* infection appears to be rare in rams but Rev I vaccination caused the development of antibodies which react with *B. abortus* antigen. The CF test for *B. ovis* appears to be specific and cross-reactions are rare, even in Rev I vaccinated animals.

INTRODUCTION

In this survey the incidence of CF antibodies to *Brucella ovis, Brucella abortus* and *Actinobacillus seminis* antigens in ram sera was investigated. Initially, relatively few sera from sheep in different parts of the country were investigated but later many sera from stud Merino and Dorper flocks in the Karoo region were collected. The incidence and significance of *A. seminis* titre will be reported later in detail by one of us (E.M.V.T.) Those given in this paper will therefore be confined to the results of tests done with *B. ovis* and *B. abortus* antigens.

It was reported previously that by using the CF test it is possible to distinguish *B. ovis* from *B. abortus* and *B. melitensis* antibodies in rabbits[1]. *B. melitensis* and *B. abortus* are known to have common antigens[2] and therefore a test using *B. abortus* and *B. ovis* antigens should distinguish between animals infected with *B. ovis* and those infected with *B. abortus, B. melitensis* or with Rev I (attenuated *B. melitensis* vaccine strain). It was therefore hoped that this survey would yield valuable information on the incidence of *Brucella* infections in rams and the effectiveness of Rev I vaccination[3, 4], which is used on a large scale in South Africa for the control of *B. ovis* infection in rams.

METHODS

Sera were collected from rams by members of the Division of Veterinary Services and sent to Onderstepoort where CF tests were carried out by the methods previously reported[1, 5] using *B. abortus, B. ovis* and *A. seminis* antigens. All sera were tested at doubling dilutions to 1/16. A titre of 1/8 (50 per cent complement fixation at 1/8 dilution) was regarded as indicating infection. Histories of the vaccination procedures and of the incidence of breeding problems were obtained from the owners by the veterinarians who collected the samples. Any flock with a history of epididymitis, abortions or a reported lambing percentage of less than 50 per cent was classed as a flock with breeding problems.

RESULTS

Sera from 5 855 vaccinated and 4 111 non-vaccinated rams were tested. Because about 12 per cent of these sera were anticomplementary or otherwise unsuitable for test purposes, these figures were reduced to 4 916 and 3 579 respectively. The sera from vaccinated rams were collected on 224 farms of which 106 had breeding problems, and those from non-vaccinated rams from 208 farms of which 56 had breeding problems. Histories of vaccination procedures were generally inadequate: farms were classified merely as vaccinated or non-vaccinated. The results of the CF tests are summarized in Table 1.

In the vaccinated animals 8,5 per cent had titres of 1/8 or 1/16 (titres regarded as indicating infection) with *B. abortus* antigens and 3,6 per cent had the same titres with *B. ovis* antigen. In the non-vaccinated group the percentages were 0,8 and 5,0 respectively.

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Table 1: CF TITRES OF THE SERA OF VACCINATED (REV 1) AND NON-VACCINATED RAMS USING **B. ABORTUS** AND **B. OVIS** ANTIGENS

Category	No. sera	B. abortus titres					B. ovis titres				
		1/16	1/8	1/4	1/2	neg.	1/16	1/8	1/4	1/2	neg.
Vaccinated	4 916	263	153	257	824	3 408	98	81	196	1 012	3 529
Non-vaccinated	3 579	16	11	33	201	3 318	110	70	140	659	2 600

It is obvious that *B. abortus* titres were far more common in vaccinated than in non-vaccinated animals. As there is no reason to believe that *B. abortus* or *B. melitensis* should be more common in the vaccinated animals. it is clear that this difference is due to titres induced by vaccination. In the non-vaccinated animals the incidence of *B. abortus* titres was 0,8 per cent. Because vaccination histories cannot be regarded as entirely reliable in a survey of this type and some of these titres may have been due to unreported vaccinations, it is concluded that the incidence of *B. abortus* and *B. melitensis* infection in these rams is very low. A survey of non-vaccinated ewes or a detailed investigation of individual cases would be required to confirm the occurrence of these infections.

In non-vaccinated rams, *B. ovis* titres of 1/8 or more were found in 5 per cent of rams and about 30 per cent of non-vaccinated flocks contained at least one animal with a *B. ovis* titre. In vaccinated flocks the corresponding figure was 3,6 per cent, which seems to indicate that vaccination had not resulted in a great deal of improvement. It must be remembered, however, that vaccination is generally applied on farms on which breeding problems occur and that the incidence of reported breeding problems was nearly twice as high on the farms on which the vaccine was used as on farms where it was not. In addition, some of the farms had only recently started vaccination programs; others were using the vaccine incorrectly. Cross-reactions which may indicate vaccination of already infected animals (see below) were also more common in the vaccinated group. When all these factors are considered, we believe that the difference in the incidence (5 percent and 3,6 per cent) in the two large groups surveyed may indicate that vaccination is a useful procedure. It is obvious, however, that on some farms *B. ovis* infection and epididymitis had persisted despite the use of vaccine for a number of years.

Cross-reactions between *B. ovis* antibodies and *B. abortus* antibodies did not play an important rôle. This was determined by making a detailed analysis of all results in which a titre of 1/8 or greater was found to either antigen. In each case the number of times in which the *B. abortus* titre was greater than less than, or equal to the *B. ovis* titre and the number of times a titre of 1/16 was found in both cases (sera not titrated to end titre) was determined. The results are shown in Table 2.

Table 2: COMPARISON OF TITRES OF SERA REACTING POSITIVELY TO **B. OVIS** OR **B. ABORTUS**

	ab>ovis	ab=ovis	ab<ovis	undetermined*
vaccinated	398	1	149	15
non-vaccinated	26	0	184	

ab = **B. abortus** titre
ovis = **B. ovis** titre
*titres of 1/16 with both antigens (end titres undetermined)

In the majority of the cases summarized in Table 2 the sera had postive titres (>1/8) to one of the antigens and negative titres (<1/8) to the other antigen. From a more detailed investigation of these results it was seen that of the 563 animals in vaccinated flocks with titres of 1/8 or more to either antigen, only 24 had titres of 1/8 or more to both antigens, i.e. 4,3 per cent of the positive sera or 0,5 per cent of the total sera tested. Of these 24 cross-reacting sera, 15 had titres of 1/16 with both antigens and in only 1 case were the titres equal at 1/8. Cross-reactions in this group could conceivably have arisen from vaccination of rams already infected

with *B. ovis* or be the result of recent vaccination with Rev I[6]. In the non-vaccinated rams, cross-reactions occurred in two sera out of 210, in which titres of 1/8 or more were obtained with either of the antigens, i.e. 1,0 per cent of the reactors or 0,06 per cent of the total sera tested. Cross reactions, therefore, do not appear to be a serious source of error in this type of survey.

DISCUSSION

From the results reported it can be seen that the CF with *B. ovis* antigen has a high degree of specificity, as cross-reactions with *B. abortus* only occur infrequently in non-vaccinated rams. Even in vaccinated rams the incidence of cross-reactions (4,3 per cent of reactors or 0,5 per cent of total sera tested) is low enough as not to interfere unduly with the usefulness of the test.

According to the results of this survey, it appears that *B. ovis* infection is widespread in South African flocks. In non-vaccinated flocks 5 per cent of all the rams tested had positive titres and about 30 per cent of non-vaccinated flocks contained at least one animal with positive titre. Vaccination appears to be a useful but not absolutely reliable method of controlling the infection. It is our experience that meticulous Rev I vaccination completely eliminated *B. ovis* infection from a number of stud flocks within a few years. Van Heerden and van Rensburg[4] also reported excellent results with Rev I vaccination. It seems likely that, where vaccination has not proved beneficial, this may have been due to faulty vaccination procedures. The vaccine should be correctly handled and stored and vaccination of rams should take place at weaning time before they have become naturally infected with *B. ovis*. Vaccination should also be used in conjunction with sound management and hygiene programs. A more detailed investigation of these points is indicated where vaccination has failed.

The incidence of *B. abortus* and *B. melitensis* infection appears to be low, but Rev I vaccination interferes with the interpretation of CF test results. A survey in non-vaccinated ewes would be required to establish the incidence of these infections.

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MORPHOLOGY OF THE FREE-LIVING STAGES OF *NEMATODIRUS SPATHIGER* WITH SOME OBSERVATIONS ON THEIR DEVELOPMENT UNDER LABORATORY CONDITIONS*

J. H. VILJOEN**

SUMMARY

1. Morphological dimensions of the undeveloped egg and infective larva of *N. spathiger* resemble those published by other workers.
2. The eggs are usually excreted in the 2 to 8 blastomere stage. On rare occasions sterile eggs or those with only one blastomere are passed.
3. Pre-parasitic development of *N. spathiger* in water is completely inhibited at freezing. At temperatures of 4 to 7° and above 39°C development is suppressed in the morula stage but between 10 to 39°C further development can take place. Hatching occurs from 21 to 36°C and is optimal at 33°C, when eggs start to hatch after 8 days incubation; after 21 days a 93 per cent hatch is possible.
4. The present observations on the life-history of *N. spathiger* incubated at 27°C correspond to a large extent with those of a previous author with the exception that hatching occurred four days earlier.
5. Low temperatures initially have a favourable effect on the subsequent development and hatching of *N. spathiger* larvae at higher temperatures. No difference appears to exist between development in the eggs stored in distilled water and those in the faecal pellet.

INTRODUCTION

The morphology of *Nematodirus spathiger* (Railliet 1896) has been studied by different workers[1-7]. These data are summarized in Table 1.

Table 1: SUMMARY OF THE DIMENSIONS OF **N. SPATIGER** EGGS AND INFECTIVE LARVAE AS OBSERVED BY DIFFERENT WORKERS (MEASUREMENTS IN MICROMETERS)

Author	Eggs				Infective larvae							
	Length		Width		Anterior end to genital primordium		Anus to tip of tail sheath		Length of oesophagus		Total length	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Railliet et al.[1]	—	200–260	—	100–110	—	—	—	—	—	—	—	—
May[2]	—	150–220	—	80–110	—	—	—	—	—	—	—	—
Tetley[3]	—	180–210	—	90–105	—	—	—	—	—	—	—	—
Kates et al.[4]	200	—	98	—	—	—	—	—	—	—	—	—
Monnig[5]	—	—	—	—	—	—	340	325–340	—	—	—	—
Dikmans et al.[6]	—	—	—	—	417	365–500	328	315–350	195	160–225	1 009	922–1 118
Thomas[7]	—	—	—		428	394–480	332	310–390	204	192–224	1 018	976–1 130

*Based on a lecture given at the Biennial Scientific Congress of the South African Veterinary Medical Association, East London, 13—17 September, 1971.

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Development in the egg before hatching was studied under laboratory conditions by Thomas [8], Marquardt & Senger [9] and by Kates & Turner [4], and their findings are listed in Table 2.

Table 2: NUMBER OF DAYS REQUIRED FOR THE EGGS OF **N. SPATHIGER** TO START HATCHING

Temperature °C	Author		
	Thomas[8]	Kates & Turner[4]	Marquardt & Senger[9]
21	22	—	—
27	—	14	—
15	—	—	24—35
20	—	—	21—24
25	—	—	10
30	—	—	7
32,5	—	—	7—8
35	—	—	8
37	—	—	8

Marquardt & Senger [9] found 30°C to be the optimum temperature for the development and hatching of infective larvae of *N. spathiger*.

They also found that development in the ova could take place between 10 and 37°C but hatching of the infective larvae only occurred between 15 and 37° C. At a temperature of 27°C development in the eggs reached the morula, tadpole, second and third larval stages in 2, 4, 5 to 6 and 6 to 14 days respectively [4].

In the present paper the results of morphological and developmental studies of a strain of *N. spathiger* from sheep in the Karoo are compared with those of other workers.

MATERIALS AND METHODS—GENERAL

A pure strain of *N. spathiger* was maintained in a group of young, susceptible host lambs for adequate supplies of *N. spathiger* eggs at all times.

a. *Collection and culturing of eggs*
McMaster faecal collection bags were attached to the hindquarters of 6 Merino wether lambs with previously confirmed natural infestations of *N. spathiger* and left in position for at least 12 hours. Cultures of *N. spathiger* eggs were prepared by using a method described by Horak as cited by Reinecke [10]. In this method a 40 per cent sugar solution is used to float the eggs into the supernatant fluid during centrifugation, instead of a saturated salt solution as advocated by Thomas [8] and Seghetti [11]. The eggs that were harvested were placed in shallow distilled water 3 mm deep in covered petri dishes and incubated at 27°C for 3 weeks until the infective larvae hatched [10]. These larvae were used to infest the first group of worm-free lambs.

b. *Worm-free lambs*
A group of 6 young Dorper lambs (Dorset Horn × Blackhead Persian) were obtained from Nelspoort in the Beaufort West District and treated with anthelmintics for cestodes and nematodes. These lambs were kept in separate hospital pens which were cleaned regularly to avoid possible cross-infestation by other sheep. From September, 1970, to July, 1971, three different groups of lambs were used to provide infested faeces for these experiments.

c. *Preparation and dosing of worm-free lambs*
The technique of Reinecke [10] was modified in injecting a single dose of 18 000 infective larvae into each lamb intraruminally, rather than dosing them orally.

d. *Egg counts*
Faeces were examined regularly and only when the faecal egg count exceeded 500 epg could enough eggs be harvested either for these experiments or for the artificial infestation of another lamb. Lambs were seldom kept for a period longer than four months, by which time egg counts had dropped to very low levels.

The following experiments were undertaken.

EXPERIMENT 1: MORPHOLOGICAL OBSERVATIONS

MATERIAL AND METHODS

A standard microscope fitted with a calibrated eyepiece was used to study the morphology of the egg from the time of expulsion in the faeces to the development of the third stage larvae.

Eggs were collected as described above, whilst larvae were obtained from the cultures described below in Experiment 3. Eggs as well as larvae were transferred to glass slides with a finely drawn pipette and killed by

adding a small drop of Lugol's iodine. Coverslips were used to cover infective larvae but not the eggs, because of the possibility of distorting the shape and dimensions of the egg. A total of 300 eggs and the same number of infective larvae were examined and the following measurements taken:—

Eggs:

(a) Mean length
(b) Mean width
(c) Mean diameter of blastomeres 8 cell stage)
(d) Mean thickness of egg shells

Third stage larvae:

(a) Total length
(b) Length of oesophagus
(c) Anterior end to genital primordium
(d) Anus to tip of tail sheath

The range was also determined for each of the measurements mentioned above.

RESULTS

The mean dimensions and range of variation of 300 eggs and infective larvae of *N. spathiger* are summarized in Tables 3 and 4. The eggs had a mean length of 218 and width of 103 μm and the infective larvae varied in length from 969—1 282, with a mean of 1 075 μm.

EXPERIMENT 2: BLASTOMERE COUNTS

MATERIAL AND METHODS

At least 100 adult female worms were collected from the small intestine of a lamb during a routine autopsy examination and immediately placed in physiological saline (6 mm deep) in a large petri dish and incubated at 33°C. The petri dish was removed after 30 min incubation and the eggs that had been laid, transferred with a micropipette to glass slides. A small amount of Lugol's iodine was added to each drop and the coverslip sealed with Glyceel (Gurr). At least 300 eggs were examined during successive collections, the number of blastomeres in each egg was counted and the findings were expressed on a percentage basis.

RESULTS

The results of blastomere counts of freshly laid *N. spathiger* eggs can be summarized as fololws:

(a) Eggs not fertilized: 7%
(b) Eggs with one blastomere: 4,6%
(c) Eggs with two blastomeres: 10%
(d) Eggs with four blastomeres: 34%
(e) Eggs with eight blastomeres: 45%

EXPERIMENT 3: RATE OF DEVELOPMENT AT DIFFERENT TEMPERATURES

MATERIAL AND METHODS

Eggs of *N. spathiger* were cultured in distilled water (3 mm deep) in covered petri dishes at different temperatures ranging from 0 to 42°C. These cultures were either kept in a refrigerator at 0, 4, 7 and 10°C, or in an incubator at 10, 21, 24, 27, 30, 33, 36, 39 and 42°C respectively. As a result of technical difficulties no experimental work was performed either at 13 or 16°C.

For every temperature mentioned above,

Table 3: SUMMARY OF THE DIMENSIONS OF **N. SPATHIGER** EGGS (MEASUREMENTS IN MICROMETERS)

Number of Observations	Length		Width		Blastomere diameter		Thickness of shell	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
300	218	173—238	103	97—119	49	32—68	2,6	1,4—2,7

Table 4: SUMMARY OF THE DIMENSIONS OF THE INFECTIVE LARVAE OF **N. SPATHIGER** (MEASUREMENTS IN MICROMETERS)

Number of Observations	Anterior end to genital primordium		Anus to tip of tail sheath		Length of oesophagus		Total length	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
300	433	356—540	331	270—416	188	103—351	1 075	969—1 282

25 petri dishes were incubated, each of them containing 150 undeveloped eggs. For 21 days, one petri dish was removed daily from the incubator and examined. During examination the following techniques were used:—

a. The contents of a petri dish were transferred to a square perspex counting chamber, a few drops of a concentrated iodine solution added and the eggs examined with the aid of a stereoscopic microscope.
b. By using the descriptions of Veglia[12] for *Haemonchus contortus*, of Kates & Turner[4] for *N. spathiger* and of Thomas[8] for *Nematodirus battus* and *Nematodirus filicollis*, eggs were grouped into different stages of development and the results expressed on a percentage basis.
c. The different larval stages were collected and at least 25 eggs were transferred to a glass slide. A coverslip was then placed on the specimen and sealed with Glyceel before examining it with a standard microscope.
d. The first moult was regarded as part of the first stage and the second moult included with the second larvae respectively. Only if the process of moulting had been completed and the larva was free from the sheath of the previous stage, was it classified in the following stage.
e. After 21 days either in the refrigerator or incubator at a constant temperature the contents of the four remaining petri dishes were pooled for the final examination.

The different stages of development in the eggs were photographed using a Wild M 20 microscope with a phototube attachment and an Icarex (Zeiss-Ikon) camera with built-in light meter. Observations were concentrated on the morphology of specimens incubated at 27°C.

RESULTS

The interval of time required by the eggs of *N. spathiger* to reach the different stages of development at different temperatures is summarized in Table 5. The stage of development reached by 21 days at different temperatures is illustrated in Fig. 1.

From the summarized and illustrated data in Table 5 and Fig. 1 the following conclusions were drawn:—

(a) Development was completely inhibited at 0°C and could only progress to the morula stage at 4 to 7° and above 39°C respectively.
(b) At temperatures of 10°C and higher, further development could take place; hatching started at 21°C and reached its maximum when temperatures varied from 27 to 33°C. At 36°C hatching decreased and at higher temperatures it was completely suppressed.

Table 5: DAYS REQUIRED FOR **N. SPATHIGER** EGGS TO REACH DIFFERENT STAGES OF DEVELOPMENT AT DIFFERENT TEMPERATURES

Temp. °C	Morula	Larval stage in eggs			Hatched	Mean Mortality
		1st	2nd	3rd		
0	—	—	—	—	—	7
4	3	—	—	—	—	2
7	3	—	—	—	—	2
10	2	3	—	—	—	1
19	2	5	9	—	—	1
21	2	3	6	12	19	4
24	2	4	6	9	12	3
27	2	3	4	7	10	2
30	2	3	4	7	10	5
33	2	2	4	5	8	2
36	2	3	4	6	10	1
39	2	3	—	—	—	3
42	2	—	—	—	—	3

The most suitable temperature for development and hatching seems to be 33°C. At this temperature some eggs had hatched within 8 days of incubation; this rose to 93 per cent after 21 days.

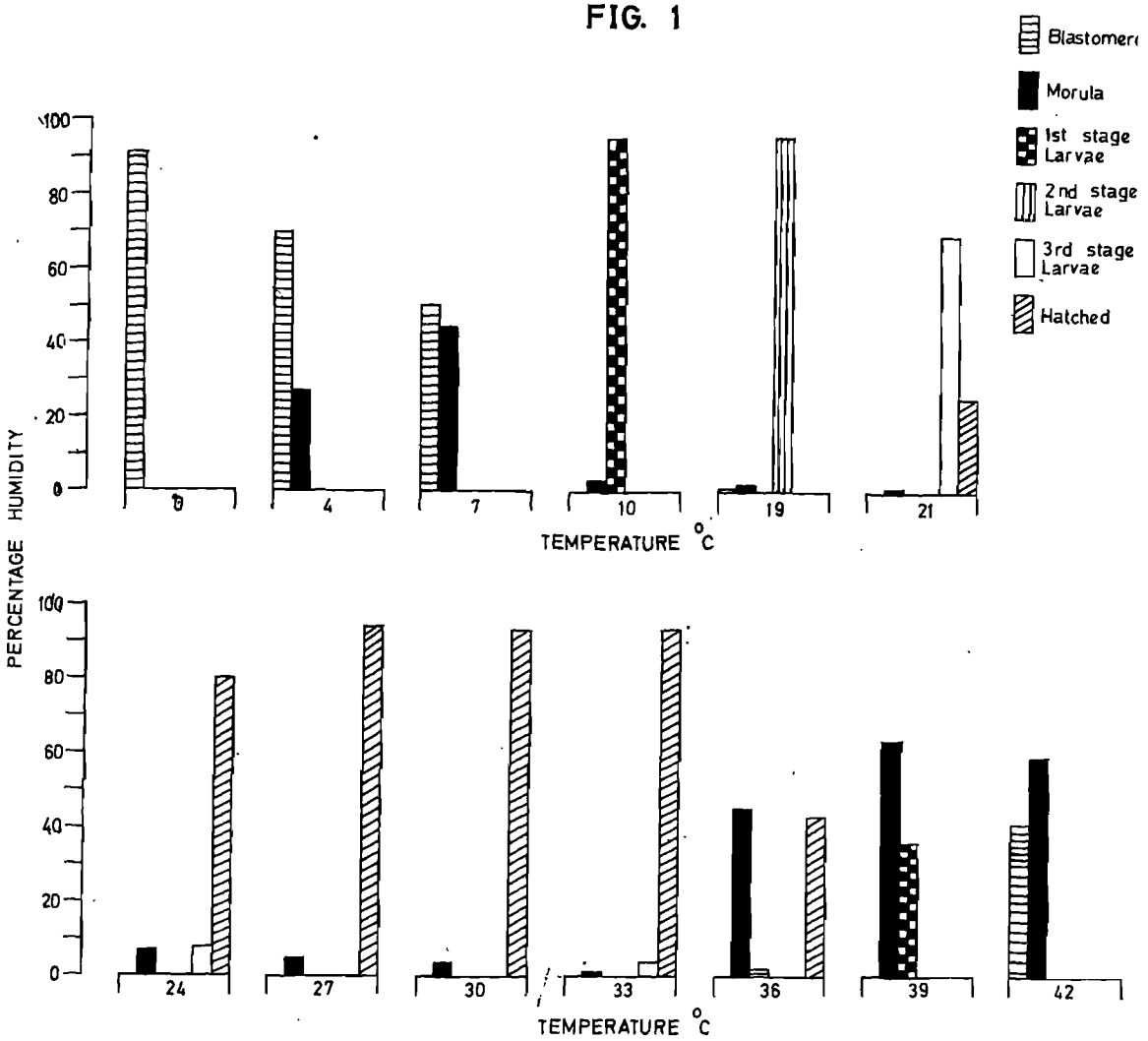
Figures 2 to 13 illustrate different stages in the development of *N. spathiger* eggs at an incubation temperature of 27°C. At this temperature, eggs of *N. spathiger* developed to the morula, tadpole, second and third larval stages in 2, 3, 4 and 7 days respectively and hatching started after 10 days' incubation (Table 5).

EXPERIMENT 4: THE EFFECT OF COLD STORAGE ON FURTHER DEVELOPMENT

MATERIALS AND METHODS

In this experiment 40 g of freshly collected faeces were divided into two samples of 20 g each. After separation of the eggs and before incubation, each sample was further

FIG. 1



divided into 25 sub-samples of 150 *N. spathiger* eggs per petri dish in order to facilitate daily examination. The one group of 25 subsamples (sample 1) was kept in the refrigerator at 0°C for 7 days before incubation, while the control group of 25 subsamples was immediately incubated at 36°C. Another experimental sample of 20 g faeces (sample 2) was left in the refrigerator at 0°C for 7 days before separating the eggs and subdividing into subsamples. This sample was moistened daily by sprinkling a small volume of distilled water over the top layer of faeces. In this way the effect of the surrounding medium on further development of *N. spathiger* eggs could be ascertained. The techniques used during the daily examination of these subsamples were the same as those described above in Experiment 3.

RESULTS

It is evident from the results summarized in Table 6 that low temperatures have a favourable effect on the further development and hatching of *N. spathiger* eggs.

In the samples previously stored at 0°C (Samples 1 and 2) twice as many eggs contained third stage larvae after 7 days' incubation at 36°C than in those of the control group incubated at 36°C throughout. After 14 and 21 days' incubation, more than twice as many eggs had hatched in Samples 1 and 2 when compared with the control. No difference could be illustrated between the development of eggs stored at 0°C in distilled water and those stored at that temperature in faeces.

DISCUSSION AND CONCLUSIONS

The dimensions of eggs and the infective larvae confirm the observations of other workers [1-7].

The eggs are usually laid in the 2 to 8 blastomere stage but occasionally non-fertilized eggs or those with only one blastomere are found.

The pre-parasitic development of eggs in a distilled water medium is completely inhibited at 0°C. At temperatures which vary from 4 to 7°C, as well as those above 39°C, development was suppressed in the morula stage, but from 10 to 39°C further development took place and hatching occurred between 21 and 36°C, with the highest percentage hatch from 27 to 33°C. At 33°C a minimum period of 8 days was required for eggs to start hatching; this temperature must therefore be regarded as optimum for larval development and hatching. Comparing these observations with those of Marquardt & Senger [9], definite similarities are noticed, especially as regards (a) the intervals before hatching at different temperatures (Table 2); (b) the optimum temperature of 30°C; and (c) the temperature range of 10 to 37°C as being the best for development of ova.

The present observations on the life-history of *N. spathiger* incubated at 27°C (Figures 2 to 13) confirm those of Kates & Turner [4] and can be summarized as follows:

a. The shell of the egg is transparent and more or less of uniform thickness. This observation does not agree with those of Tetley [3] and Railliet & Henry [1], who detected thickenings at the poles. Blastomeres are usually homogeneously granular and a nucleus can be detected as a light area in the centre. A hyaline membrane is visible at each egg pole.

b. On the second day of incubation at 27°C, 23 per cent of all eggs were still in the blastomere stage whilst 77 per cent had developed to the morula stage.

c. On the third day 10 per cent had morulae but 88 per cent had developed to the tadpole stage or beyond. The tadpole stage was determined by comparison to Veglia's [12] observations in *H. contortus*. This stage was included in the first larval stage to facilitate classification. At this stage no internal organisation could be recognized but larvae were very active within the shell.

d. On the fourth day of incubation the cuticle of the first moult was first seen. During the same day 84 per cent of all larvae had freed themselves of this cuticle, i.e. they had moulted and were then at the second stage of development. These larvae were very sluggish and only capable of infrequent and slow movements.

e. After 7 days the internal structure became visible, as well as the second larval sheath, and at the end of this day 10 per cent of the larvae had completed their moult and were then classified as third stage larvae. These larvae showed considerable activity.

f. During the next two days more larvae developed to the third stage and at the end of the ninth day, 95 per cent of the larvae were at this stage.

g. Hatching started on the 10th day and gradually progressed until the 16th day, when more than 90 per cent of the larvae were swimming free in the water. This observation is not in agreement with that of Kates & Turner [4], who found that in a culture at 27°C hatching only started after 14 days (Table 2).

h. During hatching the larvae usually emerged through one side of the egg membrane. The very loose and wide cuticle of the first moult was then scraped off and left behind within the egg shell as has been noted with other *Nematodirus* spp. [8, 13].

If eggs of *N. spathiger* are exposed to low temperature (0°C), whether in distilled water or in faeces, before being cultured at a higher temperature (36°C), subsequent development and hatching are influenced very favourably. This phenomenon can be compared with a similar process of sensitization by low temperatures, before incubation at higher temperatures, that was considered necessary for the hatching of both *N. battus* and *N. filicollis* [14, 15].

Table 6: THE EFFECT OF COLD STORAGE ON THE FURTHER DEVELOPMENT AND HATCHING OF **N. SPATHIGER** EGGS INCUBATED AT 36°C

Period of incubation days	*Sample	Blastomere %	Morula %	Larval stage in eggs %			Hatched %	Mortality %
				1st	2nd	3rd		
7	1	—	3	3	40	53	—	—
	2	2	2	5	35	55	—	1
	Control	—	57	8	13	20	—	2
14	1	—	7	3	—	8	82	—
	2	—	2	7	—	10	81	—
	Control	—	48	9	—	7	35	1
21	1	—	3	3	—	2	89	3
	2	—	3	2	—	4	89	2
	Control	—	45	2	—	—	43	10

*Sample 1: Eggs separated from faecal sample and kept at 0°C for one week, thereafter incubated at 36°C.
Sample 2: Eggs not separated from faecal sample and kept at 0°C for one week, thereafter incubated at 36°C.
Control: Eggs separated from faecal sample and immediately incubated at 36°C.

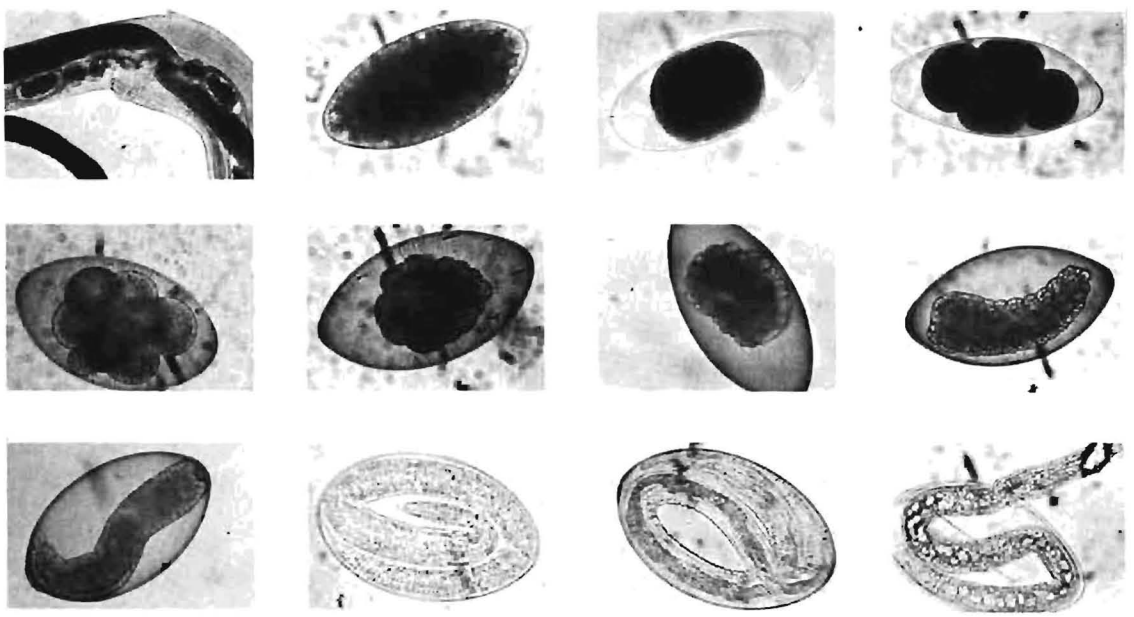


Fig. 2. Photomicrograph of ovijector of female **N. spathiger** showing eggs before excretion.
Fig. 3. Non-fertilized egg.
Fig. 4. Egg containing one blastomere.
Fig. 5. Egg containing four blastomeres.
Fig. 6. Egg containing eight blastomeres.
Fig. 7. Transition between blastomeres and morula stages.
Fig. 8. Photomicrograph of morula stage.
Fig. 9. Tadpole stage classified as early first stage larvae.
Fig. 10. First stage larvae.
Fig. 11. Transition between first and second stages classified as first stage.
Fig. 12. Second stage larva after mechanical rupture of egg shell.
Fig. 13. Third stage larva just before hatching, showing cuticles of first and second moult.

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A CONTRIBUTION TOWARDS THE DIAGNOSIS OF TRICHOSTRONGYLOSIS IN FLOCKS OF SHEEP UNDER FIELD CONDITIONS

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SUMMARY

To arrive at a more accurate yet simpler and more practical method of estimating worm burdens in flocks of sheep, correlations were made between faecal worm egg counts and worm counts done at autopsy for five genera of Trichostrongylidae on eleven flocks of five sheep each, the flocks being kept on three different types of veld. The egg count done on each flock as a whole yielded a more reliable estimate of the total worm burden of the flock than a total worm count done on one randomly selected member of that flock. In the case of infestation by *Haemonchus*, the egg count gave more reliable results than the worm count done on two members of the flock; in the case of *Nematodirus* the egg counting technique even proved superior to worm counts done on three randomly selected members of the flock.

INTRODUCTION

Trichostrongylosis of grazing animals presents a number of specific problems:

1. It is a combined flock- and pasture condition.
2. Control measures should be instituted before ill effects are obvious.
3. The requisite anthelmintics are expensive and curative success is often not obvious to the farmer.
4. Livestock are practically never free of parasitic helminths. The degree of infestation needs to be established and this determines the line of therapeutic action.
5. The degree of infestation varies considerably between individuals of the same flock.
6. A complete survey of the incidence of trichostrongylus in a flock is impossible due to the number of stock involved. The results of sampling are erratic due to uneven distribution patterns of worm incidence.

On farms most therapeutic measures are carried out without accurate knowledge of flock infestation with Trichostrongylidae. Considerable expense and a great deal of work are involved. Veterinary supervision by continuous survey of the incidence could save a great deal of expenditure and effort. Such a survey necessitates suitable diagnostic procedures for the study of the dynamics of worm populations in flocks of sheep. No known procedure fufils all requirements completely and the aim of the present investigation is to measure how closely known diagnostic procedures represent worm incidence in flocks.

The advantages and disadvantages of the following techniques for determining the incidence of trichostronglylosis in a sheep flock are:

(a) *Worm count at autopsy:* The method provides an accurate count of worms in the individual sheep, including the immature stages. But is is time-consuming, requiring 2-8 working hours per animal. The method requires the sacrifice of the animal(s) selected from the flock. This may be unacceptable to the stock owner and renders repeated use of the individual impossible. It may not give an adequate reflection of the degree of infestation of the flock as a whole.

(b) The counting of worm eggs in faecal samples: The method is less laborious and 6—12 egg counts can be completed in one working hour. Approximately the same time is required for differential counts of larvae from faecal cultures. Individuals

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subjected to the test can be used again. On the other hand, the egg count of the individual sheep varies considerably with respect to the number of worms present. Burdens of immature worms cannot be assessed.

Both techniques thus incorporate sources of error. The discrepancy between the individual worm count at autopsy and the flock incidence can be reduced by repeating the counting operations on further individuals of the same flock. The cost of the sheep and the work involved set a limit to the practicability of such repetitions. The egg counting technique involves two sources of error: the discrepancy between the egg count and worm count and that between the worm count of the individual and of the flock. Repetitions within the same flock are more practical, as no sheep need be killed and considerably less labour is required. The discrepancy between egg and worm count of the individual animal is expected to be reduced when utilizing counts of several animals of the same flock. This may give fairly accurate information on the worm incidence of the whole flock.

MATERIALS AND METHODS

Five sheep each from 11 different flocks kept on three different types of grazing were used. Sheep and pastures were naturally infested and the flocks were not subjected to any anthelmintic treatment for at least five weeks prior to the trial.

Faecal samples were collected from the rectum of each sheep. These were examined by the "MacMaster Counting Technique"[1] with the following modifications. Four chambers of 0,15 ml fluid were examined when the total egg count per gram of faeces (epg) of the sample was 900 or higher. Eight chambers were examined when the total count was 450—900 epg and 12 chambers were examined when the total count was less than 450 epg.

Droppings of some individual sheep were soft but formed. The correction factor of multiplying such counts by 1.5, suggested by Levine & Clark[2], was used in these instances.

During the process of counting *Nematodirus* eggs were distinguished from the small strongyle eggs. The latter were assigned to the four genera present: *Haemonchus, Ostertagia, Trichostrongylus* and *Oesophagostomum,* on a percentage basis according to differentiations of third stage larvae hatched from faecal cultures. The number of larvae differentiated depended on the egg count. In the case of an epg of 500 or higher, one larva was differentiated for every 5 epg. In the case of an egg count between 100 and 500 epg, 100 larvae were differentiated. In the case of an egg count of less than 100 epg, only as many larvae were differentiated as the number of eggs present per gram.

All animals were subsequently sacrificed and their total worm burden established by the technique suggested by Reinecke[3].

The first step was to establish whether the discrepancy between the egg count of the flock (of five animals) and the worm count of the same flock was smaller than that between the egg count and the worm count of an individual sheep. For this test one sheep was selected at random from each of the 11 flocks. The correlation between the egg count and worm count of these 11 individuals was calculated for each worm genus. The correlation between the total egg count and the total worm count of each flock was subsequently calculated for each worm genus and the results compared with the former.

RESULTS

The results of comparing egg counts and worm counts of individual sheep and those of the total flocks of five sheep are shown in Table 1.

All subsequent evaluations were made on the basis of worms per sheep in order to facilitate interpretation. The "expected number of worms" (ENW) was compared with the "number of worms found" (NWF). The ENW was calculated as the arithmetic mean of the worm count of one, subsequently two, three and four sheep of each flock. The sheep were selected by drawing lots.

For the second section, the comparison of flock egg counts with the NWF regression lines were calculated for each worm genus using the egg counts and worm counts of all individual sheep. The results were plotted on graphs and studied for possible deviations from the general trend of individuals of the flocks grazing on certain veld types. When such trends could not be found, pooling of all results appeared to be justified. Subsequently, the ENW was calculated for each flock and each worm genus using the total egg counts and the regression lines.

The NWF is the arithmetic mean of the worm count of the five sheep of each flock.

Table 1: COMPARISON OF THE CORRELATIONS BETWEEN THE EGG COUNTS AND THE WORM COUNTS OF INDIVIDUAL SHEEP (a) AND TOTAL FLOCK OF 5 SHEEP (b)

Genus	Correlation Factor "r"		P-values	
	(a)	(b)	(a)	(b)
Haemonchus	0,3008	0,8825	N/S*	0,001
Ostertagia	0,7398	0,8867	0,01	0,001
Trichostrongylus	0,9055	0,9131	0,001	0,001
Oesophagostomum	0,4608	0,7966	N/S*	0,005
Nematodirus	0,6574	0,8337	0,005	0,001

*Not significant

A narrower correlation exists between epg and worm count for all genera when using total counts of the flock than when using counts of individual animals.

The ENW is correlated with the NWF in Table 2.

The correlation between egg counts of the flock and worm counts for all genera are closer than the correlation between the worm count of one randomly selected sheep and the worm count of the flock. In the case of the genus *Haemonchus*, it was even closer than the correlation between the worm count

Table 2: SAMPLE CORRELATION COEFFICIENT "r" AND SIGNIFICANCE THEREOF FOR THE CORRELATION BETWEEN "EXPECTED NUMBER OF WORMS" AND "NUMBER OF WORMS FOUND"

Expected number of worms calculated from:	Worm Genus:	"r"	"t"	P
worm count of 1 sheep per flock	**Haemonchus**	0,7858	3,812	0,005
" " " 2 " " "	"	0,9197	7,025	0,001
" " " 3 " " "	"	0,9830	25,22	0,001
" " " 4 " " "	"	0,9956	31,88	0,001
egg count of the flock	"	0,9824	15,78	0,001
worm count of 1 sheep per flock	**Ostertagia**	0,7049	2,982	0,05
" " " 2 " " "	"	0,9590	10,15	0,001
" " " 3 " " "	"	0,9892	20,25	0,001
" " " 4 " " "	"	0,9953	30,83	0,001
egg count of the flock	"	0,8921	5,922	0,001
worm count of 1 sheep per flock	**Trichostrongylus**	0,8483	4,807	0,001
" " " 2 " " "	"	0,9869	18,35	0,001
" " " 3 " " "	"	0,9848	17,01	0,001
" " " 4 " " "	"	0,9938	26,82	0,001
egg count of the flock	"	0,9131	6,718	0,001
worm count of 1 sheep per flock	**Nematodirus**	0,4534	N/S	N/S
" " " 2 " " "	"	0,3435	N/S	N/S
" " " 3 " " "	"	0,7411	3,311	0,01
" " " 4 " " "	"	0,8415	4,673	0,005
egg count of total flock	"	0,8334	4,523	0,005
worm count of 1 sheep per flock	**Oesophagostomum**	0,6929	2,899	0,025
" " " 2 " " "	"	0,9454	8,71	0,001
" " " 3 " " "	"	0,9873	18,65	0,001
" " " 4 " " "	"	0,9800	14,78	0,001
egg count of total flock	"	0,7973	3,963	0,005

of two sheep selected at random and the flock. In the case of the genus *Nematodirus* it was closer than the correlation between the worm count of three randomly selected sheep and the flock.

Information on the discrepancy between worm counts from one, two, three and four sheep and the worm count of the flock (of five sheep) was regarded as desirable as well as on the discrepancy between flock egg counts and flock worm counts. This has been calculated from the 95% confidence intervals to the regression lines for some salient worm counts (Table 3).

DISCUSSION

The data are based on the findings in a limited number of flocks from a comparatively small area. In spite of this, definite trends are indicated which may prove significant.

The correlation between flock egg counts and flock worm counts for all genera was more significant than the same correlation between these counts obtained from randomized individuals of each flock. This result conforms to expectations. It is also reasonable to expect a further narrowing of this correlation when increasing the number of

Table 3: NUMBER OF WORMS (PER SHEEP) THAT CAN BE ESTIMATED WITH 95% CONFIDENCE IN A FLOCK WHEN FINDING THE NUMBER OF WORMS SPECIFIED AT AUTOPSY OF 1, 2, 3, OR 4 SHEEP OR WHEN FINDING THE NUMBER OF WORM EGGS SPECIFIED IN THE FLOCK

Genus	Type of Examination	Worms (or epg) found	expected deviation in flock
Haemonchus	autopsy 1 sheep	1 000	572
	„ 2 „	1 000	328
	„ 3 „	1 000	95
	„ 4 „	1 000	75
	egg count of flock	6 572	1 000 ± 169
Ostertagia	autopsy 1 sheep	5 000	1 907
	„ 2 „	5 000	1 171
	„ 3 „	5 000	583
	„ 4 „	5 000	393
	egg count of flock	536	5 000 ± 2 025
Trichostrongylus	autopsy 1 sheep	20 000	8 295
	„ 2 „	20 000	2 518
	„ 3 „	20 000	2 774
	„ 4 „	20 000	1 770
	egg count of flock	5 050	20 000 ± 7 073
Oesophagostomum	autopsy 1 sheep	100	63
	„ 2 „	100	25
	„ 3 „	100	12
	„ 4 „	100	15
	egg count of flock	932	100 ± 59
Nematodirus	autopsy of 1 and 2 sheep		very large variations
	autopsy 3 sheep	500	245
	„ 4 „	500	211
	egg count of flock	68	500 ± 257

individuals from which observations are made.

Flocks of five sheep used in the present study are infinitely smaller than sheep flocks under general farming conditions. Considerable discrepancies must be expected to exist between the worm count of a sample of 5 sheep and a total flock of, say, 500 sheep. This could be reduced by increasing the size of the sample and examining 10 or even 25 sheep. Such an increase in sample size is impractical when using the worm counting technique at autopsy. It is, however, practical for the egg counting technique. A suitable pooling method of faecal samples from several sheep for the egg counting technique could be developed, thereby further reducing the amount of work involved in making a flock diagnosis.

To what extent the discrepancy between egg count and worm count in larger flocks is reduced, remains speculative at the moment. Indications are that the correlation between the worm egg counts of 25 sheep and the worm counts of a flock of 500 sheep may be as significant as the correlation between the worm counts of 5 sheep and the flock worm counts. Whether this is correct, warrants further investigation. If confirmed, it would prove the egg counting technique superior to the method of counting worms at autopsy for establishing the flock incidence of Trichostrongylidae, in that it requires less time and no sacrifice of stock.

This method still has the disadvantage of giving no information on the incidence of immature stages. The extent of this drawback is determined by the proportion of immature stages to the adults found. Viljoen [4] found 800 835 adult stages and 152 319 fourth stage larvae of Trichostrongylidae in 410 sheep carrying naturally acquired worm infestations. His information on this proportion is scanty as far as the genus *Ostertagia* is concerned. In the present investigation 107 560 mature and 35 398 immature *Ostertagia* specimens were found at autopsy. The majority of the immature worms found by Viljoen belonged to the genus *Nematodirus;* 30 084 immature stages of the genera *Haemonchus, Trichostrongylus, Ostertagia* and *Oesophagostomum* were found as compared with 726 158 adults. This is less than 10%.

Taking these figures into consideration, it may be concluded, that, generally speaking, the immature stages of *Haemonchus, Ostertagia, Trichostrongylus* and *Oesophagostomum* do not contribute a very significant proportion to the total worm burden. It is quite likely, therefore, that the egg counting technique will give reliable evidence concerning the incidence of these genera of Trichostrongylidae.

Nematodirus needs special consideration. Viljoen found 122 235 fourth stage larvae as compared to 182 237 adults, a proportion supported by the present findings. In individual animals, infestation rates with immature stages of this genus were often higher than those with adults. Special allowance must be made, therefore, when using the egg counting technique for establishing the worm load in the case of *Nematodirus* spp.

The egg counting technique offers the advantage of using the same individual sheep over and over again. This eliminates variations within the flock. This advantage is considerable for repeated studies. Repetitions are necessary in surveys of the degree of worm incidence, in direction of control measures and in the investigation of re-infestation rates.

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THE EFFICACY OF CAMBENDAZOLE AGAINST CESTODE AND NEMATODE INFESTATIONS IN SHEEP AND CATTLE

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SUMMARY

The anthelmintic efficacy of cambendazole, dosed at 20 or 25 mg/kg liveweight, was determined against naturally acquired cestode and nematode infestations in sheep and cattle.

In sheep, cambendazole was highly effective against *M. expansa* and fourth stage larvae and adults of *H. contortus, O. circumcincta, T. colubriformis* and *N. spathiger*. It apparently had no effect on *S. hepatica* or *C. tenuicollis*.

In cattle, cambendazole was effective against *Moniezia* spp. and highly effective against adult *H. placei, Cooperia* spp., *Trichostrongylus* spp. and *O. radiatum*.

INTRODUCTION

The efficacy of a new anthelmintic, cambendazole, isopropyl 2-(4-thiazolyl)-5-benzimidazolecarbamate, against nematode infestations in sheep and cattle has recently been reported [1, 2]. This anthelmintic, however, is not only effective against nematodes but exhibits marked activity against certain cestodes.

The present paper reports the results of controlled anthelmintic trials against naturally acquired cestode and nematode infestations in sheep and cattle.

MATERIALS AND METHODS

Sheep

Experiment 1. Twelve Dorper lambs, under three months of age and running on lucerne pasture in the Skeerpoort district of the Transvaal, were selected on the presence of visible tapeworm segments in faecal samples. They were transported to the laboratory and housed and fed under conditions calculated to preclude the acquisition of further helminth infestation.

The lambs were allocated to two groups using tables of random numbers and six were treated *per os* with cambendazole at a dosage rate of 25 mg/kg liveweight. Thirteen and fourteen days later all the lambs were slaughtered and the contents of the abomasa and small intestines washed on sieves with 150 μm apertures. The contents of the sieves were examined macroscopically for scolices, segments, strobila and adult *Haemonchus contortus* and the volume of water displaced by the tapeworms was recorded.

Experiment 2. Eighteen worm-free Merino lambs and one Dorper lamb were placed on an irrigated grass/clover pasture in the Hennops River district of the Transvaal for 47 days. They were then removed from the pasture and housed and fed under conditions that precluded the further acquisition of helminth infestation. Four Dorper lambs from the Skeerpoort district were added to their number.

For three days prior to treatment faeces were collected from each of the lambs and examined for tapeworm segments and eggs, and faecal worm egg counts were done on nematode eggs. On the day prior to treatment one Merino lamb was slaughtered as an indicator control. The remaining lambs were randomly allocated to two groups, each group containing two Merino lambs negative for cestode infestation on faecal examination and two Dorper lambs from Skeerpoort.

Thirteen of the lambs were treated intraruminally with cambendazole at 20 mg/kg liveweight. Two hours after treatment faecal collecting bags were attached to the treated lambs and the bags' contents examined periodically for a period of 48 hours for tapeworm segments. All the sheep were slaughtered one week after treatment.

At slaughter the abomasal ingesta were washed on a sieve with 37 μm apertures and the mucosa was digested with a pepsin/HCl

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solution. After thorough scraping of the mucosa and removal of all visible tapeworms the small intestinal ingesta and scrapings were placed in a modified Baermann apparatus in a waterbath to assist in the recovery of worms [3, 4].

The tapeworms were counted macroscopically; those which had the original terminal segment were counted separately and recorded as non-gravid tapeworms. The nematodes present were counted microscopically; the Dorper lambs from Skeerpoort were not examined for nematodes as they had been treated with thiabendazole prior to inclusion in the trial.

Experiment 3. Thirty-one Dorper lambs were run on the irrigated grass/clover pastures at Hennops River for two months. Their faeces were examined on three occasions for the presence of tapeworm eggs or segments.

They were then housed under worm-free conditions, randomly divided into two groups and on the day of the last faecal examination 25 were treated intra-ruminally with cambendazole at 20 mg/kg liveweight. Faecal collecting bags were hung on all the sheep and the contents examined 24 hours later for the presence of tapeworm segments. The sheep were slaughtered seven to nine days after treatment.

At slaughter the viscera were removed and allowed to lie for two hours. The small intestine was then separated from the mesentery and severed from the large intestine and placed in a large bucket. A warm 0,9% NaCl solution was poured through the small intestine [5], at the same time drawing the intestine through tightly applied fingers, thus forcing the fluid down the intestine until it flowed from the severed ileum. All visible cestodes were collected from the fluid in the bucket before the fluid was sieved on a sieve with 150 μm apertures.

The small intestine was opened and thoroughly washed and the washings sieved. The mucosa was then scraped and the scrapings sieved. The ingesta in the large intestine were also sieved. The contents of the various sieves were examined macroscopically for cestodes.

Cattle

The freshly collected faeces of seventeen Africander type calves, eight to sixteen months of age, originating from the Viljoenskroon district of the Orange Free State, were examined on three occasions for the presence of cestode and nematode eggs. The calves were randomly divided into two groups and on the day of the final faecal examination 11 were treated intra-ruminally with cambendazole at 20 mg/kg liveweight.

All the calves were slaughtered five to seven days after treatment. With the exception of the first half of the small intestine, which was processed in a waterbath after removing all visible cestodes and then sieved on a sieve with 37 μm apertures, the remainder of the gastro-intestinal ingesta was washed on sieves with 150 μm apertures.

The scolices of tapeworms and the nematodes of the large intestine were counted macroscopically while the abomasal and small intestinal ingesta were examined micro- and macroscopically for nematodes.

RESULTS

Sheep

The results are summarized in Tables 1 to 3.

Table 1: **SHEEP: EXPERIMENT 1.** CESTODE AND **H. CONTORTUS** RECOVERIES

Sheep No.	Cestodes recovered		Volume in ml Moniezia only	Haemonchus contortus
	M. expansa	A. centripunctata		
Untreated controls				
22	2	0	Not done	79
33	19	0	250	184
52	7	4	122	28
89	9	2	128	116
91	6	5	16	10
119	17	0	97	8
Treated with Cambendazole				
53	0	0	—	0
88	2	0	<1	1
110	0	0	—	0
120	0	0	—	0
124	0	0	—	0
129	0	0	—	0

Experiment 1. All the control lambs were infested with *Moniezia expansa* while three were also infested with *Avitellina centripunctata*, these lambs also harboured between 8 and 184 adult *H. contortus*. Only one of the treated lambs was infested; it harboured two immature *M. expansa* and one adult *H. contortus*.

The livers of two of the control lambs were examined for *Stilesia hepatica* and both were found to be infested, while two of the three livers examined in the treated group were also infested.

Table 2: **SHEEP: EXPERIMENT 2.** CESTODE AND NEMATODE RECOVERIES

Sheep No.	M. expansa recovered			Nematode epg at treatment	Nematodes recovered							
					H. contortus		O. circumcincta		T. colubriformis		N. spathiger	
	Non-gravid	Gravid	Total		4th	Adult	4th	Adult	4th	Adult	4th	Adult
Indicator Control												
80	8	21	29	Not done	1 752	431	30	60	0	395	2	5
Untreated Controls												
24	5	15	20	11 833	5 210	2 531	0	400	3	1 027	0	10
51	2	14	16	10 833	1 810	2 255	0	95	11	1 188	6	15
61	1	0	1	357	3 045	542	515	105	0	804	0	0
72	3	19	22	4 867	1 727	1 006	28	60	0	609	19	7
90	7	9	16	10 133	3 420	1 306	115	245	2	872	3	18
91	0	0	0	1 700	2 050	751	0	85	1	492	2	0
133	1	13	14	9 800	5 537	1 469	118	370	7	1 222	1	3
132	3	1	4	Not done—Dorper from Skeerpoort								
194	6	5	11	Not done—Dorper from Skeerpoort								
Treated with Cambendazole												
15	0	0	0	1 100	10	0	0	0	0	0	0	0
28	0	0	0	25 300	0	0	10	0	0	0	0	0
46	0	0	0	7 200	0	0	0	0	0	0	0	0
67	0	0	0	1 600	0	0	0	1	0	0	0	0
77	0	0	0	2 100	5	0	1	1	0	1	0	0
84	0	0	0	5 833	0	0	0	0	0	0	0	0
85	0	0	0	5 900	0	2	0	1	0	0	0	0
99	0	0	0	10 667	0	0	0	0	0	0	0	0
107	0	0	0	2 633	25	30	0	0	0	2	0	0
141	0	0	0	1 267	0	0	0	2	0	0	0	0
191	0	0	0	7 067	0	0	0	1	0	0	0	5
156	0	1	1	Not done—Dorper from Skeerpoort								
159	0	0	0	Not done—Dorper from Skeerpoort								

4th = Fourth stage larvae

epg = Eggs per gram of faeces

Experiment 2. With the exception of one lamb, which was not infested and another which harboured only one non-gravid *M. expansa*, the control lambs were infested with non-gravid and gravid *M. expansa*. Both the former lambs had also been negative on faecal examination.

Only one of the treated lambs harboured a single scolex of *M. expansa*, the others were all negative. Two of the treated lambs, although passing segments in their faeces on the day of treatment, passed no segments after treatment and were negative for tapeworms at slaughter. It is possible that the worms had been digested after treatment or that they had been excreted in the interval between treatment and the hanging of the faecal collecting bags. The two lambs which had been negative for cestode segments and eggs on faecal examination prior to treatment both passed segments after treatment.

The control lambs were also infested with fourth stage larvae and adults of *H. contortus, Ostertagia circumcincta, Trichostrongylus colubriformis* and *Nematodirus spathiger*. Treatment was highly effective against both stages of the above species.

Two of the treated lambs died from peritonitis resulting from the intra-ruminal treatment, while a third succumbed to pulmonary oedema of unknown origin. As they were processed for worm recovery immediately after death, their worm burdens are included in the results recorded in Table 2.

Experiment 3. Five of the six untreated lambs were infested with adult *M. expansa* and one of them harboured one immature worm. The sixth sheep, which had been negative on the final faecal examination, had no cestodes. Sheep 453, which had also been negative at the final faecal examination, harboured 10 *M. expansa*.

Nineteen treated sheep passed segments after treatment and were negative for cestodes at slaughter. The other six treated sheep, which passed no segments after treatment, and incidentally were also negative on the final faecal examination before treatment, also harboured no cestodes at slaughter.

All 29 cestodes in the control sheep were recovered after running the warm saline solution through the intestine and no other scolices were present on subsequent examination of the intestine.

Table 3: **SHEEP: EXPERIMENT 3.** CESTODE RECOVERIES

Sheep No.	Day of treatment		Segments in bags after treatment	*M. expansa* recovered			Volume in ml
	Cestode eggs in faeces	Segments in faeces		Non gravid	Gravid	Total	
Untreated controls							
435	+	–	–	0	10	10	12
450	–	–	–	0	0	0	0
453	–	–	–	1	9	10	28
458	+	+	+	0	1	1	1
461	+	–	–	0	2	2	50
466	+	–	–	0	6	6	20
Treated with Cambendazole							
	+	+	+	0	0	0	—
407	–	–	–	0	0	0	—
414	+	–	+	0	0	0	—
416	+	+	+	0	0	0	—
423	–	–	–	0	0	0	—
424	–	–	+	0	0	0	—
428	–	–	–	0	0	0	—
430	+	+	+	0	0	0	—
431	+	–	+	0	0	0	—
440	–	–	–	0	0	0	—
444	+	+	+	0	0	0	—
446	–	–	–	0	0	0	—
447	+	–	+	0	0	0	—
449	+	–	+	0	0	0	—
451	+	–	+	0	0	0	—
463	–	–	–	0	0	0	—
464	–	–	+	0	0	0	—
468	+	–	+	0	0	0	—
469	–	–	+	0	0	0	—
470	+	–	+	0	0	0	—
473	+	+	+	0	0	0	—
479	–	–	+	0	0	0	—
484	+	–	+	0	0	0	—
485	+	–	+	0	0	0	—
487	–	–	+	0	0	0	—

A large number of the treated sheep was also infested with *Cysticercus tenuicollis*. At slaughter some of these cysts were stripped from the mesentery and placed in a warm saline solution. Clearly visible contractions of the cyst wall indicated that the cysticerci were still alive.

Cattle

The results are summarized in Table 4.

Control Calves 18, 34 and 361 were infested with *M. expansa;* Calves 29 and 53 were infested with *Moniezia benedeni* and Calf 12 was uninfested.

Three of the treated calves were still infested at slaughter. Calf 27 had a mixed infestation of *M. expansa* and *M. benedeni,* while Calves 43 and 52 were infested with *M. benedeni* only.

The untreated calves were also infested with *Haemonchus placei, Cooperia* spp., *Trichostrongylus* spp. and *Oesophagostomum radiatum.* Treatment was higly effective against the nematodes present.

DISCUSSION AND CONCLUSIONS

Sheep

A total of 22 sheep was used as controls in the three experiments. At slaughter 20 of these sheep were found to be infested with cestodes and harboured a total of 233 tapeworms: a mean of 11 tapeworms per sheep. The 44 treated sheep harboured only three tapeworms between them.

Because of the difficulty of assessing the degree of cestode infestation prior to slaughter in naturally infested sheep, or of recovering and counting scolices expelled after treatment, it is impractical to use controlled or

Table 4: **CATTLE:** CESTODE AND NEMATODE RECOVERIES

Calf No.	Cestode eggs at treatment	*Moniezia* spp. recovered			Volume in ml	Nematode epg at treatment	Adult nematodes recovered			
		Non-gravid	Gravid	Total			*H. placei*	*Cooperia* spp.	*Trichostrongylus*	*O. radiatum*
Untreated Controls										
12	–	0	0	0	—	200	275	557	0	0
18	+	0	1	1	52	150	199	362	2	18
29	+	16	28	44	15	1 650	1 158	9 110	3 670	355
34	+	0	3	3	42	1 700	930	4 412	175	145
53	+	1	1	2	8	150	98	1 336	30	89
361	–	0	2	2	39	150	20	11 037	1 380	113
Treated with Cambendazole										
3	–	0	0	0	—	1 100	0	0	0	0
17	+	0	0	0	—	400	2	3	0	0
20	+	0	0	0	—	350	3	4	0	2
23	+	0	0	0	—	600	0	2	0	11
27	+	2	1	3	2	50	1	11	1	0
36	+	0	0	0	—	1 800	4	20	0	0
37	+	0	0	0	—	400	0	0	0	3
43	+	0	1	1	<1	250	32	4	0	18
52	+	0	1	1	<1	400	0	3	0	16
247	+	0	0	0	—	300	0	6	0	0
357	+	0	0	0	—	1 250	0	25	2	63

epg = Eggs per gram of faeces

critical anthelmintic trials, which require comparisons between numbers of helminths recovered, to determine efficacy. A stricter, but more simply applied criterion is to compare the number of sheep still infested after treatment with the number of infested sheep treated.

In the present trials, 38 sheep were treated with cambendazole at 20 mg/kg liveweight and only one remained infested at slaughter, thus 97,4 per cent were cleared of infestation. If, from the total, one were to exclude the eight sheep which passed no segments after treatment and were negative at slaughter, 96,7 per cent of the remaining 30 sheep were cleared of infestation. Cambendazole apparently was ineffective against *S. hepatica* and *C. tenuicollis*.

At the same time efficacy in excess of 90 per cent was obtained against the fourth stages and adults of a number of nematode species.

Cattle

Cambendazole dosed at 20 mg/kg liveweight was not as effective against cestodes in cattle as it was in sheep, as only 72,7 per cent of the treated calves were cleared of infestation: a higher dosage level seems indicated. The efficacy against nematodes was excellent.

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THE EFFICACY OF THIABENDAZOLE AGAINST IMMATURE AND ADULT *DICTYOCAULUS FILARIA* IN SHEEP

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SUMMARY

The controlled anthelmintic test was used to assess the efficacy of thiabendazole administered at 88 mg/kg liveweight against artificially induced immature and adult infestations of *Dictyocaulus filaria* in sheep.

Thiabendazole was 69,6 per cent effective against third and fourth stage larvae one to eight days old at the time of treatment.

Against fifth stage and adult worms, nine to 33 days old at treatment, thiabendazole was 84,1 per cent effective.

INTRODUCTION

The effect of thiabendazole against naturally acquired adult infestations of *Dictyocaulus filaria* in sheep has been described by Robinson[1] who used the controlled anthelmintic test as a means of assessing efficacy.

Walley[2] has used critical tests for the evaluation of anthelmintics against naturally acquired and induced infestations of *D. filaria*. In his test the expelled worms were collected in a plastic bag attached to a tracheotomy tube. The controlled anthelmintic test has been employed by Gibson & Parfitt[3] in artificial infestations with *D. filaria*. Both they and Walley infested lambs on a single occasion and then treated them at set periods thereafter, anthelmintic efficacy being determined against worms of a particular day of age at the time of treatment.

The present paper describes controlled anthelmintic tests against immature and adult *D. filaria* in which multiple infestations were employed to cover the immature and adult phases of the life-cycle as suggested by Reinecke[4].

MATERIALS AND METHODS

Forty-five Dorper lambs, approximately nine months old, were purchased in an arid region, where the chances of lungworm infestation were minimal, and transported to the laboratory. They were drenched with thiabendazole at 88 mg/kg and housed and fed under worm-free conditions.

Immature worms

Twenty-two of the lambs were infested daily for eight days with larvae of *D. filaria*. On the day following the last infestation, 11 lambs were treated with thiabendazole at 88 mg/kg liveweight administered intra-

Table 1: **EXPERIMENTAL DESIGNS.** IMMATURE AND ADULT WORMS

Immature Worms		Adult Worms	
Day	No. of larvae administered	Day	No. of larvae administered
		−33	100
−8	110	−30	100
−7	105	−27	100
−6	105	−24	105
−5	105	−21	85 Withdraw 1st L.V.C.
−4	95	−18	90 Introduce 2nd L.V.C.
−3	85	−15	100
−2	90	−12	95
−1	90	− 9	95
Total	785	Total	870
0	Treat 11 sheep Slaughter 1 Indicator control	0	Treat 11 sheep
24—26	Slaughter 21 sheep	3—6	Slaughter 21 sheep

L.V.C. = Larval viability control.

The first larval viability control was slaughtered on Day—11 and the second on Day—5.

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ruminally by means of a trochar and cannula. One lamb was slaughtered at the same time to serve as an indicator control. The remaining sheep were slaughtered 24 to 26 days after treatment.

The experimental design is summarized in Table 1.

Adult worms

Twenty-one of the remaining 23 lambs were infested with larvae of *D. filaria* on nine occasions at three day intervals. The other two lambs were infested on either four or five occasions at three day intervals and were slaughtered as larval viability controls.

Nine days after the last larval dose, 11 lambs were treated intraruminally with thiabendazole at 88 mg/kg liveweight. All the lambs were slaughtered three to six days after treatment. The experimental design is summarized in Table 1.

Autopsy procedure

At slaughter the mesenteric, hepatic and thoracic lymph nodes were collected and all excess fat removed. They were finely sliced with scissors and placed in a stainless steel pan containing a warm 1,2% solution of NaCl.

The trachea and bronchial tree were opened with scissors as far as possible into the pulmonary tissue and were thoroughly washed with a 1,2% saline solution. The washings were added to the contents of the stainless steel pan. The lungs were cut into approximately 1 cm square cubes and these were added to the pan. A metal sieve was placed on top of the lung cubes and 1,2% saline solution was added until the base of the sieve was covered and all the lung cubes submersed.

The pan was placed in a waterbath and incubated at 42°C for four hours. Thereafter the contents of the pan were washed over a sieve with 37 μm apertures and the lung cubes were discarded after careful washing to remove any adherent worms. The material trapped on the sieve was subsequently examined microscopically and the worms counted.

RESULTS

Immature worms

The total numbers of worms recovered from the control and treated sheep are ranked in Table 2.

The indicator control harboured nine third stage and 86 fourth stage larvae of *D. filaria*. With the exception of one sheep, which had 339 fourth stage larvae, the remainder of the controls had zero to five larvae in this stage of development and 201 to 356 adult worms with a mean total burden of 316 worms.

Table 2: WORM RECOVERIES IN THE TRIAL AGAINST IMMATURE WORMS

Numbers and developmental stages of **D. filaria** recovered from

Indicator Control		Untreated Controls			Treated Sheep		
3rd	4th	4th	Adult	Total	4th	Adult	Total
9	86	1	201	202	3	46	49
		1	217	218	0	58	58
		0	273	273	0	75	75
		1	273	274	5	84	89
		5	276	281	3	88	91
		2	289	291	1	99	100
		2	300	302	4	98	102
		0	311	311	10	103	113
		2	314	316	1	114	115
		339	356	695	0	116	116
					0	148	148
			Mean	316		Mean	96
						%Eff.	69,6

3rd = Third stage larvae
4th = Fourth stage larvae

The treated sheep had zero to 10 fourth stage larvae and 46 to 148 adult worms with a mean total burden of 96 worms. The efficacy calculated from a comparison of the mean total burdens was 69,6 per cent.

Adult worms

The total numbers of worms recovered from the control and treated sheep are ranked in Table 3.

Fourth stage larval burdens in the control and treated sheep varied from 5 to 39, and zero to 28 worms respectively, while adult burdens ranged between 50 and 321, and one and 59 worms respectively. The mean total burden of the controls was 201 worms and that of the treated sheep 32 worms, a mean efficacy of 84,1 per cent.

Table 3: WORM RECOVERIES IN THE TRIAL AGAINST ADULT WORMS

Numbers and developmental stages of **D. filaria** recovered from

Viability Controls			Untreated Controls			Treated Sheep		
3rd	4th	Adult	4th	Adult	Total	4th	Adult	Total
12	9	162	14	50	64	1	2	3
3	7	25	16	67	83	0	4	4
			5	120	125	3	1	4
			9	133	142	9	10	19
			16	172	188	11	11	22
			11	186	197	22	16	38
			19	244	263	18	22	40
			39	251	290	28	15	43
			31	293	324	14	30	44
			17	321	338	10	35	45
						26	59	85
				Mean	201		Mean	32
							%Eff.	84,1

3rd = Third stage larvae
4th = Fourth stage larvae

DISCUSSION

The discussion on the life cycle is based on results made available in a personal communication by Verster, Collins & Anderson.

Because of the rapidity with which the larvae reach the fifth stage and for the purpose of testing anthelmintics, it is more convenient to consider the third and fourth stages as a single entity than to split them into two separate stages as suggested by Reinecke [5] for anthelmintic tests against gastro-intestinal nematodes.

Although most of the worms have reached the fifth stage and a few are starting to migrate to the lungs by the eighth day of infestation, it is still practical to consider eight days as the transition period from immature to adult worms. Until eight days after infestation, the majority of worms is present in the mesenteric lymph nodes, thereafter ever increasing numbers migrate to the lungs. Thus treatment eight days after initial infestation is aimed at immature worms in extra-pulmonary sites and treatment thereafter at fifth stage and adult worms present in the lungs.

The indicator control slaughtered in the trial against immature worms was infested with third and fourth stage larvae only. This confirmed that the sheep treated when their infestations were one to eight days of age were infested with immature worms.

The mean efficacy of 84,1 per cent obtained against adult worms is similar to the 86,1 and 84,6 per cent recorded by Robinson [1] in two separate trails against natural infestations of adult *D. filaria*.

Although the technique employed was satisfactory for the recovery of adult worms, it was poor for the recovery of very young worms. This can be seen from the small number of worms recovered from the indicator control and from the second larval viability control slaughtered within seven days of the last two larval infestations in the trial against adult worms.

The mean number of worms recovered from the controls in the trial against adult worms was considerably lower than that of the controls in the trial against immature worms. As the former sheep had received nearly 100 larvae more than the latter during the period of infestation, the difference is even more significant. This difference is possibly due to development of resistance to reinfestation and a reduction in the number of worms already present during the prolonged period of infestation employed. This appears to have been the case in two sheep, in particular, from which only 64 and 83 worms were recovered. The worm burdens, however, were satisfactory from the point of view of determining anthelmintic efficacy.

ACKNOWLEDGEMENTS

Dr. R. K. Reinecke is thanked for advice on the experimental design and Mr. J. P. Louw and Mrs. S. M. Raymond for their technical assistance.

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Jl S. Afr. vet. Ass. 43 (1): 110—111 1972

CLINICAL NOTE KLINIESE AANTEKENING

TREATMENT OF LUXATION OF THE TARSOMETATARSAL JOINT OF THE DOG

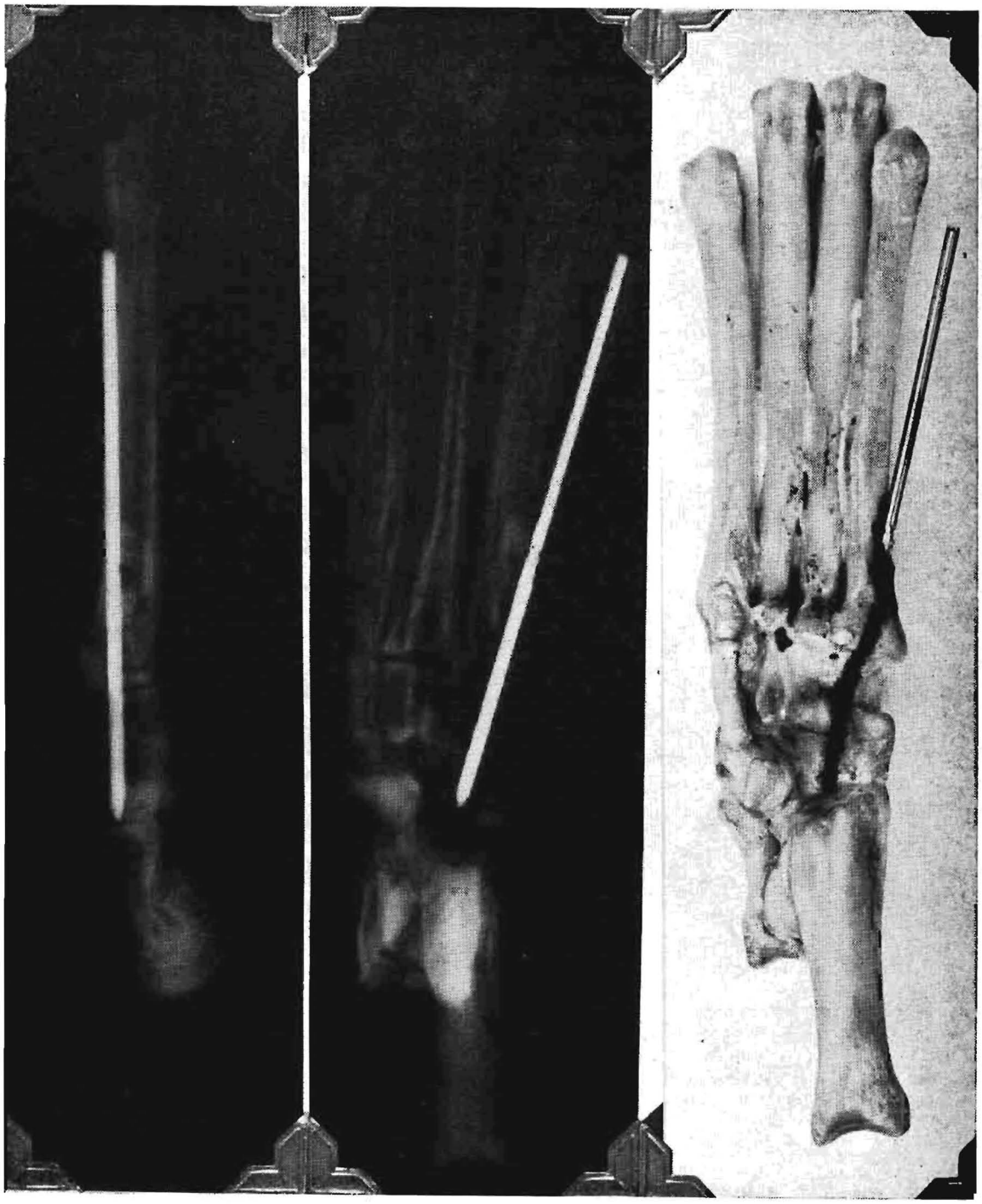
In response to an enquiry by Dr. C. G. N. Trace for clearer directions on the surgical procedure for treating luxation of the tarsometatarsal joint of the dog (P. H. le Roux, 1971, *Jl S. Afr. vet. med. Ass.* 42 : 195), a photograph in plantar view and roentgenographs in plantarodorsal and lateromedial views are reproduced here. The pin is notched at the point on a skeletal preparation of the left tarsometatarsus of a dog as prepared by the Department of Anatomy.

Fig. 1. Plantar view of canine left tarsometatarsal skeleton with pin in position, as indicated by the black line. Note notch on pin.

Fig. 2. Roentgenograph of canine left tarsometatarsal skeleton with pin. Plantarodorsal view. (Breakage in metatarsal bone V has been repaired, causing density in the roentgenograph as artefact).

Fig. 3. Roentgenograph of canine left tarsometatarsal skeleton with pin. Lateromedial view.

Photograph: A. M. du Bruyn, Section Photography, Onderstepoort. Roentgenographs: D. de Vos, Department of Surgery, Onderstepoort.



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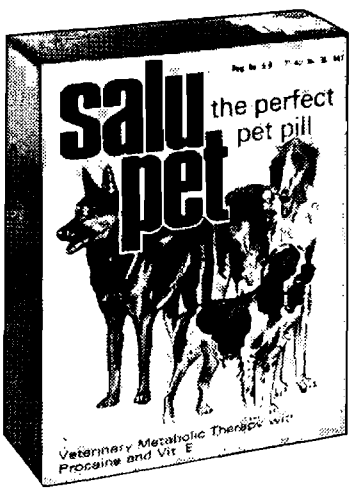

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In Memoriam

WYLE PHILIPPUS STEFANUS SNYMAN

Op 21 Junie 1971, twee maande voor sy 73ste verjaardag, is „Philip", soos hy deurgaans by sy kollegas en vriende bekend was, op Pietermaritzburg oorlede.

Omdat hy nooit self daarvan gepraat het nie, was dit nie alom bekend dat hy vir etlike jare 'n kroniese hartleier was en selfs 'n ernstige hartoperasie ondergaan het 'n jaar of wat voordat hy tuig in die Staatsdiens neergelê het nie.

Hy is op 22 September 1898 te Heidelberg, Transvaal, gebore, die jongste seun van wyle ds. en mev. P. S. Snyman. Hy was pas 'n jaar oud met die dood van sy vader.

Sy vroegste skoolopleiding was aan die C.N.O. skool, Heidelberg. Met die verhuising van die familie ongeveer tien jaar later na 'n plaas in die distrik Rustenburg in die omgewing van Koster, waar sy stiefvader, mnr. Johannes Eloff, geboer het, is hy na Sunnyside-skool, Pretoria, tot 1914 en vandaar na die Hoërskool Ermelo waar hy aan die einde van 1918 sy skoolloopbaan voltooi het. Alvorens hy in 1920 na Pretoria vir Universiteitsopleiding gegaan het, het hy vir 'n tyd lank op Sheepmore, in die distrik Ermelo, skoolgehou.

Hy was onder die eerste groep kandidate om vir die kursus in Veeartsenykunde in te skryf toe die Fakulteit van Veeartsenykunde aan die Transvaalse Universiteitskollege te Pretoria aan die begin van 1920 tot stand gekom het.

Ten spyte van al die probleme wat die Fakulteit in die aanvangsjare ervaar het, waardeur talle studente reeds in die eerste twee jaar van die kursus uitgesak het, het Philip egter met sy studies volhard en was hy een van die eerste sewe studente wat onder leiding van daardie voortreflike en alombekende wetenskaplike, Sir Arnold Theiler, die graad in Veeartsenykunde in die jaar 1924 verwerf het. In 1940 is die graad D.V.Sc. van die Universiteit van Suid-Afrika aan hom toegeken vir 'n verhandeling oor hondsdolheid.

In 1925 aanvaar hy 'n aanstelling as Staatsveearts in die Afdeling Veeartsenyvelddiens, eers in Pietersburg en daarna te Nongoma, Noord-Zoeloeland. 'n Jaar later is hy na Durban, alwaar hy met inspeksiepligte in verband met die vleisuitvoerhandel belas was. Vervolgens het hy diens gelewer as Staatsveearts te Greytown, was hy verbonde aan die Navorsingsinstituut, Onderstepoort, vanaf

1929 tot 1934 en het hy ook as lektor opgetree.

In 1934 is hy terug na Velddienste met bevordering tot Onder-Direkteur in bevel van die Oranje-Vrystaat, en tot Assistent-Direkteur met Pretoria as hoofkwartier in 1946.

By sy uitdienstreding weens bereiking van die ouderdomsgrens in 1959, het dr. Snyman reeds in die hoedanigheid van Adjunk-Direkteur (tans Direkteur) die hoogste sport van verantwoordelikheid in sy Afdeling behaal.

Hy was geken as iemand sonder vrees, wat nooit geaarsel het om verantwoordelikheid te aanvaar as hy eenmaal tot die oortuiging geraak het dat sy handelswyse en gevolglike besluite onder omstandighede die regte was nie. Sy kennis en praktiese aanleg by die bestryding van dreigende, gevaarlike veesiektes was altyd van onskatbare waarde vir sowel die leiding wat dit aan lede van sy personeel besorg het, asook vir bemoediging van die betrokke vee-eienaars, wat noodsaaklikerwys beperkings, wat op hulle gelê was, moes verduur en handhaaf. Ter erkenning van sy besondere bydrae tot die bestryding van bek- en klouseer by wyse van beplande bedrading van die Kruger-Wildtuin, is daar op die grens van die Wildtuin by Krokodilbrug 'n koperplaat deur sy kollegas en personeel op 1 Mei 1959 aangebring.

Na sy aftrede uit die Staatsdiens, het die oorledene hom op boerdery toegespits en wel op Corriesrust, 'n deel van die ou van Rooyenfamilieplaas in die distrik Greytown, Natal. Terselfdertyd het hy hom steeds beywer in belang van georganiseerde landbou en het as voorsitter van die plaaslike Boerevereniging, asook as lid van die Grondbewaringskomitee gedien.

Weens swak gesondheid moes hy boerdery staak en het hy in September 1970 na Pietermaritzburg verhuis, alwaar hy 10 maande later oorlede is.

In 1928 is Philip met Wynansie van Rooyen van Corriesrust getroud en uit die huwelik is twee seuns en 'n dogter gebore, laasgenoemde waarvan hom reeds vooraf gegaan het. Aan Wynansie en haar twee seuns, Fanus en Frankrinus, betuig ons ons innige meegevoel.

J. G. W.

In Memoriam

LATE BRIAN PETER RUDOLPH

Born on 26 April, 1940, at Pretoria, Brian Peter Rudolph was educated at Pretoria Boys High School and C.B.C. He matriculated in 1959 and proceeded to the University of Potchefstroom. After completing his first year, he was admitted to Onderstepoort and graduated in 1965. At Onderstepoort he was class captain for two years running and took a keen interest in various sporting activities.

After qualifying he went into private practice and opened up in Kempton Park. In 1966 he married Margaret Elphinstone, whom he had known for many years.

He took a great interest in wild birds of which he had an extensive knowledge. Because of his love of nature, one of his greatest ideals was to develop his own farm.

On the 8 October, 1971, after five years of hard work, and six weeks after he had taken a partner, he was tragically killed in a motor accident near Kempton Park.

To Margaret and his three sons, Peter, David and Roger, we extend our deepest sympathy, while we mourn the loss of a colleague and friend.

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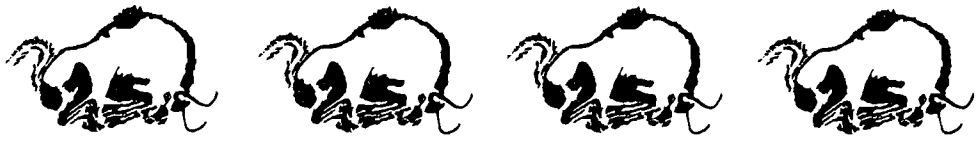

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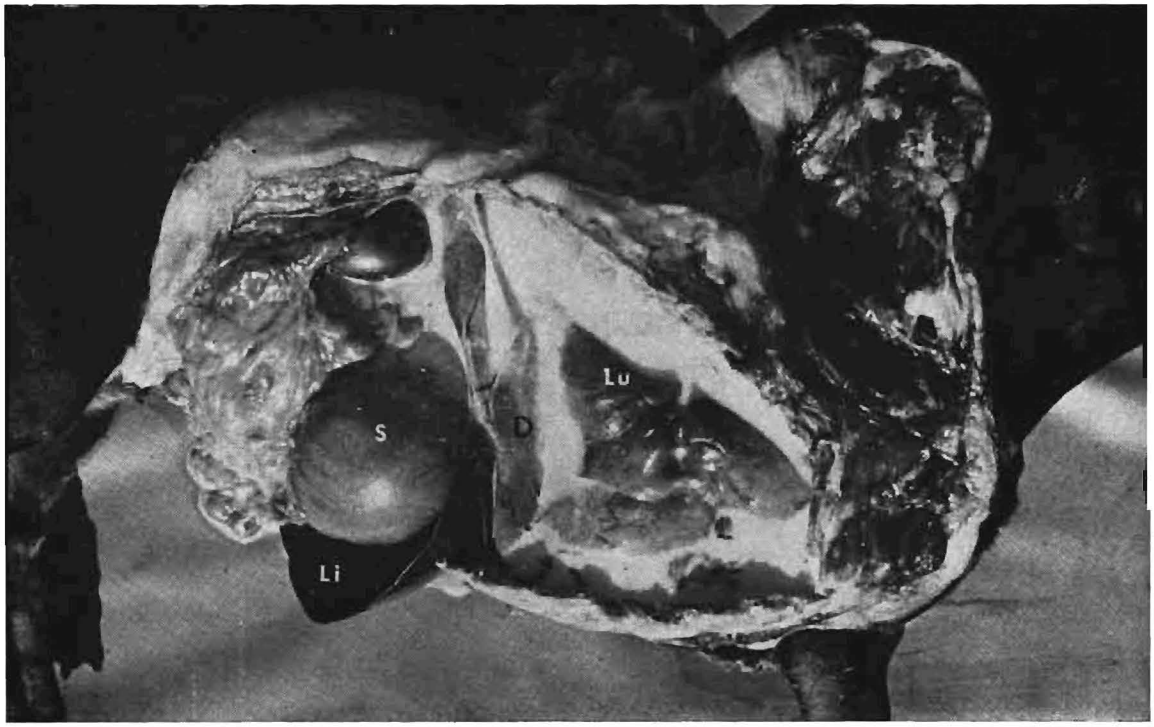

CHYLOTHORAX IN A DOG

A two-year-old female Dachshund developed dyspnoea soon after being run over by an automobile. The animal was brought to the Onderstepoort Outpatients Clinic for examination two days after the accident. Clinical examination revealed abnormal lung sounds and severe dyspnoea; death occurred before radiographic examination of the thorax could be performed.

Necropsy revealed approximately 1 500 ml of milky white liquid in the thoracic cavity, pleural hyperaemia, and bilateral pulmonary atelectasis. The fluid clotted soon after the thorax was opened; fat was extracted from the supernatant by the addition of ether. Based on its appearance, coagulation, and the fat extraction, it was identified as chyle and a diagnosis of chylothorax was made. Leakage from the right or thoracic lymphatic duct coult not be identified because of the large amount of chyle present in the mediastinal tissues.

Neoplasia in the mediastinum or trauma are the usual causes of rupture of these lymphatic ducts. The poorly defined nature of the ducts makes determination of the aetiology other than neoplasia difficult. Trauma was the assumed cause of leakage in this case. It is interesting that only about 72 hours were required for this large amount of chyle to accumulate and cause death.

In the figure the carcass is seen from the right. The right forelimb and rib cage have been reflected and the right abdominal wall has been removed.

L = Lung; D = Diaphragm; S = Stomach; Li = Liver.

Submitted by: R. C. Bartsch, Section of Pathology, Veterinary Research Institute, Onderstepoort.

CHILOTORAKS IN 'N HOND

'n Tweejarige Dachshundteef het dispnee ontwikkel kort nadat sy deur 'n motor omgery is. Twee dae na die ongeval is sy na die Kliniek vir Buite-pasiënte te Onderstepoort gebring, alwaar abnormale longgeluide en swaar dispnee vasgestel is. Sy is dood voordat die borskas roentgenologies ondersoek kon word.

Nadoods is ongeveer 1 500 ml melkerige, wit vloeistof in die borskas gevind met pleurale hiperemie en bilaterale kollabering van die longe. Die vloeistof het kort na die oopmaak van die borskas gestol, en vet kon d.m.v. eter uit die bodrywende sug geëkstraheer word. Op gronde van die voorkoms van die vloeistof, die stol daarvan en die vetekstraksie, is dit as kyl geïdentifiseer en 'n diagnose van chilotoraks gestel. Lekkasie van die borsbuis of regter limfbuis kon nie vasgestel word nie, omrede die groot hoeveelhede kyl in die mediastinale weefsels.

Neoplasie van die mediastinum of trouma is gewoonlik die oorsaak van ruptuur van hierdie buise. Die dunwandigheid van hierdie buise maak bepaling van die etiologie moeilik, behalwe in geval van neoplasie. Trouma is aanvaar as oorsaak in hierdie geval. Dis interessant dat soveel kyl binne 72 uur kon aansamel en die dood veroorsaak.

In die afbeelding word die karkas van regs gesien. Die regter voorpoot en ribbekas is omgeklap en die regter buikwand is verwyder.

Lu = Long; D = Diafragma; S = Maag; Li = Lewer.

Ingestuur deur: R. C. Bartsch, Seksie Patologie, Navorsingsinstituut vir Veeartsenykunde, Onderstepoort.