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THE JOURNAL OF THE S.A.V.M.A. is owned and published by the South African Veterinary Medical Association, of which it is the official organ. It appears quarterly and is devoted to matters of veterinary importance generally.

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## „TIERAERZTLICHE HOCHSCHULE, WIEN“ — 200-JARIGE BESTAANSVIERING

DIE SAVMV GEËER

Ter geleentheid van sy tweehonderdjarige bestaansviering in 1968, het die „Tierärztliche Hochschule" te Weenen, Oostenryk, 'n praggedenkbundel in 1970 die lig laat sien. 'n Eksemplaar hiervan is onlangs deur bemiddeling van prof. dr. D. R. Osterhoff by terugkeer van sy oorsese langverlof beskikbaar gestel.

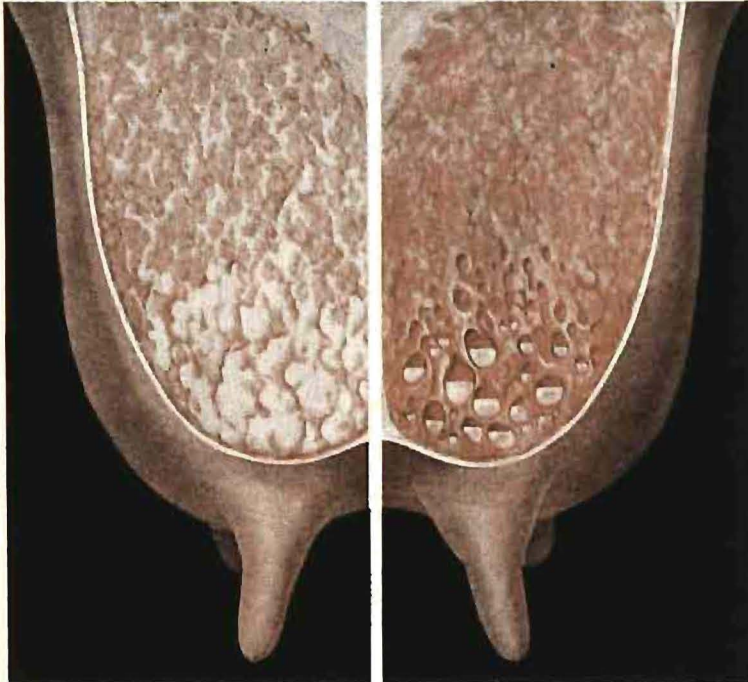
Op die voorblad van die teks verskyn 'n faksimile van die vergulde oorkonde wat die gelukwense en waarderingsgroete van die SAVMV, in Afrikaans en onder die hand van sy destydse President, dr. A. F. Tarr, aan die „Tierärztliche Hochschule" oordra. Van tallose soortgelyke dokumente wat by die feesverrigtings oorhandig is, is dit die enigste een wat afgebeeld is, en dit in so 'n ereposisie. Dit word met waardering aanvaar as 'n pluimpie vir die Vereniging en 'n gebaar wat die agting van ons Oostenrykse kollegas jeëns die beroep in Suid-Afrika weerspieël.

### CORRIGENDUM

Vol. 42 No. 1 p. 78, in the article by Giesecke & van den Heever on "Losses caused by Mastitis to Industrial and Fresh Milk Producers in the Republic of South Africa", for "...loss of milk amounting to 773 million kg or 32.5% read: "...loss of milk amounting to 583 million kg or 24.6% ...."



# Spring mastitis voor eer u pasiënt nog „Moeë” kan sê



Die illustrasie toon aan hoe nuwe Metibiotic-Mastitisskuim, as dit ingespuut word, die uier-kwart volkome vul.

In teëstelling hiermee vul die gewone olierige middels net die speen en die onderste gedeelte van die uier. Hulle kom nooit met die boonste agterkwart in aanraking nie.

**Nuwe METIBIOTIC is 'n selfgedrewe skuim.**

**Slegs 'n skuim kan die hele kwart vul.**

**En dan daar bly om sy taak te verrig!**

Daar is talle puik antibiotika vir mastitis. Maar konvensionele metodes van inspuiting ná melkery laat die middels nie versprei nie; hulle dryf net in die speen en onderste gedeelte van die uier rond. Die geneesmiddel bereik gewoonlik nie die besmette boonste gedeelte van die uier vir 'n volle melksiklus nie.

**Nuwe Metibiotic- aërosol-gedrewe matitisskuim** bereik onmiddellik die boonste gedeelte van die uier. Metibiotic bevat ook Tween, 'n verspreidings- en emulgeringsmiddel wat die antibiotikum dra na versamelbuise en alveoli wat voorheen moeilik bereik is.

**Geen „uitmelkery” voor behandeling nie.** Met konvensionele behandeling word 'n groot hoeveelheid van die geneesmiddel dikwels „uitgemelk” voordat dit die besmette weefsel bereik. Metibiotic se onmiddellike verspreidingswerking laat die volle dosis werk voordat „uitmelkery” kan plaasvind.

**Beproeft formule, verminder weefselbeskadiging.** Aktiewe bestanddele sluit in:

**Twee beproefde antibiotika** (penisillien-G-prokaseien 100.000 eenhede, dihidrostreptomisien 300 mg.) en een kortikosteroïde (prednisonasetaat U.S.P. 4 mg.) om infeksie te beheer en inflammasie te stil. Behandeling is vinniger, weefselbeskadiging minder, koste is laag.

## **NUWE METIBIOTIC**

**Waarskuwing:** Melk wat tydens behandeling, en oor 'n tydperk van 72 uur (6 melkbeurte) ná die laaste behandeling van die dier verkry word, is nie geskik vir menslike verbruik nie.

**Verpakking:** Enkeldosis-houers, doos met 12.



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Editorial

EDITORIAL POLICY

Redaksioneel

The Editorial Committee of the Journal of the South African Veterinary Medical Association consists of eight members elected at the Annual General Meeting. By accepting the task entrusted to them, they have more or less voluntarily chosen the company of printer's devils in favour to that of family, friends or cabaret stars during evenings, are forced to slog punctuation marks instead of golf balls during week-ends and to angle for factual inconsistencies and grammatical errors instead of trout and bass. Always under extreme pressure "to get the Journal out on time," the Committee, nevertheless, has to make time to consider its task in wider perspective and to re-define editorial policy. In this respect frank reactions from members of the Association, laudatory or critical, would be most welcome to serve the Committee as guide. Nothing is more disconcerting than emptying into a void. Uncritical acceptance of the Journal and formal votes of thanks at Annual General Meetings could well lead to self-delusion of excellence. Readers from adjoining territories and overseas, not necessarily members of the Association, could also play an important role in this respect. Distance should not lend enchantment: lack of knowledge of local conditions is more than made good by the greater opportunity of unprejudiced and unbiased evaluation in terms of international standards.

The Journal has been, and will continue to be, the official mouthpiece of the Association, insofar as it serves as publication medium for the scientific observations and findings of more general or specific veterinary interest, and as disseminator of information amongst all interested parties, whether they be members, subscribers, or regular or occasional readers. Contributions are not limited to members of the Association, nor necessarily to members of the veterinary profession. Perusal of previous issues bears this out.

Because of its geographical location and because of the unique position and developmental role of South Africa in this part of the continent, the Journal should also act as home and "foster-mother" to those organisations of veterinary nature in South-

ern Africa, whose internal affairs as yet do not make regular publication of their own journal practicable. On the other hand, the existence of the Journal of the SAVMA should not, and will not, act as deterrent to the publication of other veterinary journals in this part of the African continent. On the contrary, this should be encouraged "Competition," if it exists or should emerge at all, must be restricted to a normal, healthy atmosphere to induce and maintain the incentive element only.

Since its inception forty-four years ago, the Journal has co-existed with the Onderstepoort Journal of Veterinary Research, which began as the Report of the Government Veterinary Bacteriologist for the Year 1903—1904, under Arnold Theiler, as official publication of the Department of Agriculture. Initially, the SAVMA Journal subsisted almost solely on contributions from members of the Department. With the evolution of private practice and the opening of so many avenues of employment for veterinarians outside the Civil Service, the Journal was ready to cater for them as well. Nevertheless, a significant proportion of contributions still comes from officials of the Department of Agriculture, despite the fact that, in keeping with modern trends, the Onderstepoort Journal of Veterinary Research no longer caters for mainly the long, monograph type of article. Although as a result of a decision by the Director of Veterinary Services, his permission to publish is no longer acknowledged at the end of each contribution by officials of the Department, the Editorial Committee nevertheless expresses its sincerest gratitude to him, and, through him, to the Director of Veterinary Research and the Director of Veterinary Services (Field), for their coöperation and generous assistance in allowing the results of work done officially to appear in this Journal.

It is most gratifying to note the increasing volume of the erstwhile trickle from State Veterinarians in the Field Services, despite the relative handicap with regard to publication imposed upon them by the very nature of their work. One sometimes wonders whether the extent of this handicap



is not often exaggerated and more imaginary than real. Research projects may not be their primary object but they, as well as colleagues in private practice, do function as the eyes and ears of the profession. As such, they have a vital role to play.

In conversation with colleagues in the front line, namely those in the Field Service and in private practice, one is so often struck by the wealth of interesting information and hypothesis they have at their command. When challenged to write, they plead lack of time and opportunity to follow up and substantiate their observations and ideas by more detailed observation, experiments and consultation of the literature. If that be so, then why not submit the brief communication instead of a full-length article? How many a disease has long escaped notice, simply because the profession as a whole had not been made aware sufficiently of a symptom complex not properly fitting into any known category? Even if a diagnosis cannot be made, the report on a particular syndrome may give others the opportunity to be on the look-out and the "back-room boys" a chance of rendering assistance in arriving at a diagnosis. Quite apart from this, the seriousness of losses in particular categories of animal production, such as neonatal mortality, losses in pig raising, etc., is not properly known and hence not adequately realized.

The Editorial Committee is fully aware that the obtaining of a degree in veterinary science by no means implies the ability to write. The Committee has taken upon itself the arduous task of assisting contributors to the best of its ability by means of constructive criticism. The personal reaction of contributors to these criticisms has been most heartening and has adequately compensated committee members for the infinite trouble taken. In this respect, the Editorial Committee is deeply indebted to those colleagues, who, as experts in their particular fields, have so willingly given of their time, energy and expertise in refereeing articles, in writing detailed critiques and so have assisted contributors and have guided the Committee in coming to fair decisions.

What has been said in the foregoing two paragraphs applies particularly to the most mute sector of the profession in South Africa, namely the private practitioner. As a result of paucity of clinical communica-

tions, the erroneous impression may have been created that the Journal is nothing more than a miniature research journal, and that editorial policy wills it so. This problem has been a cause for concern to the Editorial Committee and the subject of a memorandum to the Council by a past chairman of the Committee. It was felt by Council that unless the issue be raised at an Annual General Meeting and a definite resolution taken, the Committee could do nothing more than appeal to clinicians. A heartening aspect has been the appearance of "Veterinary Clinician". The incentive taken by those veterinarians is most laudable and the Editorial Committee wishes them every success. The Journal of the SAVMA now occupies a position of "extreme centre" in the scene of South African veterinary journals. Coöperation between the editorial bodies concerned has already lead to mutual referral of articles, with author's consent, whenever these were deemed more suitable for a particular journal.

Returning to the concept of a more "outward" policy, it may be mentioned that the Editorial Committee has decided to approach veterinarians and veterinary organisations of surrounding territories with the invitation to use the Journal as publication medium in the spirit previously outlined. It was also felt that this Journal could well be the medium of bringing all the veterinary work done on this part of the African continent to the attention of the world outside. For this purpose, it is intended to run abstracts of all such publications. Already the work published in the Onderstepoort Journal is being abstracted. Other journals are being approached for the necessary permission. The present international distribution list of the SAVMA Journal is under scrutiny with the object of increasing its effective circulation; consideration is being given to having French, German, Spanish and Portuguese summaries added to articles published in this Journal to facilitate its international use.

At the "home front", branches of the Association have been requested to screen papers delivered at branch meetings for submission to the Journal. Should these branches wish to appoint recorders for this purpose, they will be coöpted to the Editorial Committee. This step, taken at the incentive of the President of the Association,



who is also a hard-working member of the Editorial Committee, has already met a favourable reception from some branches.

Two groups of veterinarians have already played a very active part in submission of papers to the Journal in relation to their total numbers, namely those in the employment of pharmaceutical houses and those concerned with wild life conservation and utilization. Municipal veterinarians are also doing a fair share. It is hoped that this "honourable mention" may act as a further incentive.

Although close association between veterinary and medical research has been a feature of the South African scene since the earliest days, only relatively recently has there been a noticeable trend for veterinarians to be associated with medical research institutes on a larger scale, mainly with the object of devoting attention to laboratory animals but at the same time forming part of research teams. With this development in view, the Editorial Committee has published most of the papers read at the "Symposium on the Production and Use of Laboratory Animals" held at Pretoria during June last year, under the auspices of the National Nutrition Institute of the Medical Research Council. To this body the Committee is grateful for permission granted to publish the papers. It is hoped that this action by the Committee will also act as an incentive.

The response of readers to the "Refresher Courses" has stimulated the Committee more actively to pursue the policy of publishing informative, up-to-date reviews.

With occasional exceptions, scant attention has been paid to veterinary politics in the Journal. By recommencing the policy of publishing editorials, at the suggestion of Dr. J. H. Mason and agreed to by other members of the Committee, this aspect could be brought into the limelight, more particularly if it were to evoke a response in the form of "Letters to the Editor". On the other hand, readers need not wait until an editorial has appeared before airing their views. Obviously, such letters are not restricted to matters of veterinary politics.

An idea mooted by the previous Secretary of the Association that the monthly circulars, issued to members only, should be up-graded to a regular monthly journal,et,

appears well worth while. Because of the shorter time lapse involved, it could increasingly serve as a source of personal and domestic information and as a platform for discussions and polemics which, for one reason or another, had best be restricted to members of the Association.

The appearance of the Journal of the SAVMA, its format, cover, position of the index, etc., have been called into question. Unless a majority opinion against any of these is raised, the Editorial Committee will continue its present procedure. The matter of the form of the references is still receiving attention. Most members consulted feel that the titles of articles referred to should be given. It would also seem desirable to have a uniform policy regarding this aspect for all veterinary publications in South Africa at least. As a new version of "Instructions to Contributors" will have to be drawn up, and the necessary time lapse will have to ensue between its issue and the acceptance of contributions in required form, any major changes can only take effect as from the next volume at the earliest (1972). Should any reader wish to offer criticisms or suggestions on any point, whether raised here or not, now is the time to act, between the issue of this number and the next (September).

In response to favourable replies from advertisers, an index to advertisements will appear immediately after the main index, as from this issue. Readers are kindly but urgently requested to mention this Journal when ordering drugs and equipment in response to advertisements: the advertiser has the right to know from which avenue he reaps the best results. In these days of imminent curtailment of advertising expenses, such a procedure is all the more important: without the support of advertisers, the Journal's finances would be direly straitened.

Wat Afrikaans betref, bevind die Redaksiekomitee hom in die moeilike posisie dat hy, tenspyte van sy sterk begeerte om Afrikaans as vaktaal te bevorder, nie sterk stappe kan doen teneinde 'n groter getal Afrikaanse bydraes te verseker nie, aangesien dit weer die internasionale gangbaarheid van hierdie Tydskrif sou verminder. Die offisiële beleid bly bestaan, dat bydraes in ingeen van die twee landstale aanvaar-



baar is en dat die keuse van medium geheel en al aan die bydraer oorgelaat word. Indien 'n Afrikaanse bydrae ingedien word, word die versoek gerig dat 'n betreklik uitvoerige Engelse opsomming die artikel vergesel, sodat die nie-Afrikaansmagtige ten volle op hoogte kan kom met die kern van die werk. Die Redaksiekomitee is bereid om hulp te verleen, indien iemand 'n bydrae wil lewer in een van die landstale wat hy

meen hy nie ten volle magtig is nie.

'n Slotgedagte, wat as vlieër ter meningspeiling opgelaat word, is die vraag of die instel van 'n Afrikaanse vaktaalrubriek ingang by lesers sal vind. Lastig vertaalbare terme en uitdrukkings sou dan onder so 'n hoof behandel kon word. Vir die taalkundige sou dit 'n handige bron wees en 'n tasbare bydrae van ons beroep tot die ontwikkeling van Afrikaans.

## BOOK REVIEW

## RESENSIE

### GOAT PRODUCTION IN THE TROPICS

C. DEVENDRA AND MARCA BURNS

R. & R. Clark Ltd. Edinburgh. 1970. Pp XII & 184, 48 illustrations and 376 References. Price £2 50.

Although issued as a technical communication of the Commonwealth Bureau of Animal Breeding and Genetics, this small volume contains much more information on goat breeds, performance, feeding and management than can normally be expected from this type of publication.

The extensive bibliography and excellent illustrations give some indication of the enthusiasm and knowledge with which the authors approached their task. The publication contains sufficient pertinent information about all the important aspects of the goat industry in the tropics to enable research worker, teacher, student and farmer to benefit from its reading.

I am not aware of comparable illustrated descriptions of goat breeds in Africa, the Near and Middle East, the Indian Subcontinent, Malaysia, Indonesia, the Phil-

ippines, China, Tibet, South America and Europe in English publications. The text is introduced with a successful attempt by the authors to show that much of the reputed destructiveness of the goat is not true. In most cases goats are introduced only after the vegetation has been destroyed by various other agents.

Throughout the book the growing importance of these multi-purpose animals in supplying meat, milk, skins and hair to an ever growing population in the tropics is stressed. I feel sure that readers will, after finishing this small volume, look upon the lowly goat with different eyes and that publication of the book will lead to greater efforts to improve goat breeds and give them an even more important place in animal husbandry in the tropics.

K. v. d. W.



## Arnold Theiler

### Memorial Lecture

### Gedenklesing

#### DIE VEEARTSENYKUNDIGE PROFESSIE EN SY PLEK IN DIE WETENSKAPLIKE ONTWIKKELING VAN SUID-AFRIKA\*

Douw G. STEYN\*\*

Geagte meneer die Rektor, professor Hofmeyr, dekaan van die Fakulteit Veeartsenykunde, dekane en professore van ander fakulteite, geagte eregaste, dames en here:

Ek wil die Fakulteit Veeartsenykunde hartlik dank vir die groot eer wat hulle my aangedoen het om my uit te nooi om die vyfde Arnold Theiler-gedenklesing te lewer tydens die viering van die vyftigjarige bestaan van die Fakulteit. Dit is vir my een van die heuglikste aande in my lewe en 'n groot eer en voorreg om iets te kan sê van, en lof te kan toeswaai aan, my eertydse hoof en een van die voortreflikste en grootste manne wat ek in my lewe geken het.

Die voorbereiding en skrywe van hierdie lesing was vir my daadwerklik 'n geestelike ervaring. Ek het die twee jaar te Onderstepoort, waar ek die groot voorreg gehad het om in noue aanraking met Sir Arnold te wees en te werk, hom leer ken en waardeer as die verwesenliking van wat ek as 'n ideale wetenskaplike navorser beskou: 'n harde werker met onuitputlike energie en entoesiasme en wye kennis, wyd-belese, vriendelik, behulpsaam en nederig in die toepassing van sy kennis en van die vrugte van sy arbeid tot voordeel van mens en dier.

Ek wil graag voldoen aan die versoek van die Fakulteit om kortliks aan te stip wat veeartsenykundige navorsing vir Suid-Afrikaanse wetenskap beteken en wat my siening is van die rol wat die veeartsenykundige professie nog verder in ons land

moet, en sal, speel, om reg te laat geskied aan sy wetenskaplike potensiaal.

Ek verwelkom so 'n versoek veral omdat dit so goed inpas by wat ek graag oor wyle Sir Arnold wil sê en ook omdat hy so 'n geweldig belangrike rol gespeel het in die ontstaan, ontwikkeling en betekenis van die veeartsenykundige wetenskap in Suid-Afrika en ook 'n belangrike bydrae gelewer het tot die mediese wetenskap.

Ek voel gedwonge om in die algemeen enkele besonderhede van Arnold Theiler se loopbaan en werk te verskaf, omdat sy hele lewe en strewe die belangrikste boustene en vrugte van sy werk gevorm het, en ook omdat daaruit sal blyk watter belangrike bydrae hy gelewer het tot die ontstaan en ontwikkeling van veeartsenykundige en mediese navorsing en aktiwiteite in ons land.

Arnold Theiler is op 26 Maart 1867, in die dorpie Frick, Kanton Argau, Switserland, gebore. Hy, sowel as ons, was gelukkig dat hy gebore is en gelewe het in 'n tydperk waarin daar groot belangstelling en aktiwiteite was op die gebied van biologiese navorsing. Reeds gedurende sy skooldae het hy baie belang in biologie gestel en was hy gelukkig om 'n onderwyser te gehad het wat hom opmerkzaamheid geleer het en ook hoe om vir homself te dink.

In 1889 het Theiler as veearts gekwalifiseer en het hy 'n praktyk in Beromunster begin. Hy kon egter geen uiting vir sy groot en ontembare gees in so 'n klein dorpie vind nie. Theiler se hele lewe en werk herinner my aan John Masefield se woorde: „Not for us are content and quiet and peace of mind, for we go seeking a city we shall

\*Arnold Theiler-gedenklesing gelewer tydens die viering van die 50-jarige bestaan van die Fakulteit Veeartsenykunde aan die Universiteit van Pretoria, 28 September 1970, in die Musalon, Universiteit van Pretoria.

\*\*Voorheen Professor in Farmakologie en Toksikologie, Fakulteit Veeartsenykunde, daarna in die Fakulteit Geneeskunde, Universiteit van Pretoria. Huidige adres: P/a Noristan Laboratoria, Privaatsak, Silverton.



never find." Theiler was lief vir lees en het veral belang gestel in reisbeskrywings deur wetenskaplikes; wat hom veral bekoor het, was Le Vaillant se beskrywing van sy reise in donker Afrika oor die Kaap. Dit het Theiler se avontuurlike gees nog meer ontevrede gemaak met sy praktyk in die klein dorpie.

Gelukkig vir hom en vir ons hier in Suid-Afrika, het die destydse Switserse Diplomatieke Verteenwoordiger in Transvaal hom in kennis gestel dat daar geen veeartse in Transvaal was nie en dat veeartsenykundige dienste dringend noodsaaklik was omdat veesiektes die Kaapkolonie en aangrensende gebied bedreig.

So het dit gebeur dat Theiler sy koffers, boeke, mikroskoop en chirurgiese instrumente ingepak en per boot na die Kaap vertrek het. Hy het op Nuwejaarsdag 1893, in Pretoria aangekom en praktyk as veearts begin. Veël werk was daar egter nie vir hom nie, want deur die jare heen het die boere noodgedwonge hul siek diere self behandel omdat geen gekwalifiseerde veeartsenykundige dienste beskikbaar was nie, ook was geld baie skaars. Verder was daar ook geen inligting aangaande heersende veesiektes beskikbaar nie, net hul name was bekend (hartwater, galsiekte, longsiekte, rooiwater en perdesiekte). Om 'n lewensbestaan te kon maak, het Theiler sy dienste as plaashulp aan die „Irene Estates" aangebied. Hier het hy goed gebruik gemaak van die geleentheid om veesiektes te bestudeer en soveel nadoodse ondersoeke moontlik in die omgewing te doen.

Daarna het hy 'n klein laboratorium in Johannesburg opgerig. Hier het omstandighede uitkoms vir Theiler gebring. In April 1893 het pokke in Johannesburg uitgebreek en op aanbeveling van die mediese gesondheidsbeampste was Theiler benoem tot „Consultant to the Rand Health Board" en is hy in bevel geplaas van entstofproduksie teen pokke. In 1895 het hy na Pretoria verhuis om in nouer kontak te wees met regeringskringe en behulpsaam te wees met die opstel van veesiekte-regulasies.

In 1896 stuur President Paul Kruger Theiler na Rhodesië om ondersoek in te stel na 'n geheimsinnige siekte wat onder beeste uitgebreek het; tesame met C. E. Gray van Rhodesië het hy runderpes gediagnoseer. Deur die groot runderpestragedie het daar gelukkig 'n ligstraal opgegaan vir die rege-

rings van verskillende gebiede in Suidelike Afrika, en het hulle besef dat georganiseerde veeartsenykundige navorsing en gekwalifiseerde veeartse onmisbaar is vir die welvaart van hul lande. 'n Direkte gevolg van die runderpesuitbreek was dat Theiler op 11 Mei 1896, amptelik ingesweer is as staatsveearts van die Z.A.R. en het hy in samewerking met Watkins-Pitchford en Verney pogings aangewend om 'n serumterapiemethode vir runderpes te ontwikkel. Intussen het Robert Koch op versoek van die Kaapse regering probeer om deur middel van inentings met gal van aangetaste beeste immuniteit teen runderpes teweeg te bring. Theiler se vervolmaakte serummetode het egter die omslagtige galmethode vervang. Van toe af was Theiler baie besig met die opstel en proklamasies van veesiektewette en met die toesighou oor die veldtog teen runderpes en ander veesiektes.

Die ingebore drang na navorsing het Theiler egter met geen rus gelaat nie, en in 1897 het hy voorgestel dat 'n navorsingsinstituut onder toesig van 'n staatsveearts opgerig word waar entstowwe teen runderpes, longpes, sponssiekte en pokke berei kon word. Hy het voorgestel dat die destydse ontsmettingstasie en stalle te Daspoort, net buite Pretoria, teen geringe koste in so 'n navorsingsinstituut omskep kon word. Theiler was egter nie 'n navorser met sy kop in die lug nie, maar het terdeë besef hoe belangrik dit is om die algemene publiek, en veral die boere, die vrugte van sy navorsing te laat pluk; daarom het hy voorgestel dat die staatsveearts die regering van raad moet bedien in alle sake rakende veesiektes en higiëne, en dat die laboratorium moet dien as bron van raadpleging en inligting vir die publiek. In Maart 1898 is Theiler deur die regering in kennis gestel dat sy voorstel aangeneem is en dat hy benoem is tot Direkteur van die Bakteriologiese Instituut; kort daarna is hy benoem tot staatsveearts verbonde aan die Staatsartillerie van die Z.A.R.

Omdat die jaar 1898 'n kwaai perdesiekte jaar was, het Theiler dadelik begin met sy navorsing op hierdie siekte. Terwyl die laboratoria te Daspoort opgerig is, het Theiler met die hulp van sy vrou, pokkiesentstof (kalflimf) by sy huis in Les Marais berei. Hier by Daspoort het Theiler se belang in veldgewasse ontstaan, kennis wat hom later met sy belang in gifplante goed te pas gekom het. As hoof van die Bakteriologiese Laboratorium was Theiler dikwels geraad-



pleeg om raad te gee aangaande mediese aangeleenthede.

As gevolg van die uitbreek van verdagte builepes in Middelburg, Transvaal, het Theiler aansoek gedoen om verskeie navorsingsinstitute in Europa te besoek, met die doel om homself op hoogte te stel met die nuutste laboratoriumapparaat en tegnieke. So het dit gebeur dat hy die Sewende Internasionale Veeartsenykundige Kongres te Baden-Baden, Duitsland, as afgevaardigde van die Z.A.R. bygewoon het. Met hierdie geleentheid het hy voorlopig verslag gedoen oor perdesiekte en sy pogings om perde teen die siekte te immuniseer. Met hierdie reis in Europa het hy verskeie institute besoek en op versoek van die Transvaalse Regering ook 'n studie gemaak van slagpale in groot stede.

Na 'n kort dienstydperk in die leer van die Z.A.R. gedurende die Tweede Vryheidsoorlog, het generaal Botha hom van militêre diens vrygestel om te Daspoort entstof teen longsiekte en pokkies te berei en sodoende ook met sy navorsing oor perdesiekte voort te gaan. Na die oorlog was die Instituut te Daspoort gekommandeer vir die leer se siek diere en was Theiler se dienste behou as Hoof van die Bakteriologiese Laboratorium. Ook het hy opgetree as konsultant vir dr. George Turner wat Mediese Gesondheidsbeampte vir Transvaal was.

Van nou af kon Theiler voortgaan met sy navorsingswerk oor veesiektes, veral die wat deur insekte oorgedra word. In 1903—1904 het Theiler bewys dat die onbekende siekte wat in die laeveldstreek by Komatipoort uitgebreek het, Ooskuskoors was en van toe af is baie navorsing oor dipstowwe gedoen, 'n gebied waarop Watkins-Pitchford in Natal baie geprester het. Aandag is ook geskenk aan die drie-dae stywesiekte en siektes deur *trypanosome* veroorsaak.

In 1905 het Theiler sy ses maande verlof in Europa deurgebring en Transvaal op die Agste Internasionale Veeartsenykundige Kongres te Boedapes, Hongarye, verteenwoordig. In 1907 het hy aandag aan bloutong onder skape geskenk en het hy vir meer navorsingsfasiliteite gepleit, d.w.s. groter en meer moderne geboue en beter ingerigte laboratoria. Generaal Botha het gelukkig die onskatbare waarde van Theiler as navorser besef en onmiddellik planne vir nuwe geboue goedgekeur. Onderstepoort is gekies vir die oprigting van die geboue omdat daar so baie muskiete en ander vlieënde

insekte was, maar veral omdat dit so goed geskik was vir navorsing oor perdesiekte. En so het dit gebeur dat Theiler en sy personeel in 1908 van Daspoort na Onderstepoort verhuis het. In die volgende jare sou hier, onder Theiler se bekwame leiding, wêreldberoemde navorsing gedoen word oor veesiektes wat deur bakterieë, protosoë, virusse en gifplante veroorsaak word en wat so n ryk oes van eer besorg het aan Theiler en die personeellede van sy instituut.

In 1909—1910 is Theiler weer met sy vyfjaarlikse verlof oorsee, met die doel om personeel, spesialiste op hul spesifieke gebiede, te werf. Hy het toe ook verskeie lande besoek.

Met Unie-wording was die veeartsenykundige organisasies van die vier provinsies onder een sentrale kontrole in Pretoria gehuisves. Van nou af was Theiler se weg gebaan om Onderstepoort te lei in die glorieryke periode wat dit so ryklik verdien het. Dit sou my te ver voer, en te veel ruimte en tyd opneem om maar net kortliks aan te stip wat Theiler en sy personeel alles te Onderstepoort nagevors en verrig het. Dit is alles noukeurig opgeteken in baie boekdele en tydskrifte.

Een baie belangrike punt wat Theiler goed besef het, was dat wat in die laboratorium ontdek is, nie sonder meer in die praktyk toegepas kon word nie en het hy laboratoria in verskillende dele van die Unie van Suid-Afrika ongerig en proefplase aangeskaf om proewe met middels op groot skaal uit te voer en om entstowwe te toets wat op Onderstepoort berei is. Ook het hy terdeë besef dat 'n biblioteek onontbeerlik is en het hy bepaal dat Woensdagaande deur hom en sy personeel benut sou word om literatuur-oorsigte te gee.

Die groot hoogtes waarna Theiler Onderstepoort gelei het, was hoofsaaklik te wyte aan sy visie, sy ongelooflike werkkrag en entoesiasme, sy organisasievermoë en die wyse waarop hy sy personeellede, en later sy studente, geïnspireer en gelei het.

Dit is goed en reg dat ek kortliks berig hoe Sir Arnold sy werksdae en week ingedeel het:

- (a) Vroeg in die oggend het hy die eksperimentele stalle en krale besoek, en ook die werf geïnspekteer.
- (b) Na ontbyt het hy al die temperatuurkaarte en vorderingsverslae van sy personeellede nagesien en ook aandag aan



sy korrespondensie en navorsingswerk geskenk.

- (c) Saans was gereserveer vir die lees van literatuur.
- (d) Drie aande in die week het hy, tesame met sy vrou, aan matesis gewy vir afleiding van al die biologiese werk.
- (e) Gedurende naweke het hy plaaslande en Bantoekwartiere besoek.
- (f) Proefdiere wat dringende en ononderbroke aandag nodig gehad het, het Theiler gedurende die nag met 'n stormlantern besoek en so kon hy uiters waardevolle en belangrike observasies doen wat anders verlore sou gaan.

Theiler het gereeld oorsese reise onderneem, navorsingsinstitute en kollegas besoek en lesings bygewoon, hoofsaaklik oor patologie, fisiologie, helmintologie en plantkunde. Theiler se belangstelling in wetenskap het gestrek vër buite die wat intiem betrokke was in navorsing op veeartsenykundige en mediese gebiede, m.a.w. hy was nie 'n geleerde barbaar nie.

Met die stigting van die Suid-Afrikaanse Mediese Navorsingsinstituut in 1912 het 'n sterk band tussen veeartsenykundige en mediese navorsing ontstaan, waarin Theiler 'n belangrike rol gespeel het. Hy het ook lid van die Suid-Afrikaanse Vereniging vir die Bevordering van Wetenskap geword, het 'n aandeel in die stigting van die Ornitologiese en Biologiese Verenigings gehad en het ook sy personeellede aangemoedig om lede van die verenigings te word.

Na die rampspoedige Spaanse griep-epidemie wat uitbreek het na die 1914—1918 wêreldoorlog, het die regering 'n kommissie onder leiding van dr. Paul Cluver, Direkteur van die Suid-Afrikaanse Mediese Navorsingsinstituut, aangestel en Theiler versoek om daarop te dien, want sy kennis en dienste was onmisbaar, ook vir mediese navorsing. Hy was ook lid van die „Government Research Grant Board for Scientific Research”. As lid van hierdie raad kon en het Theiler onskatbaar veel vir wetenskaplike navorsing gedoen, want daarvoor was sy hand, ook as Direkteur van Onderstepoort, nooit gesluit nie.

Intussen het die werk op Onderstepoort in so 'n mate toegeneem dat dit onder verskillende afdelings moes gegroepeer word, bv. patologie, protosoölogie, bakteriologie, biochemie, helmintologie, entomologie, virologie en toksikologie (gifplante). Spesialiste

in hierdie vakke moes in die begin vanuit ander lande ingevoer word.

In 1919 is Theiler aangestel as „Director of Lamsiekte Research” en 'n hele span navorsers was hom behulpsaam met die soek na die oorsaak van die siekte. In 1920 kon Theiler rapporteer dat die resultate van hul ondersoek bewys het dat 'n hele reeks gebeurtenisse, beginnende met 'n hunkering na fosfaat op fosfaatarm-weiding en die kou van bene, lamsiekte veroorsaak. In 1927 het dit dr. E. M. Robinson, bakterioloog te Onderstepoort, geluk om die skuldige kiem, *Clostridium botulinum*, te isoleer.

Met die vernaamste veesiektes of gekontroleer of aan die afneem, met die beskikbaarheid van bloutong- en perdesiekte-entstof en van haarwurm- en knoppieswurm-middels en met 'n welopgeleide en geïnspireerde personeel, het Theiler na 'n roemryke loopbaan in 1925 afgetree as Direkteur van Veeartsenykundige Navorsing.

Deur sy belang in lamsiektenavorsing was dit onvermydelik vir Theiler om ook 'n intensiewe studie te maak van osteodistrofiese siektes, soos stywesiekte, osteomalasie en osteofibrose. Toe hy in 1927 afgetree het, het hy met 'n groot versameling noukeurig uitgesoekte bene na Basel, Switserland, vertrek waar hy as gas van die patoloog, R. Rössle, sy navorsing verder voortgesit het.

Terwyl hy in Basel was, het die Australiese regering hom uitgenooi om hulle te gaan adviseer i.v.m. veteriniere- en voedingsprobleme, met die organisasie van veeartsenykundige dienste en met die oprigting van 'n veeartsenykundige opleidingsinrigting. Die pos van 'n „Director of Animal Health” om sy voorgestelde program in werking te stel is hom aangebied, maar weens gesondheidsredes kon hy dit nie aanvaar nie. In 1929 was hy terug in Switserland en het hy in sy woonstel voortgegaan met sy navorsing oor beensiektes.

Hy het sy besoeke aan ander lande voortgesit. In die vroeë dertiger jare het hy te Weybridge, London, saam met sy eertydse vriend en kollega, dr. H. H. Green, gewerk, om verdere navorsing oor gebreksiektes te doen. Hierin was daar ook noue samewerking tussen hom en dr. P. J. du Toit en dr. A. I. Malan van Onderstepoort. In Augustus 1934 het hy die twaalfde Internasionale Veteriniere Kongres in New York as Switserse verteenwoordiger bygewoon. Op sy weg terug het hy Durban aangedoen en



op sy reis deur die land ou vriende in Suid-Afrika besoek. Dr. P. J. du Toit het Theiler sy ou laboratorium op Onderstepoort aangebied en so het dit gebeur dat hy begin 1935 weer terug was in sy laboratorium waar hy vir soveel jare met soveel vreugde en vrug gearbei het. In 1936 het Theiler na Europa vertrek waar hy weer baie vriende en kollegas besoek het.

Op 24 Julie 1936 is hy aan 'n hartaanval oorlede.

In the short time at my disposal, I have done my best to do justice to the life and work of one of the most honourable and most famous adopted sons of South Africa. Theiler would have been the first to acknowledge that he would never have achieved such success and fame had it not been for the eminent and faithful services rendered by his well-selected professional and technical staff. It is self-evident that the Onderstepoort Veterinary Research Institute and the Division of Veterinary Services, which controls field veterinary services, took the highest lead and accomplished the most in the fields of research and control of stock diseases, but I dare not omit to pay tribute to the valuable contribution made by members of the farming community, technical assistants, stock-inspectors and Bantu assistants, in our efforts to detect and combat the causes of the many stock diseases in our country.

In the investigation made into the cause of lamsiekte, it was the farmers who supplied the information that they had noticed that in many cases a beast was paralysed the day after it had chewed bones. The animal and the farmer gave us the clue to the cause of lamsiekte. Plant names like "gifblaar, gousiektebossie, stywesiektebossie, vuursiektebossie, vermeerbossie and krimpsiektebossie" tell us that the farmer had already identified the poisonous plants concerned and it remained only for the veterinarian to prove that the farmers' observations were correct. In particular I would like to express my personal appreciation to farmers for all the assistance and advice I received from them in the course of my investigations into plant poisoning in stock.

I first met Sir Arnold Theiler in October 1925, when I visited Onderstepoort. He offered me a post as pharmacologist and I assumed duty on November 9, 1925. I had already become acquainted with Sir Arnold's research at Onderstepoort during my student

years in Vienna, Austria, from 1922—'25. I realized that South African stock diseases differed greatly from those in Europe and that I had to pay particular attention to them. Consequently, I obtained as many publications on Onderstepoort work as was possible.

Biochemistry had always fascinated me and this was the reason that my attention was drawn particularly to Theiler's work on mineral metabolism. My interest in mineral metabolism was further stimulated by lectures on this aspect of metabolism by my professor of cattle diseases, Professor Reisinger, at the Veterinary College in Vienna. Reisinger naturally referred to Theiler's research and publications on mineral metabolism and criticized some of his views, especially on rickets, osteomalacia and osteofibrosis. I also closely followed the polemic on mineral metabolism which was carried out in the "Tierärztliche Rundschau".

Obviously, much attention was paid to Theiler's work and views. It did not take me long to realize that Theiler's experiments were very carefully and scientifically planned and executed and that his findings and conclusions were based on sound scientific grounds. It was with this knowledge and appreciation of Theiler's work that I commenced duties at Onderstepoort.

As stated previously, Theiler's day started very early in the morning and I often made use of the opportunity to accompany him on his early morning inspections and to discuss his research with him. It was on these inspections that I learned to appreciate the man and the scientist, Arnold Theiler. I was deeply impressed by his personality, sincerity, friendliness, wide knowledge, enthusiasm and scientific approach to all problems. It was impossible not to be inspired by him.

#### THE DEVELOPMENT OF THE FACULTY OF VETERINARY SCIENCE AT THE UNIVERSITY OF PRETORIA

From what has been said before, it is clear that, not only overseas but also in South Africa, the extreme value of well-trained veterinarians and well-organized veterinary services was greatly appreciated. Onderstepoort had delivered eminent services, but locally trained veterinarians were indispensable to effective research and



the running of an efficient veterinary service. To the farming community goes the credit of first providing the impetus in this direction. In 1914 the question of training veterinarians locally in order to supply the special needs of research into South African stock diseases was discussed for the first time in the Senate of the Transvaal University College. On that occasion the advisability of the institution of "Veterinary and Agricultural Courses" was discussed; in 1915 representations were made to the Government. The outcome was that, in 1916, it was decided to establish an agricultural faculty; attempts were made to teach veterinary science in that faculty. In 1917 the University College of Stellenbosch offered Theiler a chair in veterinary science, but Professor A. M. Bosman immediately took steps to retain him in Pretoria by offering him a similar post at the local University College in the Faculty of Agriculture. In 1918 a chair in veterinary science was instituted at the Transvaal University College but, as Theiler did not accept it, P. R. Viljoen was appointed.

The Faculty of Agriculture soon realized that an independent Faculty of Veterinary Science was urgently needed in order to train fully qualified veterinarians. Professor P. R. Viljoen was the driving force in having such a faculty established at the Transvaal University College. Following on representations made by the Council of the University College of Transvaal, the Government decided to establish a veterinary faculty as an integral part of the University College, the first two years to be taught at any university institution in the Union of South Africa and the last three years at Onderstepoort. Theiler was appointed Director of Veterinary Education and Research and was requested by the Prime Minister to draw up a syllabus. Theiler's recommendations for new buildings at Onderstepoort were duly accepted. These included extensions to the existing pathology building, anatomy, a second post-mortem building, new staff houses and a student's hostel.

The first appointment to the newly established faculty was made on June 24, 1920, in the person of P. R. Viljoen, provisionally as Professor of Anatomy. On July 20, 1920, the appointment of six professors and two lecturers was announced. The teaching staff then consisted of the following persons:

#### *Professors:*

Sir Arnold Theiler (Pathology)

P. J. du Toit (Hygiene and Infectious Diseases)

H. H. Green (Biochemistry)

W. H. Andrews (Physiology)

P. R. Viljoen (Medicine)

G. v. d. W. de Kock (Anatomy)

#### *Lecturers:*

E. M. Robinson (Hygiene)

C. P. Neser (Medicine)

Sir Arnold was elected the first dean and remained in that post until his retirement at the end of 1927. He also distinguished himself as a past master and leader in teaching. None of his students left Onderstepoort without being highly inspired by him and by the knowledge he had imparted to them. The first group of eight students qualified at the end of 1924, obtaining the B.V.Sc. degree. In the course of time more courses and more degrees were instituted. At present 45 students are being trained annually at Onderstepoort with the intention of raising the number to 90 in 1973.

At present the following post-graduate degrees are offered:

(a) M. Med. Vet. (12 different specialities)

(b) D.V.Sc.

(c) Diploma in Veterinary Hygiene

From the beginning of 1971 two more M.Med.Vet. degrees will be awarded.

Four medals, eight prizes, one donation and two bursaries are awarded annually to veterinary students specifically by different organisations.

### THE FUTURE ROLE OF VETERINARY SCIENCE IN THE REPUBLIC OF SOUTH AFRICA

From what I have said before, it is clear that Theiler was a pioneer not only in all fields of veterinary science but also in some fields of medical science and that he forged



a link between veterinary and medical science. This link has stood both sciences in good stead in the past, with the promise of even greater benefits in future. Theiler's research and activities have brought ample proof of the great benefits to be derived from close co-operation between the two sciences concerned. More examples of achievements in the field of veterinary science and their contribution to medical science, more pertinently as far as the zoonoses are concerned could be quoted; the following examples will suffice to illustrate my point:

1. Rabies was first diagnosed in South Africa by Dr. P. J. du Toit in 1929.
2. The life-cycle of *Schistosoma matthei* was worked out at Onderstepoort.
3. Rift Valley fever was diagnosed for the first time in South Africa in 1951.
4. Wesselsbron virus was identified by Onderstepoort for the first time in 1955.
5. In the fields of hygiene (abattoirs), nutrition, toxicology and pharmacology as well as in the study of cancer, infectious diseases and zoonoses, veterinary science is indispensable to medical science.

In addition the services of veterinarians are being increasingly sought by medical research institutions.

### AN APPEAL

In conclusion I would like to appeal urgently to the South African Veterinary Medical Association to place on full record the life and work of Arnold Theiler, truly one of the most remarkable and most famous men and most famous scientists our country has thus far produced. His achievements are an everlasting monument to the greatest veterinarian that has ever lived. The fruit of his labours have greatly benefited not only our country but the whole world. The following list of the many honours bestowed on Theiler by many countries is proof of my contention:

1. Dr.h.c.: University of the Cape of Good Hope, 1911.
2. Dr.h.c.: University of Syracuse, U.S.A. 1923.
3. Dr.h.c.: University of Berne, Switzerland, 1923.
4. Dr.h.c.: University of Cape Town, 1935.

5. Dr.h.c.: University of Utrecht, Holland, 1936. (Simultaneously with General Jan C. Smuts and General J. B. M. Hertzog).
6. Honorary D.V.Sc. degree: University of South Africa, 1925.
7. Honorary D.Sc. degree: University of the Witwatersrand, 1935.
8. Honorary Professor of Tropical Medicine, University of Pretoria, 1936.

### Medals:

1. Bronze medal and Grant: South African Association for the Advancement of Science, 1908. (First recipient).
2. Senior Captain Scott Medal, South African Biological Society, 1918.
3. Gold Medal, Society for Exotic Pathology, Paris, 1927. (First Award).
4. Budapest Gold Medal for Research in Veterinary Science, 1934. (Second recipient).
5. Gold Medal of the Royal Agricultural Society, England, 1934.
6. Private Gift and Personal Souvenir from King George V and Queen Mary.

### Other honours

1. C.M.G.—1907.
2. K.C.M.G. — 1914, for distinguished services to Agriculture and Veterinary Science. First colonial veterinarian to be knighted.
3. Chevalier de l'Ordre de la Couronne, Belgique, 1912.

Theiler was Honorary Member of, and correspondent to, numerous learned societies in South Africa and other countries (some 23 in number). He was Honorary Member of the Afrikanerkring, Honorary Fellow of the Royal Society of South Africa, Honorary President of the South African Biological Society in 1929, Life President of the South African Association for the Advancement of Science, and Honorary Life President of the South African Veterinary Medical Association.

Clearly, the South African Veterinary Medical Association would be failing in its duty if it were not to record in full the life and work of Sir Arnold Theiler. It would constitute a most interesting record and history of the development of veterinary services and veterinary education and of certain aspects of medical science in South Africa. It would be a most valuable piece of Africana.



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## REVIEW

## OORSIG

## REFRESHER COURSES IN PHARMACOLOGY

## 3 THE INTRACORPOREAL DISTRIBUTION OF DRUGS

W. L. JENKINS\*

## INTRODUCTION

In the previous articles in this series it was noted that, whatever the route of drug administration, the molecules will ultimately diffuse through the capillary or lymphatic endothelium and will thus enter the blood stream.

There are a number of possible fates which circulating drug molecules may subsequently suffer. Firstly, the unbound drug may become bound to a blood constituent or it may freely diffuse out of the blood and become distributed to a greater or lesser extent in various organs or tissues. During this process, and throughout the period of sufficient concentration of drug molecules in the biophase, they will react with their specific receptors to bring about a characteristic effect. Biotransformation of the drug by appropriate enzyme systems located in a variety of tissues usually prepare the foreign compound for excretion but the time required for this process may vary considerably for different substances. Finally, the unchanged drug or its metabolites are excreted from the body via a number of possible routes. A schematic representation of these considerations is presented in Fig. 1.

The intracorporeal distribution of drugs constitutes an important facet of pharmacotherapeutics and the more important aspects will be reviewed here.

## GENERAL CONSIDERATIONS

After absorption, drugs are disseminated throughout the body in the blood stream. Generally speaking, a dynamic equilibrium

becomes established very rapidly between the blood and lymph and the tissues or other body fluids. This is not always the case, however, and the important exceptions include adipose tissue with its poor blood supply, bone, keratinous tissues and those organs protected by the so-called blood-tissue "barriers", e.g. the blood-brain barrier, the placental barrier, the mammary barrier, the joint capsule barrier, etc.

Most drugs are not distributed equally throughout the body but tend to be sequestered at particular sites. Compounds that permeate freely through cell membranes become distributed throughout the body water, whereas drugs which pass through capillary endothelium but are not capable of traversing cell membranes become distributed throughout the extracellular fluid. Furthermore, some high molecular weight substances, e.g. Evans blue dye, when injected intravenously, are almost wholly confined to the circulating plasma. The *apparent volume of distribution* of a drug is the fluid volume in which it seems to be dissolved and this value may be determined experimentally.

In addition to the equilibrium of free drug which may become established between the various body fluid compartments, drugs may also become bound to specific components of various cells, tissues or fluids. These loci are known as "storage depots" or "sites of loss"; a compound bound in this manner is pharmacologically inert but remains in equilibrium with unbound drug and thereby maintains an effective concentration at its site of action.

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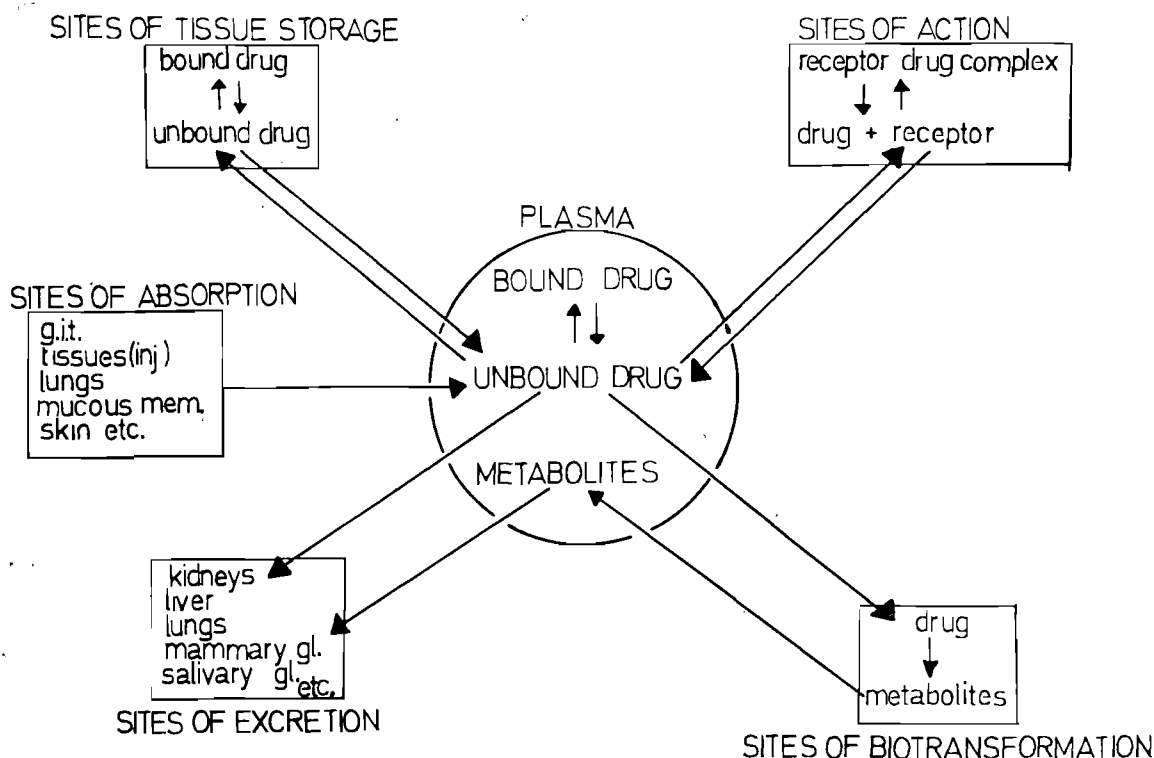


FIG. 1. The possible fates of a drug in an animal's body.

#### FACTORS DETERMINING THE SEQUESTRATION OF DRUGS

##### *Differences in pH on either side of cellular membranes*

The principles discussed in the first article of this series on the movement of drugs across body membranes play a major role in determining the intracorporeal distribution of drugs. Most drugs, being weak electrolytes, penetrate cells by simple diffusion in the nonionized form in proportion to their lipid water partition coefficients and become distributed between the body compartments in proportion to the pH gradient of the fluids.

In general, basic drugs tend to accumulate in regions of low pH and, conversely, acidic drugs tend to accumulate in regions of high pH. This distribution, however, is always dependent on the ability of a drug to penetrate the barrier between the regions and this in turn is especially dependent on the lipid solubility of the compound.

Although large pH differences between body compartments do exist, e.g. plasma

(7.4) and gastric juice (1.4), it is more common to contend with rather small pH gradients, e.g. the intracellular fluid (7.0) and extracellular fluid (7.4), or cerebrospinal fluid (7.3) and plasma (7.4). Nevertheless, even such small differences in pH across a boundary membrane may lead to an unequal drug distribution. It is noteworthy in this respect that changes in the acid-base balance within the body may lead to significant alterations of drug concentration in certain tissues. This important concept is schematically illustrated in Fig. 2.

##### *Differences in binding on either side of cellular membranes*

During the dispersion of a drug throughout the body fluid compartments and tissues it often becomes bound to various macromolecular components. While in this bound state, a drug cannot freely cross a body barrier and is thus usually pharmacologically inactive, although it does remain in dynamic equilibrium with unbound drug.

The drug molecules may become attached to protein or they may form non-diffusible



# LIPID MEMBRANE

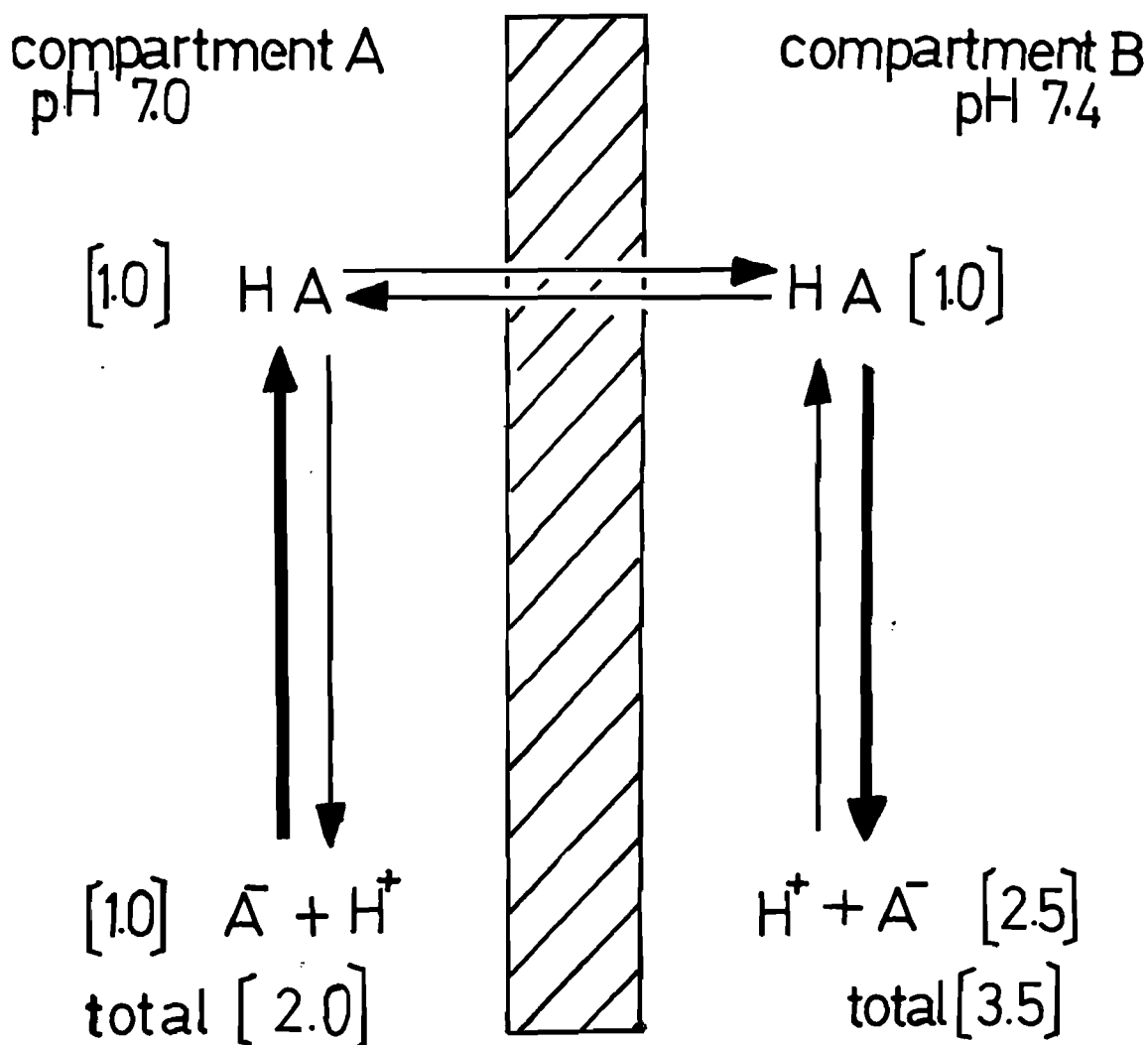


FIG. 2. Distribution of an acidic drug (pKa 7.0) between two body compartments.

compounds with other substances. They may also become attached to specific binding sites in particular tissues. The nature of this binding may vary from powerful covalent bonds, dipole-dipole bonds, hydrogen bonds or van der Waal's bonds.

The degree of binding may vary considerably between different drugs and between different tissues respectively. This situation often leads to an unequal drug distribution between the tissues and fluid

compartments. This fundamental concept is schematically illustrated in Fig. 3.

## *Lipid solubility*

The lipid solubility of a drug plays a major role in determining its distribution in the body. Besides the ability of compounds with high lipid water partition coefficients to diffuse readily across cellular membranes as noted earlier, they may also undergo solution in the lipid stores of the body.



### Active transport

A drug which is transported across a membrane by a specialized transport process will become unequally distributed across that membrane. Cells capable of this function include renal tubular epithelium, hepatocytes and gastro-intestinal epithelium.

### SEQUESTRATION OF DRUGS IN VARIOUS BODY FLUIDS AND TISSUES

#### *The binding of drugs to plasma proteins*

As a compound is absorbed into the blood stream, it may become reversibly bound to a greater or lesser extent to the plasma proteins or erythrocytes. This binding then facilitates further absorption by

lowering the concentration of free drug in the aqueous phase of the plasma.

Although several fractions of the plasma proteins may interact with drug molecules, by far the most important contribution is made by plasma albumin. Furthermore, it does generally appear that acidic drugs seem to be more highly bound than basic drugs. There are a number of additional factors, however, which determine the extent of plasma protein binding by a particular compound. Amongst the more important considerations are plasma pH, the concentration of the drug, a limitation of the number of binding sites available, the possibility of

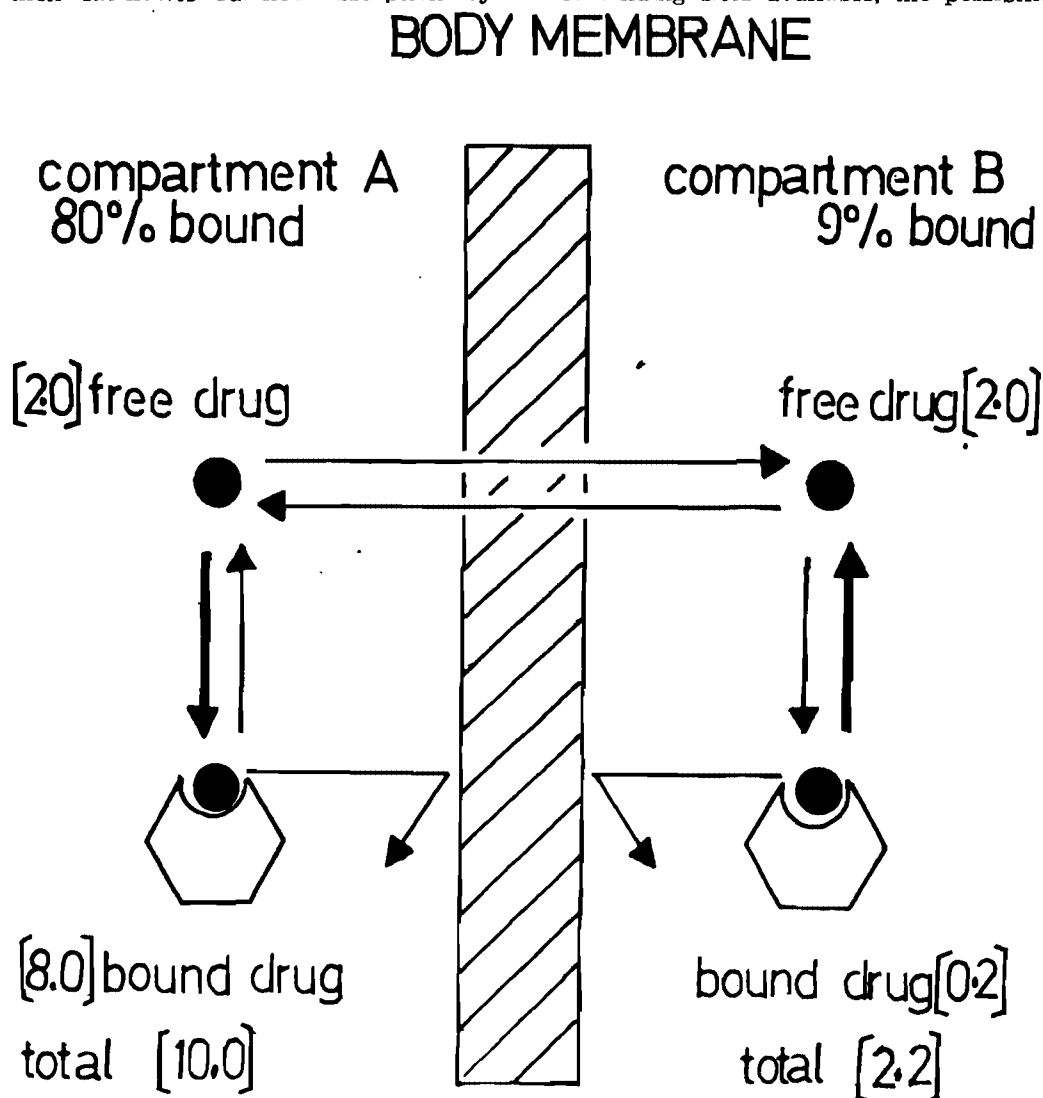


FIG. 3. Distribution of a drug which becomes bound within two adjacent body compartments.



displacement from these binding sites by drugs with a greater affinity and finally species differences.

Examples of compounds which may become highly bound to plasma albumin include salicylates, sulphonamides, phenylbutazone, barbiturates, coumarins, indanediones, cardiac glycosides, anionic dyes, iodinated anions, penicillins, streptomycin, tetracyclines, chloramphenicol, etc. In addition many natural substances may also be bound to albumin, e.g. bilirubin, uric acid, ascorbic acid, thyroxine, fatty acids, calcium, zinc, etc. The other plasma protein fractions which may play a role in binding various compounds are the alpha- and beta-lipoproteins and the metal-binding globulins.

There are some significant therapeutic consequences of this binding of drugs to plasma proteins. Some drugs could not be administered unless plasma protein bound, e.g. dicoumarol is so poorly soluble at a pH of 7.4 that it would crystallize out unless it was 98 per cent bound. Drugs with a high affinity for plasma proteins usually have lengthy plasma half lives and thus a prolonged therapeutic effect. An extreme example of this is suramin, which confers protection against trypanosomiasis for three months or more. Finally, the clinical effect of a drug may be radically increased, sometimes to a dangerous or even lethal level, if a second drug with a higher affinity displaces the first from plasma albumin. An example of this important type of interaction is the displacement of penicillin by phenylbutazone, salicylates and sulphonamides. A more dangerous interaction encountered in man is the displacement of coumarins and indanediones by the same group of drugs noted above. Methotrexate can also be displaced by acidic drugs and this represents a potentially lethal interaction. Another example of this problem is seen in the displacement of bilirubin by sulphonamides from plasma albumin in the neonate leading to the kernicterus syndrome.

#### *The binding of drugs to blood cells*

A variety of drugs have been shown to penetrate erythrocytes at rates roughly related to their lipid water partition coefficients. Once again the determinant factors are drug concentration, molecular weight and lipid solubility. Nevertheless, the rate of entry of organic and inorganic anions greatly exceeds that of equivalent cations.

There is as yet no entirely satisfactory explanation for this unusual permeability of the erythrocyte to anions.

Some compounds become localized within red cells as a result of their binding to haemoglobin, which is known to carry an excess cationic charge. Examples of such drugs include sulphaguanidine and phenol red.

Phagocytic leukocytes may also play a role in the distribution of drugs, especially those injected in the form of a suspension or as an emulsion in oil.

#### *The binding of drugs to tissue components*

There are numerous known examples of specific tissue components which bind certain drugs. A few of these examples will serve to illustrate the concept.

A site of loss of highly basic substances may be the nucleoproteins or other basophilic components of tissues, whereas oxytetracycline and chlortetracycline become localized in the liver and kidney mitochondria. Some drugs become bound to the highly ionic groups of the mucopolysaccharides and are thus often stored in connective tissue.

There are a few compounds which are incorporated into keratinous tissues, e.g. arsenic and griseofulvin, but in this case the drugs are not freely exchangeable and remain in the bound state.

Bone may become a storage site for certain heavy metals, e.g. lead and radioactive strontium, and the tetracyclines also become deposited in bone in association with calcium.

The biogenic amines are usually stored in the sympathetic neurones, chromaffin cells and mast cells. Exogenous amines and related substances, however, may also be taken up into these cells.

Many drugs accumulate in the liver and kidney in the course of biotransformation and excretion.

Body fat, which may represent as much as 20 per cent of the body weight, is a very important storage site for drugs of high lipid solubility, e.g. the anaesthetic gases, thiobarbiturates, dibenamine, phenoxybenzamine, etc. A practical application of this feature of drug distribution is encountered in inducing and maintaining anaesthesia with thiopentone. Single administration of this compound produces brief anaesthesia as it rapidly becomes sequestered



ed into adipose tissue. If the anaesthetic is administered repeatedly, however, the capacity of the fat to dissolve the drug becomes saturated and dangerous blood levels may result. This principle also applies to anaesthetic gases.

The chlorinated hydrocarbon insecticides (DDT, BHC, aldrin, dieldrin etc.) are also stored in fat, often for considerable periods of time. If the fat is utilized, these compounds may be released into the body.

#### *The passage of drugs into the central nervous system*

In the domesticated animal species, the brain usually constitutes a small proportion of the total body weight but receives a very large blood supply. Notwithstanding this rich circulation, drugs do not always readily penetrate the CNS. In fact, a complete spectrum of diffusibility exists, i.e. from drugs which enter very rapidly to compounds which equilibrate rather slowly, and finally to the many substances which do not penetrate brain tissue at all.

The unusual features regarding drug passage into the CNS were recognized over sixty years ago and the concept of a barrier became established. A drug may enter the central nervous tissue by two distinct routes. Firstly, by diffusion through the capillary endothelium and surrounding astrocyte sheath into the extracellular fluid of the brain. This represents the so-called "blood-brain barrier". Secondly, a drug may be secreted with cerebrospinal fluid by the choroid plexus and may then penetrate the ependymal layer of the ventricles into the brain substance. This latter route is known as the "blood-cerebrospinal fluid barrier".

The blood-brain barrier does not represent an absolute blockade but depends on a quantitative rather than a qualitative difference in capillary permeability as compared with that in other tissues. This difference in permeability is based on a morphological feature peculiar to the brain, namely, the close application of the astrocyte processes or "feet" to the basement membrane of the capillary endothelium. This glial sheath appears to be about 85 per cent complete. Thus a drug which enters the tissues of the CNS must first traverse the rather porous capillary endothelium and then the lipoproteinaceous glial membranes prior to its reaching responsive neurones.

The blood-CSF barrier is located at the

epithelial lining of the choroid plexus. Cerebrospinal fluid is similar to a plasma ultrafiltrate in many respects but the changes in ionic concentration and the secretion of CSF against an experimentally imposed pressure gradient suggest an active transport mechanism. The fluid flows through the ventriculocisternal system and ultimately bathes the surfaces of the brain and spinal cord. This arrangement really represents a third barrier, namely, a "cerebrospinal fluid-brain barrier". The CSF leaves the brain via the venous blood sinuses in the arachnoid villi. It is noteworthy that drugs may enter the CSF either by way of the choroid plexus or by diffusion directly across capillaries into the interstitial fluid.

The factors which are primarily responsible for determining the rates of drug diffusion into the brain are those which were discussed at length in the review of the principles governing the passage of drugs across body membranes. The three major considerations are once again the degree of ionization at the particular pH on either side of the barrier, the lipid solubility of the drug and the concentration of unbound drug present in the plasma.

There are a number of general observations which must be mentioned at this point. Different drugs penetrate various parts of the brain more easily than others. Drugs have been shown to enter the grey matter of the cortex far more readily than the white matter; this appears to be related to a greater blood supply and thus a higher delivery rate of drug to the tissues. The heavy myelination of white matter in general also impedes the free diffusion of drugs. By contrast, there are certain areas of the brain which are particularly permeable and these structures may actually become stained by some injected dyes. The more noteworthy areas involved are the area postrema, neurohypophysis, pineal body and certain parts of the hypothalamus. The basic reason for this increased permeability has not been finally established but it does seem as if the glial sheath surrounding the blood vessels is less evident in these particular areas.

It is important to note that within the CNS, the neurones, interstitial fluid and CSF may come to equilibrium with plasma at quite different rates. In addition, some drugs are selectively taken up in the CNS,



which is the reason for their predominant central action. Examples of such compounds include chlorpromazine and imipramine.

Drugs may leave the CNS by a number of possible routes. Firstly, they may diffuse back across the blood-brain barrier into the capillaries. Nevertheless, compounds which become distributed into the CSF may leave the brain by bulk fluid flow through the arachnoid villi or they may diffuse across the lipid boundaries of the blood-cerebrospinal fluid barrier. Furthermore, certain compounds, both anions and cations, may leave the CSF by specialized active transport processes.

There are a number of significant pharmacotherapeutic consequences related to the passage of drugs into and out of the CNS; some of these will be briefly enumerated:

- (i) In the neonate, myelination is not complete and therefore drugs may enter the brain in abnormally large quantities.
- (ii) Highly ionized or polar water-soluble compounds will not penetrate the CNS, whereas low ionization, low plasma protein binding and fairly high lipid water partition coefficients are properties that confer ready penetration. Thus, of the antibiotics, chloramphenicol attains a concentration in the CSF of about 30 per cent that in plasma, whereas penicillin, streptomycin and the tetracyclines do not cross the normal brain barriers to any appreciable extent. Similarly, amongst the sulphonamides, sulphadiazine reaches a concentration in the CSF about 75 per cent that of the plasma level, whereas other sulphonamides penetrate to a lesser degree due to plasma protein binding and unfavourable pKa values. This concept can be further illustrated by the following comparative examples. Atropine penetrates the CNS but methylatropine does not and while neostigmine also does not enter the CNS, the organic phosphate insecticides do penetrate the brain. Finally, injected noradrenaline is excluded from the CNS, but its precursors dopa and dopamine do cross the blood-brain barrier.
- (iii) Direct injections into the CSF of compounds which do not normally enter the brain substance often produce unusual reactions. Examples of these effects in-

clude the convulsions produced by penicillin and tubocurarine, and the soporific state which results when adrenaline is injected directly into a lateral ventricle.

- (iv) Pathological conditions may radically alter the permeability of the brain barriers. Increased permeability may result from traumatic injuries, meningitis, encephalitis, occlusive vascular lesions and neoplastic processes. Furthermore, conditions such as uraemia, hepatic coma, intoxications, severe allergic reactions and cranial irradiation may also lead to higher drug concentrations in the CNS.
- (v) There are a number of miscellaneous substances which may increase the permeability of the blood-brain barrier. These include bee venom, cobra venom, diodrast, saponins, bile salts, etc. By contrast, certain dyes appear to have the opposite effect, namely, to decrease permeability.

#### *The passage of drugs across the placenta*

The passage of drugs across the placental barrier into the foetal tissues has two very significant aspects. Firstly, the harmful actions of drugs on the embryo, including teratogenic effects, must always be considered; secondly, a knowledge of the effects on the foetus of drugs used during parturition is of vital concern in obstetrics.

Unfortunately, the knowledge regarding the placental barrier is still very incomplete. There are a number of reasons for this but the greatest problems associated with this type of research are the species differences in placentation and the extreme difficulty in performing meaningful experiments. Nevertheless, a few generally accepted facts may be noted.

There seems to be little doubt that the factors responsible for the diffusion of drugs across body membranes, namely, degree of ionization, lipid solubility, molecular size, plasma protein binding, etc. are once again the major determinant considerations in placental transfer. On the other hand, endogenous substrates are transferred by carrier-mediated transport systems across the placenta. The placental blood flow may also be a limiting factor and it appears that the fastest equilibrium possible between foetal tissues and a constant maternal blood level of a drug requires at least 40 minutes in women.



Examples of compounds which readily penetrate the placenta and enter the foetal blood stream include the anaesthetic gases and vapours, barbiturates, salicylates, ethanol, some sulphonamides, quinine, pethidine and other drugs of high lipid solubility. Those substances which are excluded from the foetus include inulin, dextran, quaternary ammonium compounds, glucuronides, etc. There are a great number of drugs which equilibrate between these extremes and at many different rates. In all these cases the possibility of species differences exists.

In respect of the placental barrier the conclusion at present would seem to be that, unless proved otherwise, it is always safest to assume that a degree of drug transfer may occur in all domesticated species.

#### *Drug penetration into the eye*

Drugs may enter the ocular fluid by two routes, namely, by diffusion and secretion through the epithelium of the ciliary body and by diffusion through the capillary endothelium of the iris into the aqueous humour of the anterior chamber. As the

compounds cross membranes during both these processes, the principles discussed previously apply once again and drugs enter ocular fluid at rates closely related to their lipid water partition coefficients.

As in cerebrospinal fluid, drugs may leave the eye by three possible routes, namely, via the normal drainage of ocular fluid, diffusion back into capillaries and by active transport through the ciliary body.

#### *Other transcellular drug depots*

Drugs may become distributed into the fluids of the gastro-intestinal tract and this often constitutes a significant drug reservoir within the body. The mechanisms involved in this case were reviewed in the previous article of this series.

Other transcellular fluids such as lymph and joint fluid represent minor but important storage depots. Unfortunately very little research appears to have been carried out on this aspect of intracorporeal drug distribution.

The features of the mammary barrier will be discussed in the review of drug excretion routes.

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## BOOK NEWS

## NUUS OOR BOEKE

### THE SUCCESSFUL PROFESSIONAL PRACTICE

ROBERT P. LEVOY

Prentice-Hall, Inc. Englewood Cliffs, New Jersey. June 9, 1970. 192 pages, \$19.95.

In his new book, *THE SUCCESSFUL PROFESSIONAL PRACTICE*, published by Prentice-Hall on June 9, 1970, Robert Levoy, Director of Professional Practice Consultants, reveals his "Six-Day Program" of tested, practice-building techniques which any professional may put to use in his own practice: how to trim an over-crowded schedule, how to free more time for each patient or client and make better case pre-

sentations, how to develop more flexibility in making appointments and in setting fees, proven methods of raising fees to increase income and ethical methods to weed out undesirable patients or clients. He gives specific methods for building the clients' and patients' confidence and shows how to avoid costly misunderstandings and disagreements.

(To be reviewed in this Journal).



# THE POTENTIAL OF GAME DOMESTICATION IN AFRICA, WITH SPECIAL REFERENCE TO BOTSWANA\*

G. P. RETIEF\*\*

## SUMMARY

Very little is done about the utilization of game animals in Botswana, except with respect to hunting and tourism. The author presents the case for the domestication of some game species, especially eland (*Taurotragus oryx*), as opposed to game cropping. The problems connected with such a scheme, the marketing of the end product, and the dangers to domestic stock are discussed.

## INTRODUCTION

In recent years, developing countries in Africa have become more and more interested in the utilization of game meat as a means of justifying conservation and game cropping<sup>1</sup>. Unfortunately this has become a controversial matter, because of possible over-enthusiasm on the part of conservationists on the one hand, and apathy and conservatism on the part of the authorities concerned on the other. In many instances the dangers to domestic livestock and man are glossed over by the protagonists of game cropping and domestication, while the same dangers are perhaps over-emphasized by the opponents to these schemes.

Cattle, sheep, goats and swine are animals exotic to Africa. They were brought into the country by the migrant populations when they first arrived here<sup>2</sup>. It must have been obvious to the new settlers in many areas in Africa, that their domestic stock were inferior in adaptability, resistance to drought and disease, and in productivity to the game animals they encountered<sup>3</sup>. Yet, man has preferred to take his own domestic animals with him, rather than attempt the domestication of new species.

Before discussing the possibilities in Botswana, it is necessary to consider whether there is in fact any need or justification

for a scheme to domesticate game animals in Africa.

The FAO report of 1966, as summarized by Crawford<sup>2</sup>, shows clearly that there is a need for new methods of food production. The lack of quality food in world supplies is of major importance. It is estimated that approximately one third of the world's population is poorly nourished, protein deficiency being the main cause of malnutrition. To correct this protein lack, a six-fold increase in animal and fish products will be required by A.D. 2000<sup>2</sup>.

One third of the world's cattle population is distributed between Africa and Asia, and yet the productivity of these regions is only about 10% of the total world output<sup>2</sup>. There is a need in Africa for a new approach to farming. Instead of trying to render the environment of domestic stock as similar as possible to the one from which they were introduced, a more logical approach would be to make use of the indigenous animals already adapted to the African environment.

About 75% of the land mass of Botswana, and large areas elsewhere in Africa, are unsuitable for cattle ranching, or at best marginal for beef production. This is because of climatic conditions, lack of surface water, and the presence of enzootic diseases. The average rainfall in Botswana is 459 mm p.a.: large areas, like the Kgalagadi, receive less than 230 mm. During the periodic drought years, thousands of domestic stock die of starvation or thirst. Certain species of game also die off in large numbers, like wildebeest (*Connochaetes taurinus*) and hartebeest (*Alcelaphus buselaphus*); these animals, like domestic stock, are dependant upon surface water for survival. They are,

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of course, not watered like domestic stock, but have to find water for themselves without getting shot in the process. On the other hand, certain plains game can survive without any drinking water, like eland (*Taurotragus oryx*) and gemsbok (*Oryx gazella*). It is among the latter species that a new domestic animal could be found.

The available natural fodder has been maintained in Africa for millions of years. This habitat is slowly being destroyed by man's interference<sup>2,4,5</sup>. Making use of animals which are primarily browsers (like eland) to complement cattle, which are primarily grazers, will result in a more balanced form of land utilization.

When export of live game or meat becomes possible through proper veterinary control of domesticated game, an additional source of foreign exchange will become available to countries like Botswana, where this commodity is at a premium.

From a sociological point of view, such a scheme could also be of value. At the moment, the Bushmen in Botswana are largely an unproductive group of the population. For centuries they have been dependant upon game for survival<sup>6</sup>. With the spread of civilization to the remoter interior areas of Botswana, they are coming into contact with other races and into conflict with game conservationists. Their special skills connected with game, the fact that they can survive under extreme drought conditions, and their knowledge of the habits of plains game in Botswana, could make them useful as herdsmen for domesticated game<sup>6</sup>.

A further inducement to start some form of game utilization must surely be the fact that the wildlife of Africa is threatened with extinction. In Botswana this threat is only confined to a few species at this stage, but if present policies continue, some species will disappear from the scene entirely, while others will only be found in isolated pockets in game reserves. Once these species are lost, they can never be reclaimed, but at the moment they may represent a tool which could be used to manage the semi-arid environments which presently are not suitable for conventional agriculture.

#### PRESENT UTILIZATION SCHEMES

At present, game cropping is practised on a fairly large scale on farms and in game reserves throughout Africa. Over 3 000

farms are already marketing game commercially<sup>2,7,8,9</sup>. Of these, the Henderson ranch in Rhodesia is perhaps the most famous. At the Queen Elizabeth Park, Uganda, hippo are cropped continuously<sup>10</sup>. Parker, of the Wildlife Services, Kenya cropped elephants in Murchison Falls National Park. Because of the limitation imposed upon the natural migration of elephants in most game reserves, they tend to destroy their own habitat if their numbers are not kept down<sup>4</sup>. In the Kruger National Park, this problem is overcome by darting old bulls with succinyl choline chloride from the air. Cropping in this way causes a minimum of disturbance in the elephant herds. A modern meat processing plant has been established in the Park.

There are at present too many problems connected with game cropping in Botswana to consider this type of utilization, except perhaps in time the cropping of elephants in the Chobe and Moremi game reserves. There seems little interference with their migratory routes at this stage, but with more and more areas in Botswana being opened up for agriculture and mining, these animals might have to be confined to the reserves in the future. Although cropping of game in Botswana is not advisable, there is certainly scope for research at least into the feasibility of domesticating certain species of game. In Rhodesia, Posselt<sup>11</sup> has already done valuable work in this connection, while in Uganda work on the utilization of eland is in progress<sup>10</sup>.

#### GAME VERSUS CATTLE

Many authors may have become over-enthusiastic about the superiority of game for ranching purposes as compared to domestic stock. However, some of the facts that have come to light over recent years are remarkable enough to justify enthusiasm. *Productivity*

Most of the data relating to productivity of game come from cropping schemes. Some of them are given here to show what can be expected from game ranching. Obviously much more research on productivity of domesticated game under ranching conditions is required.

In Kenya, the year-long standing crop from cattle on unimproved range land is 11 000 to 16 000 lb per square mile. This compares with a year-long standing crop from wild ungulates on unimproved savan-



Table 1: COMPARISONS OF YIELD OF WILD AND DOMESTIC ANIMALS ON DIFFERENT TYPES OF LAND\*

Land	Yield in terms of:	Land grazed by:	
		Domestic Sheep, goats, cattle	Wild Eland, wildebeest
Savannah	Live-weight gain per animal in lb per day	0,11—0,30	0,13—0,53
Savannah	Year-long standing crop in lb per morgen	36,5—52,8	296,4
Bush	Year-long standing crop in lb per morgen	5,8—25,4	99,1
Depleted land	Year-long standing crop in lb per morgen	29,2	58,6
Final effect:		Land deteriorated	Land recovered

\*Adapted from Talbot<sup>12</sup> and Crawford<sup>2</sup>.

nah in Tanzania of 37 000 to 70 000 lb per square mile and 90 000 lb per square mile in Kenya <sup>2, 13</sup>.

It has been calculated that the income from game cropping on the Henderson ranch (50 square miles) would be £5 500 per year, while the total game meat yield would be 118 300 lb<sup>14</sup>. Cattle on the same farm could only yield 94 500 lb per year. As other areas in Africa sustain much higher populations, the Henderson ranch could probably carry even greater numbers of game.

Reference to Table 1 will show that the live weight gains from cattle sheep and goats are in the region of 0,11 to 0,30 lb per day on savannah. This compares closely to findings on East African range land of 0,3 lb per day for cattle <sup>2, 14</sup>, and 0,12 lb for sheep<sup>16</sup>. According to Boyazoglu<sup>17</sup>, the live

Table 2<sup>15</sup>: GAME POPULATION IN SEVERAL AREAS IN AFRICA

Area	Biomass (lb weight per square mile)
Henderson Ranch Rhodesia	18 700
Wankie National Park	153 000
Serengeti-Mara	28 000
Nairobi National Park	76 000
Queen Elizabeth Park	107 000
Albert National Park	32 000 to 135 000

weight gains of *Bos indicus* cattle on bushveld in South Africa varies from 1 lb per day on average veld to 1,5 lb on good veld. This compares quite favourably with the available data for eland.

The Russian workers<sup>18</sup> also demonstrated that the eland can be fattened. A group of young eland aged from 2 to 4 years had a mean weight gain of 70,6 kg (155,6 lb) with an average daily increase of 785 g (1,7 lb) over a three month period. In South Africa five gelded eland bull calves aged 5—10 months had an average daily weight gain of 1,13 lb per day with a food conversion efficiency of 6,83, while cross-bred oxen gained 3,0 lb per day with an efficiency of 5,0<sup>19</sup>. However, these data are too limited to give more than an indication of the eland's potential under feedlot conditions.

#### Early maturity

It is usual practice in the semi-arid regions to market cattle at the age of 4 years<sup>3</sup>, as it takes them that long to reach full size and to gain condition. This depends upon the breed and the prevailing conditions. Eland and the larger antelopes require 3 to 4 years to reach maturity<sup>2</sup>, and will slaughter out at an estimated dressed weight of 600 lb<sup>14</sup>. This compares favourably with a

Table 3: LIVE WEIGHT GAINS OF ELAND

Type of Eland	Gain per day in lb	Age	Live end-weight	Author
Wild	0,54	—	—	Talbot <sup>12</sup>
Domestic	1,51	one year	1 000—1 600 lb	Posselt <sup>11</sup>
Domestic	1,1	one year	—	
Domestic	0,75	second year	2 000 lb	Treus & Krevchenko <sup>18</sup>



mean dressed weight of cattle at the Botswana Meat Comsission's factory at Lobatse of 501 lb during 1969<sup>20</sup>.

The mean age at first oestrus with conception of eland as observed in South Africa, South West Africa, Rhodesia and Russia, was 30,7 months with a range of 18 to 48, compared with Africander cattle with a range of 18 to 30. Nevertheless, the calving interval for eland ranged from 371 days on the highveld of South Africa to 336 days in Rhodesia<sup>11, 19</sup>, compared to 591 to 759 days for Africander cattle<sup>19</sup>.

*Slaughter weight and fat content of carcasses*

The slaughter weight percentage of wild antelopes is in the region of 50 to 62%<sup>2</sup>. The usual figure given for cattle off the veld is 50%, and it may reach as high as 60%. At the lower levels, cattle carcasses carry an 18% to 20% fat content and at the higher levels the figure may be as high as 30% and over<sup>21</sup>, whereas wild antelopes only carry 2% to 5% fat<sup>2, 22</sup>. The important aspect of animal production is protein and not fat; African herbivores produce a 70 to 80% lean carcase compared to 50% in the case of domestic species<sup>10</sup>.

The quality of fat also differs markedly in animals grazing on grassland and those browsing on bush. Carcase fat from cattle and grassland game species, like the Uganda kob and topi, have a low proportion of essential fatty acids with a non-essential fatty acid content of over 90%<sup>23</sup>. In a woodland environment the carcasses of African ungulates had an essential fatty acid content of 30 to 50%<sup>23</sup>.

It has been shown that elephants living in a grassland environment have a high incidence of atheroma, with about 44% of the aortic area containing calcium deposits. The incidence of atheroma in elephants from diverse woodland areas was negligible<sup>23, 24</sup>. The essential fats are known to be important in the health of vascular tissue, and the richest source of these fats is in oil-rich seed material which is prevelant in bush and tree

vegetation<sup>10</sup>. This could possibly explain th high incidence of heart disease in Western communities where grassland agriculture is practised, as compared to the low incidence in developing countries where goats and game provide the essential fatty acids. This concept is borne out by the arguments of Sinclair<sup>23, 25</sup>, who states that atheroma is a process of essential fatty acid deficiency. Despite the highly controversial nature of the cause of vascular disease, it makes biological sense to select and manage animals which produce high quality lean with respect to protein, essential fats, minerals and vitamins, as opposed to production of large amounts of non-essential fats. There is a growing awareness that the production of lean meat, rather than fat, should be the guiding principle in animal production.

*Milk production*

The work on eland milk in the Ukraine has brought some remarkable facts to light<sup>18</sup>. Four eland bulls and four cows were imported from Africa to the Askaniya-Nova Zoological gardens in 1892. The original stock have grown to 408 (in 1968) with no new blood being introduced. In spite of this intensive inbreeding, very few undesirable factors have emerged. They were fed rations indentical to those supplied to the cattle on the station. Studies on milk production started in 1950. The record milk production was 7 litres per day, with a total production per lactation of 637 litres. This was from a cow not selected or bred for milk production. The highest recorded butterfat content was 14% with a range of 9,1 to 12,6%. The albumen content (6,9 to 8,7%) was also about double that of domestic cow's milk. The milk seems to have remarkable keeping qualities. According to Russian workers, quoted by Crawford<sup>2</sup>, eland milk incubated at 37°C remained edible for three months. This remarkable claim, if substantiated, could be of importance in Botswana with its hot climate, long distances and poor refrigeration facilities.

Table 4: CHEMICAL COMPOSITION OF ELAND<sup>18</sup> AND DOMESTIC COW'S<sup>19, 26</sup> MILK

Animal	S.G.	Dry matter	Fat	Protein	Lactose	Ash
Eland	1,0342	22,14	9,5	5,9—9,7	3,7	1,1
Domestic cow	1,027—1,034	12,0	3,8	3,4	4,9	0,72
Africander cow	—	—	2,82	4,07	5,59	—



It is obvious from the above table that the proportion of nutrients in eland milk is much higher than in cattle, but it must be remembered that the mean milk production per lactation of eland in Russia was only 990 lb<sup>19</sup>, as compared to 2892 lb produced by Africander cows.

#### *Hardiness and resistance to drought*

From personal observation of eland in Botswana, there remains little doubt that eland can do without surface water indefinitely, given suitable food to browse. Eland and gemsbok are peculiarly adapted to high temperatures and water restriction in the following ways<sup>27</sup>. Body temperature increases with ambient temperature to limit evaporative skin loss; by lowering their metabolic rate, the amount of metabolic heat produced is lowered, and evaporation again reduced; by comparison with cattle, their lungs extract more oxygen from the air, limiting respiratory water vapour loss<sup>27</sup>. Eland void faeces of low moisture content: this enables them to gain more formed water<sup>3</sup>. Eland milk also contains less water than domestic cow's milk (Table 4).

It has been shown that an eland needs about 5.5 litres of water per 100 kg body-weight daily. It was also found that the acacia leaves and pods usually ingested by an eland per day provides 5.3 litres moisture per 100 kg bodyweight. Eland, therefore, can obtain all their moisture requirements from browsed vegetation. The net result is that eland conserve four times the amount of water as compared to grazing cattle<sup>27</sup>. All domestic stock are, of course, heavily dependant upon drinking water.

#### *Land management and utilization*

Through a system of biological separation, i.e. different types of animals feeding on different types of vegetation, the land in Africa has been efficiently and naturally utilized over at least two million years. This natural crop rotation is much more viable than a monoculture of cattle<sup>23, 28, 29, 30</sup>. It was highly successful, but it is now in the process of being disrupted; the breakdown can be attributed to human interference<sup>13, 23, 31, 32</sup>, which disturbs the diversity which used to exist. The imbalance of primary essential nutrients produced<sup>23</sup>, leads to destruction of animal populations and habitat, as, for example, the annual dying off of migrating wildebeest and hartebeest in Botswana.

It has been argued that when game is ranched "domestically", it could just as easily lead to "over-browsing", thus depleting what little edible fodder remains in drought years in the semi-arid regions. Bush, however, with its deeper root system, can last much longer in a drought than grassland. Browsers, like eland, can be used to complement cattle on a ranch, thus making it unnecessary to keep as many cattle. Being essentially browsers, they would not compete with cattle for grazing.

#### *Resistance to enzootic diseases*

Various diseases like malignant catarrhal fever, trypanosomiasis, *Geddoelstia* infestation and African swine fever cause havoc among domestic stock, but are inapparent in wild animals. This, unfortunately, may lead to "carrier" states in wild animals which may constitute a danger to domestic stock. The whole question of "carriers" is a controversial one. In many instances in the past game have been labelled as "carriers" of certain diseases on the flimsiest of evidence. In many cases they were blamed for outbreaks of disease merely because they happened to be in the area and conceivably could have come into contact with domestic stock. Lately, work is being done on this question, especially in regard to foot and mouth disease in Botswana and Rhodesia<sup>33, 34</sup>, but the picture is certainly not clear<sup>35</sup>. Although eland may be fully susceptible to diseases like foot and mouth, rinderpest and East Coast fever, there is, to my knowledge, no evidence that they can act as carriers of any of the major epizootic diseases, with the possible exception of sleeping sickness in man<sup>19</sup>.

Cattle, sheep and goats are adapted to the environment from which they were introduced and possess limited ability to adapt to new ones<sup>3</sup>. Through good animal husbandry and the efforts of veterinary authorities, their range has been extended, but in some instances disease may offer absolute barriers. In Africa, for instance, 4.15 million square miles of land is unsuitable for cattle ranching due to trypanosomiasis. This represents 37% of the total land area in Africa<sup>3</sup>. The various species of wild animals which are able to survive in these areas were ruthlessly slaughtered in the past in an effort to make the area safe for familiar, but unsuitable, domestic animals. Fortunately this policy of wholesale slaugh-



ter has now largely been abandoned in Botswana<sup>36</sup>.

Those areas, at present unsuitable for domestic animal ranching, could possibly be used for game ranching if proper veterinary control was exercised to ensure their safety for human consumption. This means some form of "domestication".

#### CHOICE OF ANIMAL TO BE DOMESTICATED

This choice will depend whether the species selected is hardier and more resistant to drought than domestic animals and yet as productive, without constituting a danger to domestic stock or man through disease or competition for grazing. The animal should also be suited to the habitat obtaining over most of Botswana, and should be able to make use of land which is unsuitable for domestic stock.

Many species of game are available and can be domesticated in Botswana, but most of them are unsuitable because they do not conform to the above requirements. The buffalo (*Syncerus caffer*) is a bovid, closely resembles cattle but is unsuitable because it appears to be prone to almost all the diseases of cattle<sup>37</sup>. It is also adapted to riverine habitats, of which there are few in Botswana. The blue wildebeest is a true carrier of malignant catarrhal fever, *Geddoelstia* infestation and is essentially a grazer. The other antelopes, except eland and springbok (*Antidorcas marsupialis*), offer no particular advantages over domestic animals.

The eland seems to have all the required characteristics. These have been described in this paper. A further advantage would be the eland's docility and ease of domestication, as compared to other antelopes with otherwise similar characteristics, like the gemsbok.

The springbok also shows distinct promise as a "domestic" animal, especially the subspecies *Antidorcas marsupialis angolensis* found in Botswana and South West Africa. This is a much larger and heavier animal than the South African variety (*A. marsupialis marsupialis*). Recent studies by Skinner<sup>38</sup> have shown that springbok can be confined by relatively low fences, and that they do not appear to compete with domestic stock for food under good grazing conditions. Apparently they prefer weeds and shrubs, but will eat grass when the former are depleted under drought conditions.

#### PROBLEMS TO BE OVERCOME

##### Parasitism

A problem, associated with some game cropping schemes, is the high percentage of parasites found in game meat, thus rendering it unsuitable for human consumption on aesthetic grounds<sup>39, 40</sup>. The echinococcus and cysticercus cysts found in game meat apparently originate from wild predators. This work<sup>39, 40</sup> was done in East Africa. To my knowledge, very little work of a similar nature has been done in Botswana. Nevertheless, from personal examination of several game carcasses of different species in Botswana, it can be stated that a similar problem does not seem to exist in Botswana. The parasites found were mostly confined to the intestines and of the tenuicollis type, while no sarcocysts or cases of cysticercosis were seen. This can of course not be regarded as conclusive evidence. A study of the extent of this problem in Botswana, therefore, would seem to be indicated. Just as in cattle, or in zoo animals, parasitism can be controlled under good management in any species. The need for good husbandry, both in the interests of the animal and in the fitness of the product for human consumption, strengthens the case for domestication to make use of the biological potential of the eland.

##### Methods of capture and management in confinement

Fourteen young eland were captured in the Makoba quarantine camp in Botswana. At first, various combinations of drugs were tried, using darts fired from a "Cap-chur" powder-charged gun. This method was soon discarded, as eland are very timid and difficult to approach without being seen. Dosages are critical, and the animals are very prone to shock, especially after being chased for miles through the bush. A much easier and more successful method was later evolved. When a group of eland was sighted they were followed at a leisurely pace in a vehicle suitable for the bushy and uneven terrain. The animals were merely kept in sight, or tracked when they moved out of sight. Usually after three quarters of an hour the whole group would come to rest in the shade of a clump of trees and allow the vehicle to approach to within 100 yards. They seemed to be reluctant to move on again, and when they did, it was at a much slower pace. The vehicle now had no diffi-



culty in keeping up with them. Within a few minutes one or more of the yearling heifers or bulls would usually peel off from the rest, or lag behind. One of these was then selected and chased at a slightly faster pace. After a very short chase, this young eland would flop down onto its sternum and allow itself to be captured and loaded onto the vehicle. None of the animals captured showed any signs of aggression or much excitement at this stage, and in most instances it was not even necessary to rope them: a firm grip on both horns was all that was necessary to control them.

This method was successful in Botswana under drought conditions when eight of the captured animals were in very poor condition. The best time of day for the operation was midday, when ambient temperatures were extremely high. Younger animals survive handling and confinement much better than older ones. Eight of the older captured animals died within two weeks of being confined. These were emaciated to start off with, and supervision of their feeding was impossible due to the distances involved from the author's home to the quarantine camp. After it was demonstrated to the handlers that the six remaining younger eland needed two 5-ton lorry-loads of bush material per day to start with, the five heifers and one bull settled down very well and gained condition. After one month they were slowly being weaned from bush material and put onto a balanced commercial stock feed in the form of cubes, dry veld hay, and a urea-molasses lick.

Eland are capable of jumping over 6 to 7 feet high fences<sup>11, 19</sup>. This would seem to pose insurmountable problems to the would-be eland rancher. However, from personal observation of wild eland it seems that the eland cow is a strongly territorial animal. A group of 20 eland cows confined in a camp of about 9 square miles, resisted strenuous efforts to drive them out in the interests of disease control. Their territorial instinct was so strong that they would not cross the boundary where the fence used to be when the fences had been broken down to facilitate the operation. During the severe drought of 1970, most of this camp was burnt out, severely restricting the browsing in the camp. The group would still not move out, with the result that they were dying of starvation, in spite of the fact that adequate

food was available in an adjacent camp. Eland bulls, on the other hand, are inclined to roam, remaining with groups of cows for shorter or longer periods. This could be of advantage to the eland farmer, as new blood would be regularly introduced into the herd. Here the question of the ownership of wildlife comes into play<sup>41</sup>.

In spite of their usual docile behaviour, eland can be aggressive towards their handlers or towards each other. Studies on dehorning would therefore seem necessary. Dehorning in turn might cause problems, because eland use their horns to break off branches of trees when they browse, but this does not usually apply to Botswana, where most of the bushes eaten by eland are stunted or within reach of a browsing eland.

### *Marketing*

In a developing country like Botswana, economic factors in a scheme of this nature must of necessity be of paramount importance. A market for eland meat could be developed in the urban areas of Botswana. The population of Botswana is used to the consumption of game meat. In a recent survey it was estimated, that 60% of all meat eaten in Botswana was from wild animals, but according to Child this figure is probably far too low<sup>41, 42</sup>. Smithers<sup>43</sup>, as quoted by von La Chevallerie<sup>44</sup>, states that eland meat is the finest of the large animal species, rather light in colour like veal, and very palatable.

Unfortunately, export of eland meat to European continental countries from Botswana is not possible at this stage, because of foot and mouth disease restrictions<sup>45</sup>. The position regarding export to South Africa is more hopeful. A live eland heifer sells for R600 (£351) in South Africa and a bull for R200 (£117). A market for game meat is being built up by the South African Parks Board through their game cropping scheme in the Kruger National Park.

If eland meat or live eland are to be marketed and exported to South Africa or other African countries, they would have to be domesticated to such an extent that they could be quarantined, mouthed and transported. From personal experience I believe that this will be possible, especially when eland bred in captivity are used. All the vaccines administered to domestic stock by the Veterinary Department could also be



given to domestic eland. If this procedure is rigidly adhered to, it should be possible to convince importing countries of the lack of danger to domestic animals and man.

#### IMPLEMENTATION

To implement the scheme, a pilot project involving the capture of a few eland cows and bulls could be started. A number of young eland heifers and bulls have already been captured in the Makoba quarantine camp by the author. These animals should be kept on a government ranch in southern Botswana, where no danger of foot and mouth disease exists at present. There, further research on fertility in captivity, susceptibility to disease, the question of "carriers", possibility of artificial insemination, dehorning, carrying capacity of Botswana bushland, and general husbandry, etc. could be done. Eventually, when a sizeable herd has been built up, sales to inter-

erted farmers and importers could commence.

#### CONCLUSION

In Botswana the unique opportunity exists to capture and domesticate eland. In that country large numbers of eland and springbok are still found outside game reserves, and most of the land is ecologically suited to these species. Much research still needs to be done on animal diseases and zoonoses, management, marketing and related problems. This research could be done in Botswana to enable the country to start using this untapped potential before it is too late.

#### ACKNOWLEDGEMENT

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## DISEASES OF FEEDLOT CATTLE

R. JENSEN AND D. R. MACKEY

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The two-fold purpose of this text is to emphasize diseases of feedlot cattle and to collect, within a single volume, current literature pertinent to that field of veteri-

nary speciality. The text is divided into chapters covering viruses, bacteria, fungi, protozoan and metazoan parasites and miscellaneous and unknown causes of disease.



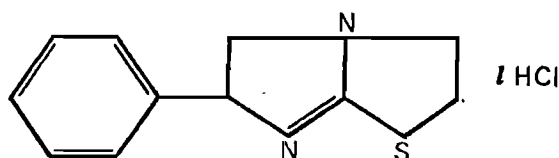
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# ROBERTSONIAN CHANGES IN THE CHROMOSOMES OF A SOUTH AFRICAN RAT POPULATION (*RATTUS rattus* L.)

W. H. GERNEKE\*

## SUMMARY

Centric fusion of four originally acrocentric pairs of chromosomes has apparently given rise to a large meta- and a submetacentric pair in the karyogram of *Rattus rattus* L. trapped in the Veterinary Faculty Buildings at Onderstepoort. This fusion (Robertsonian change) has reduced the classical rat chromosome number of 42 to 38 in the Onderstepoort species. Indications are that the population is uniformly affected, existing as a "38 chromosome race".

## INTRODUCTION

Chromosomal polymorphism has been reported recently in the sex chromosomes of the laboratory rat, *Rattus norvegicus* var. *albinus*<sup>1,2</sup> of *Mus musculus*<sup>3</sup> and of the Syrian hamster *Mesocricetus auratus*<sup>4</sup> as well as in the autosomes of the common shrew, *Sorex araneus*<sup>5</sup>. Intrapopulational chromosomal polymorphism caused by Robertsonian changes was found in *Gerbillus pyramidum*<sup>6</sup>. Autosomal polymorphism was also seen in some house rats, *Rattus rattus* L. in the Kusudomari and Misima districts of Japan<sup>7</sup>, in *Rattus norvegicus*<sup>8</sup>, as well as in the blue wildebeest, *Gorgon taurinus*<sup>9</sup>, in African pygmy mice, *Mus minotoides*, in *Mus triton* and *Acomys minous*<sup>10, 11</sup>.

At the request of Prof. Jorge Paulette-Vanrell of the Department of Morphology of the Regional Medical Faculty of São José do Rio Preto, São Paulo, Brasil, a study of the chromosomes of South African representatives of the common house rat *Rattus rattus* L. was undertaken to determine the possible occurrence of chromosomal polymorphism.

## MATERIAL AND METHOD

A dozen wild rats, all of agouti colour and the two sexes equally represented, were trapped over a period of two months in

some of the buildings of the Veterinary Faculty, Onderstepoort for this investigation. They were anaesthetized with chloroform, killed and bone-marrow was collected from the ribs, sternum and femurs. Spreads were prepared according to a method previously described<sup>9</sup>. Initially very few spreads were obtained, due, apparently, to lack of mitoses during day-time. Only after a subcutaneous injection of 1 ml of 0.1% solution of colchicine had been given and the rats sacrificed 3 hours later, were large numbers of suitable spreads (Figs. 4 and 5) obtained. About 50 spreads of each of the six colchicine treated rats were studied. No tissue cultures were made. Skins and skulls of the rats were submitted to the Zoology Department of the Transvaal Museum, Pretoria, where their identity as being *Rattus rattus* L. was confirmed.

## RESULTS

Karyogram analyses (Figs. 1—3) gave a constant diploid chromosome count of 38, four less than the normally accepted number. Occasional deviations from this number were regarded as artefacts. The autosomes and sex chromosomes of the accepted classical rat karyogram of *Rattus norvegicus*<sup>12</sup> could be matched with comparable ones of the karyograms constructed from the observed spreads except in the case of four pairs of the classical pattern. In their stead, a metacentric and a submetacentric pair (numbers 1 and 4 in figs. 1—3 respectively) were present. These did not fit anywhere in the classical pattern. Occasionally an individual chromosome or even a homologous pair (fig. 3 No. 3) with a secondary constriction was observed. Due to the scarcity of this phenomenon, it was not considered to be of any significance.

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# PLATE 1

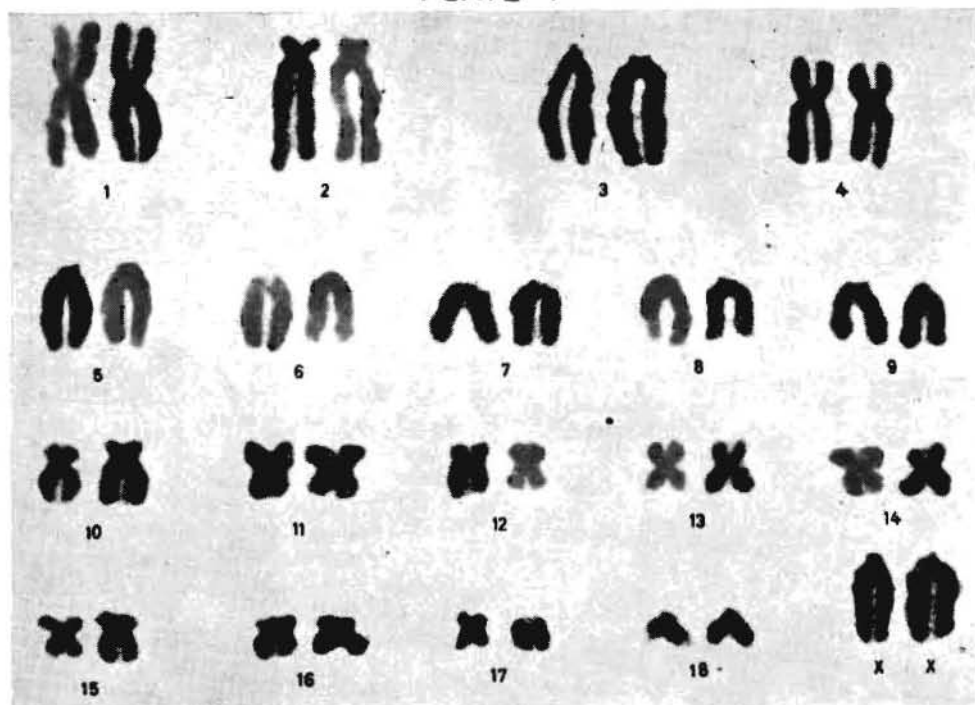


Fig. 1

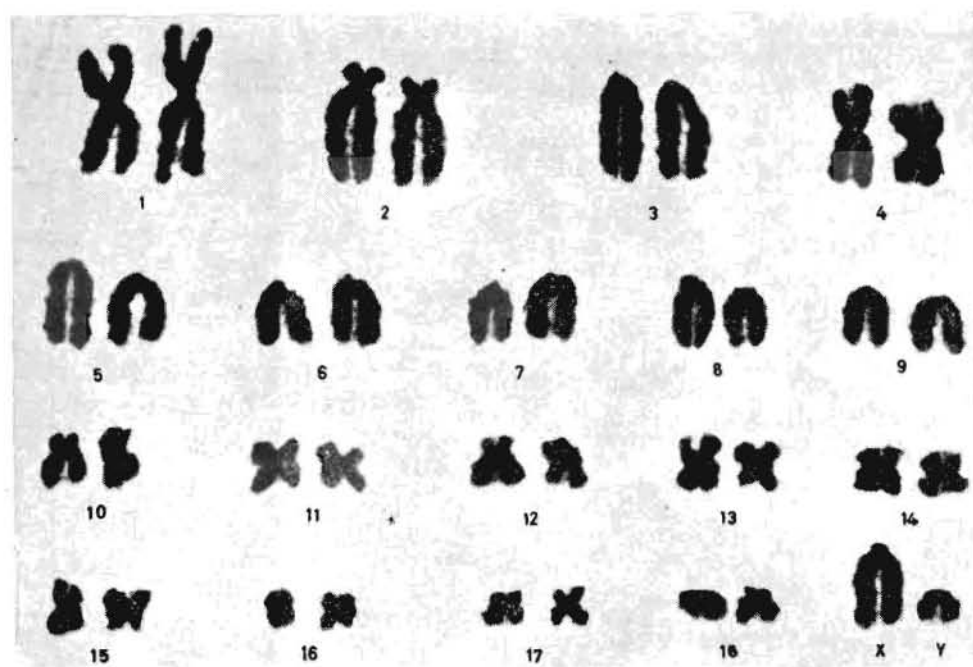


Fig. 2

FIG. 1. Karyogram of a female specimen of *Rattus rattus* L.  
 FIG. 2. Karyogram of a male specimen of *Rattus rattus* L.



## DISCUSSION

If the karyogram of *Rattus norvegicus*<sup>12</sup> is considered as the standard one for rats, then the karyogram of *Rattus rattus* L., as reported from Japan has deviated from it by polymorphism affecting chromosome No. 1<sup>7</sup>, as well as by the lack of one small metacentric pair and by having in its stead an additional acrocentric pair. The latter features were not specifically mentioned by the author, but could be deduced by study of their figs. 7—10. The most acceptable explanation for the differences revealed in the karyogram of the Onderstepoort population (Figs. 1, 2) is that centric fusion of four originally acrocentric pairs of chromosomes had occurred to produce a metacentric and a submetacentric pair, i.e. a Robertsonian change had taken place, thereby reducing the diploid number from 42 to 38. It is an excellent example of intraspecific polymorphism affecting a circumscribed population uniformly. Whether this change has occurred more extensively in South Africa remains to be determined.

The mechanism of Robertsonian change (centric fusion) although generally acknowledged, is still not clearly understood. A reciprocal translocation, with long legs translocated to one centromere and short legs to the other, with subsequent loss of the resultant smaller chromosome, would be logically acceptable. It still leaves the question open concerning the effects of such a loss. This loss would be minimal if the small translocated legs were heterochromatic. Fusion in the strictest sense would result in a chromosome with two centromeres: this does not happen. Centromeres could possibly fuse with loss of the terminal chromosomal legs of acrocentrics: this would result in a larger centromere, which also has not been observed. The details of this mechanism, therefore, still remain to be elucidated.

The question now arises whether one is justified in accepting the karyogram of *Rattus norvegicus* as the standard for *Rattus rattus*. Whatever the answer, comparison of the Japanese<sup>7</sup> and South African findings in themselves indicate that chromosomal polymorphism occurs in *Rattus rattus*. This raises the questions whether the observed deviations are indicative of further speciation, or whether they merely are the result

of mutations with no apparent selective benefits? No morphological modification of the phenotype seems to have occurred in *Rattus rattus*; possible physiological and/or behavioural changes have not been investigated. According to Swanson<sup>13</sup>, the centric fusion type of translocation appears to be a particularly prevalent manner of chromosomal change in animals. Apparently such changes can take place without phenotypic modifications.

On the other hand, Robertsonian changes have been mentioned as a possible mechanism in the speciation of *Ovis aries*<sup>14</sup> ( $2n=54$ , with six metacentric chromosomes, which would, on isochromosome formation, give 60 acrocentrics equivalent to say the karyogram of *Capra hircus*). First indications of such changes have also been noticed in the blue wildebeest *Gorgon taurinus*<sup>9</sup> as well as in *Gerbillus pyramidum*<sup>6</sup>.

Answers to these questions would only be obtained once a world-wide investigation into the karyology of the rat has been carried out, especially in its native habitats: tropical Asia for *Rattus rattus* and the Northern temperate region of Asia for *R. norvegicus*<sup>15</sup>. With the present state of our knowledge the rat certainly poses as an internationally distributed species undergoing different chromosomal aberrations and therefore most interesting from an evolutionary point of view.

Since this article was submitted for publication, Dr. T. C. Hsu, Professor of Biology, University of Texas has informed me that two chromosomal races of *Rattus rattus* do exist, one with a basic diploid number of 42 and the other with 38. The former inhabits all Orient, the Asiatic portion of Eurasia, while the latter is found in Europe, Australia, South America and as he expressed it "probably in Africa". This article confirms the existence of the 38 chromosome race in Africa, at least in the vicinity of Onderstepoort.

## ACKNOWLEDGEMENTS

Sincere appreciation is expressed towards Miss P. Peens and Mrs. N. Vosloo for their ever-willing help in doing the chromosome counts and towards Prof. H. P. A. de Boom for his constructive criticism on reading the manuscript.



# PLATE 2



Fig.3



Fig.4



Fig.5

FIG. 3. Karyogram of a male rat with a secondary constriction in chromosome pair no. 3.

FIG. 4. Spread from which fig. 2. was compiled.

FIG. 5. Spread from which fig. 1 was compiled.



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# MILK FEVER IN A LARGE JERSEY HERD

## 1. THE INCIDENCE OF THE CONDITION

P. C. BELONJE\* AND K. VAN DER WALT\*\*

### SUMMARY

A milk fever survey was conducted on a large Jersey herd over a period of nine years. The over-all incidence was 17,5% (837 lactations) whereas in cows in their third lactation and older the incidence was 24,8% (585 lactations). Only one affected cow was recorded younger than in her third lactation.

There was a marked variation in annual and monthly incidence suggesting a seasonal effect.

In the group of cows which developed milk fever at some time during their lives, there was no difference in the average milk and butterfat production preceding normal parturition and that associated with milk fever. Moreover, there was no difference in the average milk and butterfat production following normal parturition and that associated with milk fever. Furthermore, except for the third lactation, the average intercalving period before milk fever tended to be longer than before a normal calving.

### INTRODUCTION

Milk fever is a fairly common condition of dairy cattle, particularly Jerseys<sup>1, 2, 3</sup>. Although a great deal of research has been conducted on the occurrence and aetiology of the condition in some other countries, little has been reported in the Republic. For this reason, therefore, a large scale investigation was conducted on a well-managed Jersey farm with adequate records.

This first article gives a general description of the farm and the herd together with the incidence of milk fever over a nine year period.

### LOCATION AND GENERAL MANAGEMENT

The 850 ha farm, on which the herd is

kept, is situated near Stellenbosch (34°S, 19°E) in a Mediterranean, winter rainfall type of climate. The average annual rainfall is 660 mm, approximately 80% of which falls between April and September. In this period, during June, the lowest average daily temperature of 12,4°C is recorded. On the other hand only about 8% of the annual rainfall precipitates during the summer months of December, January and February, with the highest average daily temperature of 21,7°C occurring during February.

The herd itself comprises about 240 stud Jersey animals of which about 110 are cows and heifers older than two years. Apart from commercial milk being produced, stud bulls and cows are also sold. The average age at first calving varies between 22 and 24 months; cows are usually sold after completing three or more lactations. Over a period of nine consecutive years an average of 93 animals was in milk per annum with a milk production varying between 3 000 and 9 000 kg per lactation.

The usual dairy concentrates are fed and summer grazing is provided on 35 ha irrigated grass clover pastures while 40 ha mixed grass pastures are utilized during winter. Moreover, about 140 ha are planted to fodder crops (oats, lupins and lucerne) and a further 145 t silage are produced annually.

Comprehensive records of each animal, the feeding systems used and the prevailing weather conditions are kept on the farm and it was from this data that the nine year survey was made. Cases of milk fever were recorded after a clinical diagnosis by a veterinarian; in certain cases this was confirmed by laboratory analyses.

\*Dept. of Physiology, Faculty of Agriculture, Stellenbosch University.

\*\*Dept. of Medicine, Faculty of Veterinary Science, Onderstepoort.



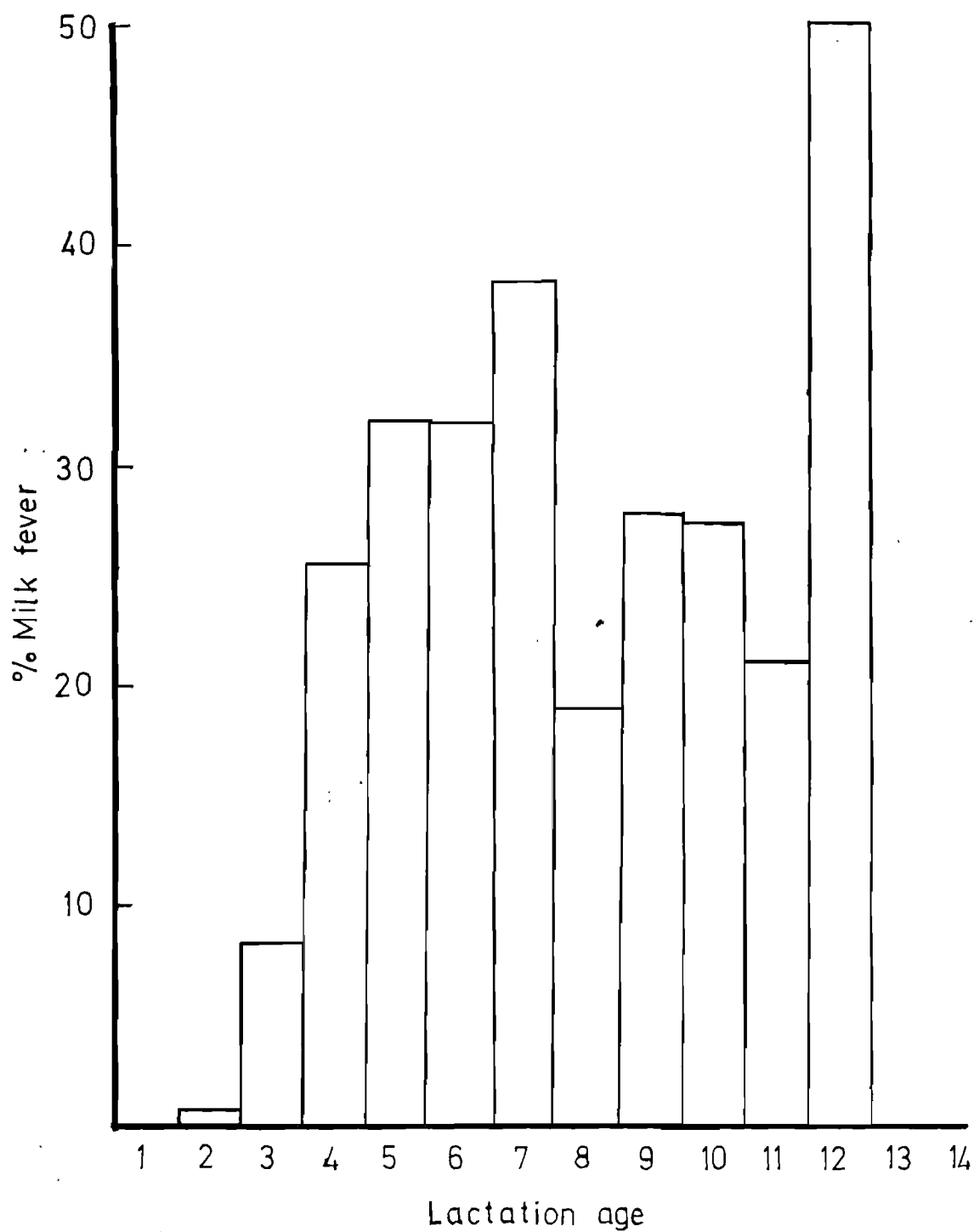


FIG. 1. The distribution of milk fever per lactation age in the whole herd.



## THE INCIDENCE OF MILK FEVER

### A. THE HERD AS A WHOLE

In this study the data from nine consecutive years were pooled. This amounted to a total of 837 lactations in which there were 14 lactation age groups. The distribution of these lactation ages is presented in Table 1.

Table 1: THE DISTRIBUTION OF LACTATION AGES DURING THE TOTAL NINE YEAR PERIOD

Lactation age	Number of animals	% of total
1	118	14.1
2	134	16.0
3	122	14.6
4	100	12.0
5	75	9.0
6	72	8.6
7	60	7.2
8	53	6.3
9	43	5.1
10	33	3.9
11	15	1.8
12	6	0.7
13	5	0.6
14	1	0.1
Total	837	

Of the total of 837 lactations, 146 were associated with milk fever, an over-all incidence of 17.4%. If, however, the percentage incidence of milk fever per lactation age is calculated, it is evident that there is a dramatic rise after the second lactation (Figure 1).

### B. THE SUSCEPTIBLE HERD

Except for one animal, there were no further cases of milk fever recorded in the first and second lactation age groups. As these two groups constitute a large proportion of the total herd data, it was decided to dispense with this bias and calculate total, annual and monthly incidence in the susceptible herd i.e. those of three lactations and above.

Of the 585 lactations in this group 145 were associated with milk fever, an incidence of 24.8%. Nevertheless, there were considerable differences in both the annual and monthly incidence over the nine year period (Tables 2 and 3). These observations suggest that there may be seasonal effect in the aetiology of the condition.

Table 2: ANNUAL DISTRIBUTION OF MILK FEVER IN THE SUSCEPTIBLE HERD (Period of nine year)

Years	No. of lactations	No. of M.F.	% M.F.
1	54	16	29.6
2	64	14	21.9
3	59	11	18.6
4	74	9	12.2
5	79	14	17.7
6	77	21	27.3
7	68	18	26.5
8	56	26	46.4
9	54	16	29.6

Table 3: MONTHLY DISTRIBUTION OF MILK FEVER IN THE SUSCEPTIBLE HERD (Period of nine years)

Months	No. of lactations	No. of M.F.	% M.F.
January	50	8	16.0
February	57	15	26.3
March	48	13	27.1
April	60	12	20.0
May	46	11	23.9
June	39	3	7.7
July	41	8	19.5
August	52	17	32.7
September	44	15	34.1
October	61	19	31.2
November	46	14	30.4
December	41	10	24.4

### C. THE AFFECTED HERD

This further subdivision includes only those cows which developed milk fever at some or other time during their lives, a total of 73 animals.

#### (i) The age at which milk fever first developed

Figure 2 shows the percentage distribution of the actual age, and Figure 3 the percentage distribution of the lactation age at which milk fever was recorded for the first time. The one animal which developed the condition at the second lactation only calved for the first time at 2 years 7 months and developed milk fever at the commencement of the following lactation 25 months later, at an age of 4 years 8 months.

#### (ii) Milk and butterfat production

The average milk and butterfat productions subsequent to a normal calving were compared with the average productions after an attack of milk fever. This was calculated from the third to the ninth lactations. As can be seen in Table 4 there were no signifi-



cant differences except in lactation 8. The reason for this is probably the small number of observations in the milk fever group which included some very high-producing animals.

In addition, the milk and butterfat production preceding a normal calving was compared with the production preceding milk fever. The averages were calculated for the lactations preceding the 3rd to the

9th calvings (Table 5). Once again there were no significant differences between the groups except in the lactation preceding lactation 9. As explained above, the reason for this is probably the small sample size which included some very high-producing animals.

(iii) *The intercalving periods*

The average intercalving periods preceding a normal parturition and those

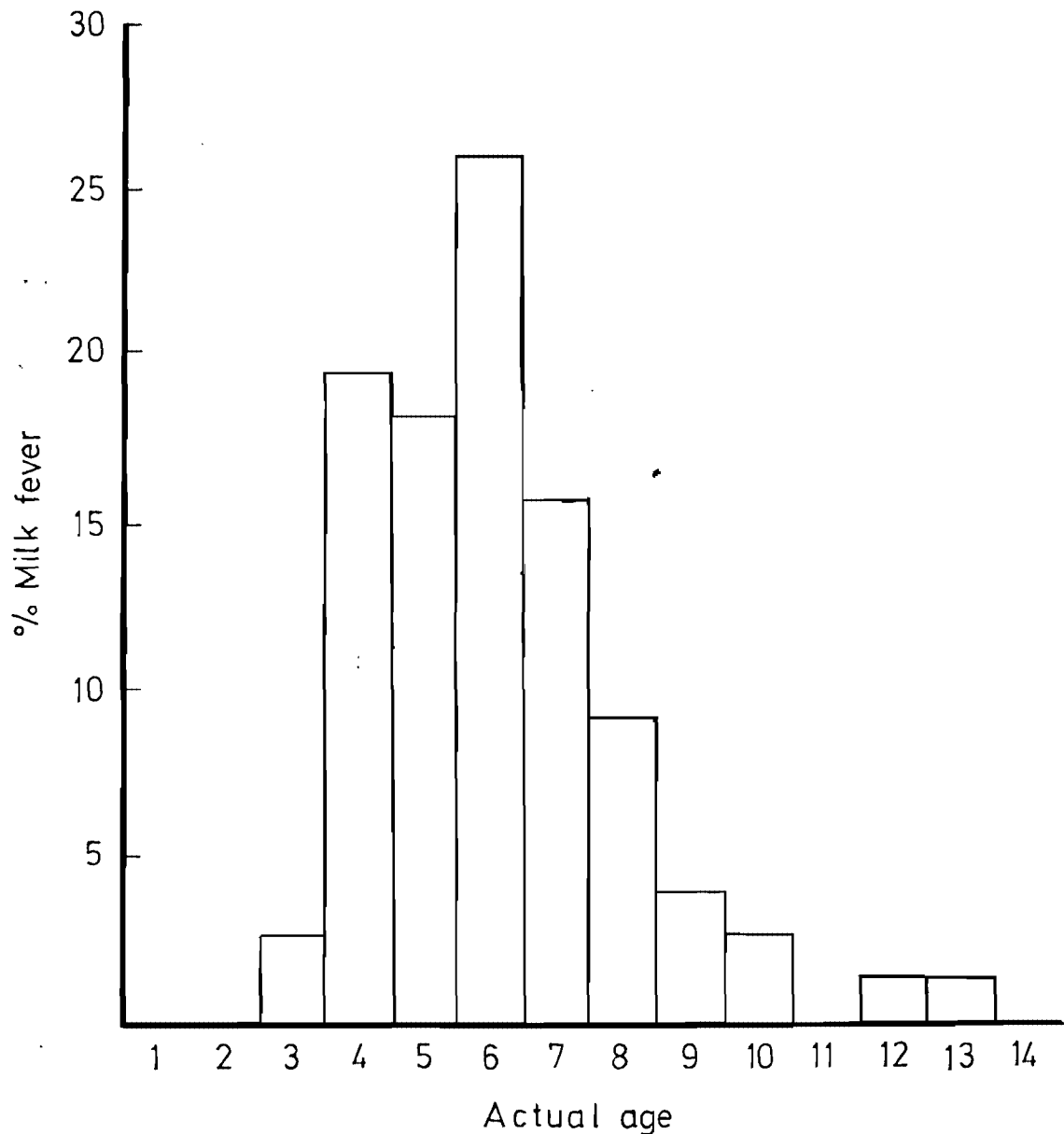


FIG. 2. The distribution of the actual age of the cows when milk fever first developed.



Table 4: THE AVERAGE MILK AND BUTTERFAT PRODUCTION FOLLOWING PARTURITIONS WHICH WERE EITHER NORMAL OR ASSOCIATED WITH MILK FEVER

Lactation age	Group	Number of observations	Average milk production lb $\pm$ SE	Average butterfat production lb $\pm$ SE
3	Normal M.F.	42	8 954 $\pm$ 299	493 $\pm$ 16
		9	8,821 $\pm$ 368	485 $\pm$ 30
4	Normal M.F.	28	9 004 $\pm$ 403	488 $\pm$ 21
		23	9 630 $\pm$ 406	525 $\pm$ 23
5	Normal M.F.	27	10 146 $\pm$ 403	544 $\pm$ 18
		21	10 427 $\pm$ 517	559 $\pm$ 27
6	Normal M.F.	24	9 763 $\pm$ 491	520 $\pm$ 8
		21	10 332 $\pm$ 393	546 $\pm$ 23
7	Normal M.F.	16	10 353 $\pm$ 677	535 $\pm$ 12
		20	9 762 $\pm$ 648	520 $\pm$ 33
8	Normal M.F.	20	8 986 $\pm$ 159	470 $\pm$ 86
		9	10 189 $\pm$ 565	536 $\pm$ 37
9	Normal M.F.	16	8 861 $\pm$ 573	465 $\pm$ 34
		11	9 073 $\pm$ 830	461 $\pm$ 38

Table 5: THE AVERAGE MILK AND BUTTERFAT PRODUCTION PRECEDING PARTURITIONS WHICH WERE EITHER NORMAL OR ASSOCIATED WITH MILK FEVER

Lactation age	Group	Number of observations	Average milk production lb $\pm$ SE	Average butterfat production lb $\pm$ SE
3	Normal M.F.	32	7 487 $\pm$ 402	428 $\pm$ 20
		9	7 403 $\pm$ 575	411 $\pm$ 32
4	Normal M.F.	23	8 808 $\pm$ 306	487 $\pm$ 18
		24	9 305 $\pm$ 518	510 $\pm$ 26
5	Normal M.F.	18	9 248 $\pm$ 460	498 $\pm$ 25
		22	9 127 $\pm$ 417	500 $\pm$ 23
6	Normal M.F.	21	10 685 $\pm$ 460	576 $\pm$ 21
		20	9 855 $\pm$ 481	528 $\pm$ 23
7	Normal M.F.	15	9 918 $\pm$ 495	522 $\pm$ 27
		17	10 111 $\pm$ 558	535 $\pm$ 26
8	Normal M.F.	14	9 938 $\pm$ 505	523 $\pm$ 24
		7	9 328 $\pm$ 802	525 $\pm$ 48
9	Normal M.F.	12	8 850 $\pm$ 498	470 $\pm$ 32
		9	10 905 $\pm$ 664	566 $\pm$ 37

associated with milk fever were calculated from the third to the ninth calvings. As can be seen in Table 6, the average intercalving periods of the unaffected animals were remarkably constant throughout. On the other hand, the milk fever groups varied considerably and except for the third lactation age, the intercalving periods were longer than normal.

#### DISCUSSION

The incidence of milk fever in this Jersey herd over the nine year period seems fairly high. Yet the incidence of 24.8% in cows, in their third lactation or older, is very close to the 22.4% in cows of similar age in Idaho<sup>4</sup>, but much lower than the 34.5% recorded at the University of Guelph<sup>3</sup>.

The age distribution appears to be



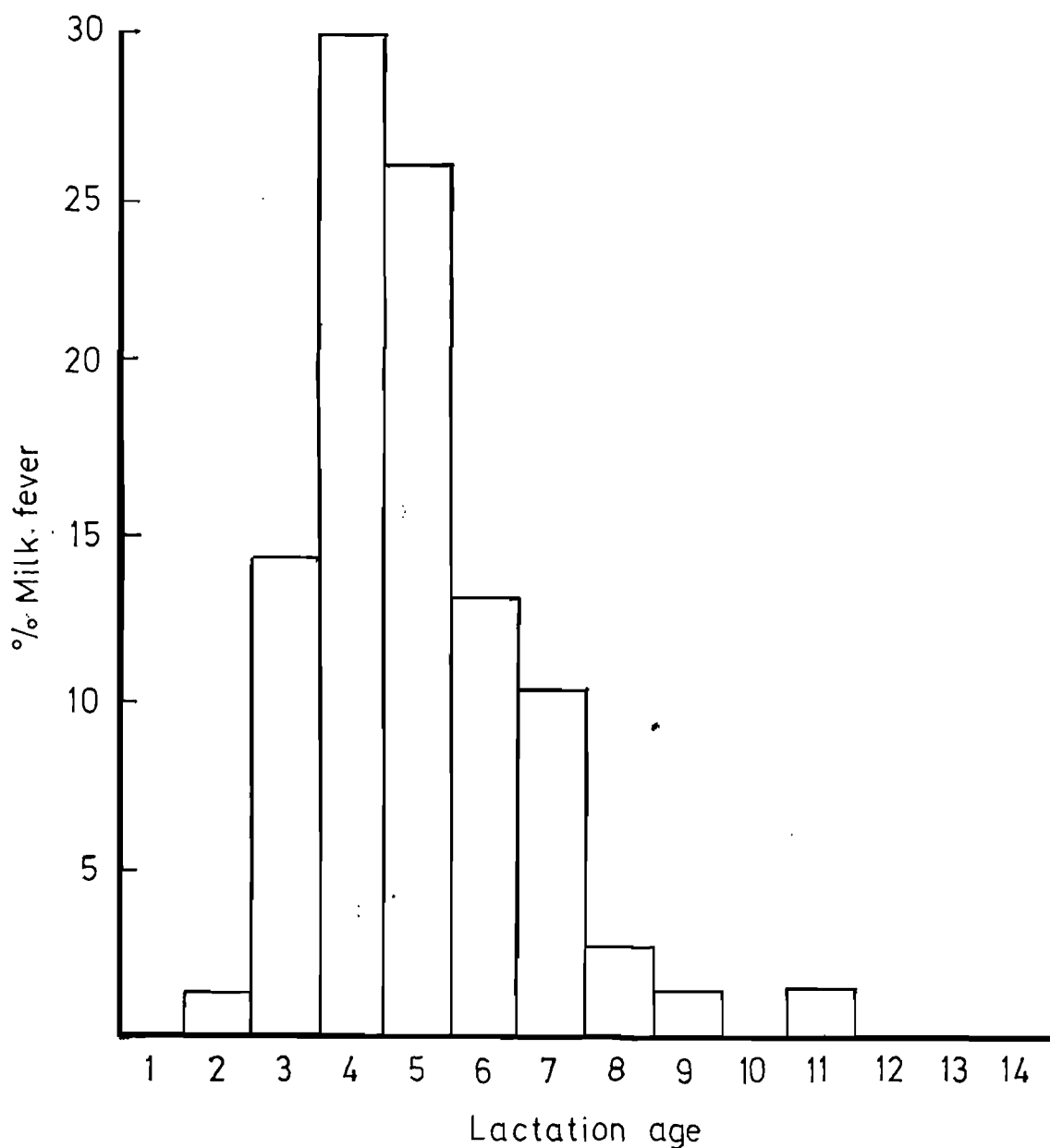


FIG. 3. The distribution of the lactation age of the cows when milk fever first developed.

typical, with a low incidence at the first two lactations, followed by a sharp increase thereafter<sup>1,3</sup>. The only cow which developed milk fever before the third lactation was an exception, as it only calved for the second time at 4 years 8 months, an indication of the effect of actual age rather than

lactation age.

The average milk and butterfat production preceding parturition did not appear to have an effect on the incidence of milk fever, neither did milk fever appear to influence the subsequent average milk and butterfat production. This finding is in



Table 6: THE AVERAGE INTERCALVING PERIODS  
PRECEDING NORMAL CALVINGS AND  
THOSE ASSOCIATED WITH MILK FEVER

Lactation age	Group	Number of observations	Average intercalving period: months $\pm$ SE
3	Normal M.F.	62	13.7 $\pm$ 0.35
		11	12.5 $\pm$ 0.37
4	Normal M.F.	43	13.1 $\pm$ 0.28
		29	13.8 $\pm$ 0.69
5	Normal M.F.	33	13.2 $\pm$ 0.33
		30	13.8 $\pm$ 0.49
6	Normal M.F.	29	13.1 $\pm$ 0.58
		25	13.2 $\pm$ 0.39
7	Normal M.F.	17	13.8 $\pm$ 0.70
		25	14.2 $\pm$ 0.57
8	Normal M.F.	23	13.4 $\pm$ 0.47
		10	16.7 $\pm$ 0.58
9	Normal M.F.	17	13.1 $\pm$ 0.48
		12	15.58 $\pm$ 0.53

general agreement with that reported by Curtis, Cote and Mills<sup>3</sup> in the Guelph herd. On the other hand Fiez, Sasser and Ross<sup>4</sup> have shown a significant increase in milk fever as higher levels of butterfat are obtained.

Fiez *et al*<sup>4</sup> found a trend in their data which suggested that milk fever increased as the non-lactating period increased. As the intercalving period includes the non-lactating period, the findings in this survey appear to substantiate this trend. The reason for this effect is obscure.

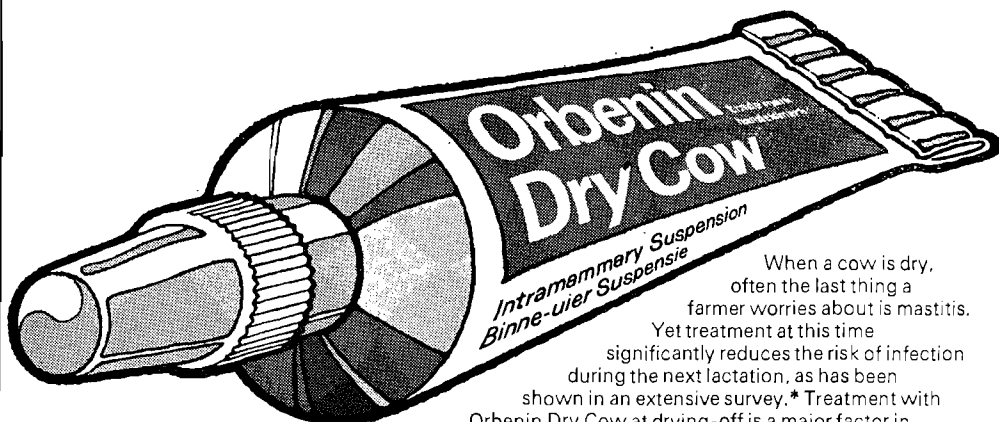
In this survey there were wide variations in the percentage incidence of milk fever recorded per month and per annum, suggesting a possible seasonal influence in the aetiology of the condition. This aspect is currently being investigated and will be the subject of a future publication.

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## DIAGNOSIS OF BOVINE MASTITIS BY RADIAL IMMUNODIFFUSION\*

M. N. MORRIS\*\* AND W. B. HOBBS\*\*\*

### SUMMARY

An effective herd mastitis control program requires a diagnostic test to facilitate the selection of cows for investigation and treatment. Direct cell counting and bacteriological methods involve more labour and skill than is practical for screening purposes. Indirect methods may not be reliable in the important range of cells from 250 000 to 1 000 000 per ml. A quantitative immunodiffusion test to detect an antigen in the milk, which indicates inflammation of the mammary gland, was applied to quarter samples from 25 cows. Large precipitin rings correlated well with the presence of pathogenic bacteria in the milk. Good correlation also existed between cell counts and precipitin ring size in 10 bulk milk samples tested. The antigen is thought to be an immunoglobulin produced by lymphocytes in the udder. Strongly positive results are visible after two hours at room temperature.

The test may be performed in the milking shed by transferring milk directly from the udder to filter paper discs.

### INTRODUCTION

Mastitis is defined as an inflammation of the mammary gland. The cause may be either microbial, chemical or physical. In any event, the secretory capacity of the mammary tissue is eventually lowered and economic losses follow. Inapparent forms of mastitis are common and no single diagnostic criterion is capable of accurately revealing the state of the udder.

The best indication of mastitis currently available is an abnormal increase of somatic cells in the milk which may be determined by direct microscopic methods<sup>1</sup>. It has been

estimated<sup>2</sup>, however, that it would be necessary to make 200 smears from a single sample to obtain 95% confidence limits with milk containing  $0.5 \times 10^6$  cells per ml. Strynadka and Thornton<sup>3</sup> found that by counting 60 fields per sample, replicate samples commonly varied by 50% of the mean. The work involved makes this technique impractical for large numbers of samples and it is common practice to take only one sample and make a single smear; this results in the inherent errors of the system being enormously multiplied. In recent years electronic cell counting of milk has been introduced and Cullen<sup>4</sup> has found the variation of duplicate counts to be of the order of only 5%. This method unfortunately involves several preparatory procedures and expensive equipment. Of the indirect cytological methods, the California Mastitis Test (CMT) is generally accepted as the best. Unfortunately, the reaction can be difficult to read in the important range of cell concentrations from 250 000 to 1 000 000 and results may also be influenced by the presence of numerous micro-organisms.

Microbiological examination is a *sine qua non* in establishing the existence and nature of infection, but Giesecke<sup>5</sup> concluded that it is unreliable as the sole means of diagnosing mastitis. Simultaneous cytological information is essential.

Under certain conditions the impracticability of taking numerous quarter samples and subjecting them to cytological and microbiological procedures makes prior selection necessary for screening purposes. Schalm does not consider his CMT adequate for selection of quarters for laboratory examination. A diagnostic test giving reliable results at all levels thus has an important part

\*Patent rights held by the South African Inventors Development Corporation.

\*\*Institute for Parasitology, P.O. Box 1035, Durban.

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to play in mastitis control programmes. We propose a simple immunological test which could be adapted for use in the milking shed to satisfy this need.

#### MATERIALS AND METHODS

##### *Preparation of antiserum*

One litre of milk showing no apparent blood or pus, but containing  $0,74 \times 10^6$  cells per ml was centrifuged and the sediment was collected and washed in physiological saline. The cells were then set in a 5% polyacrylamide gel in a 10 ml hypodermic syringe. The gel was extruded through an 18 gauge needle into physiological saline and washed by repeated centrifugation. A rabbit was given a total of four subcutaneous injections, each of 2 ml of the fragmented gel, at the base of each limb. Six weeks later a single intramuscular injection of 0,5 ml of washed cellular material in Freund's complete adjuvant was given in one hind limb. Four weeks later the rabbit was bled via a marginal ear vein and serum was prepared and used directly for immunodiffusion tests.

##### *Milk*

For diagnostic purposes, whole milk was used without any further preparation. Individual quarter samples of about 5 ml were taken from 25 randomly selected cows, and bulk milk samples were taken from the tanks of 10 commercial producers; five of the latter were sour on arrival in the laboratory.

For investigational purposes the milk was processed as follows:

- a) Dilutions of reactive milk were made, using non-reactive milk as diluent.
- b) Whole milk was separated into a cellular and a cell-free fraction by gentle centrifugation.
- c) The cell-free fraction was separated into two portions. One was heated to  $100^\circ\text{C}$  for 10 minutes and one to  $56^\circ\text{C}$  for 20 minutes.
- d) Infected milk was cultured on blood agar and the bacterial growth was extracted into physiological saline.
- e) A smear of an infected, reactive milk was stained with fluorescein isothiocyanate-conjugated rabbit antiserum by conventional methods.

In addition, colostrum was collected from each quarter of an apparently healthy cow for a period of five days *post partum*, and

four specimens of randomly selected bovine serum were collected at the local abattoir.

##### *Immunodiffusion tests*

Quantitative tests were performed using a single radial diffusion technique<sup>6</sup>. The rabbit antiserum was incorporated in 1% agarose (Seravac)\* and poured onto a microscopic slide to give a slab approximately 2 mm thick. Circular wells 2 mm in diameter were punched at 1,5 cm centres and sealed with a little molten agarose. Each well was filled with milk and the slide was left horizontal at room temperature. Precipitin rings were observed using dark field oblique illumination. After 24 hours, photographs were taken from which diameters of rings were measured using a micrometer, and the areas were calculated and recorded taking the area of the wells into account. Areas over 300 units were considered definitely positive and those under 100 were negative. In one experiment the sample wells were replaced by filter paper discs 2 mm in diameter which were wet with milk direct from udder and placed on the gel.

Qualitative determinations were performed using conventional methods. The Ouchterlony double diffusion technique was used with wells 2 mm in diameter and 5 mm apart and immunoelectrophoresis was performed on microscopic slides in 0,5% agarose\* gel with pH 8,2 barbitone buffer.

##### *Cell counts*

Milk smears were made with a 0,01 ml loop according to the method of Prescott and Breed<sup>1</sup>. Up to 100 fields were counted in the investigational work, but for diagnostic screening purposes,  $3 \times 10$  fields were counted. An average of one cell per field represents a total concentration of  $0,5 \times 10^6$  cells per ml.

##### *Bacteriology*

Milk was cultured on Edwards medium for streptococci and on mannitol salt agar for staphylococci. Subsequent identifications of organisms were made by conventional methods.

Qualitative gel-diffusion using the rabbit antiserum and milk containing pathogenic bacteria and an excessive number of cells, showed a single major band and in some cases one minor band close to the serum wall. Immunoelectrophoresis showed that the major antigen migrated slightly towards the anode. Gel-diffusion tests with the fractions

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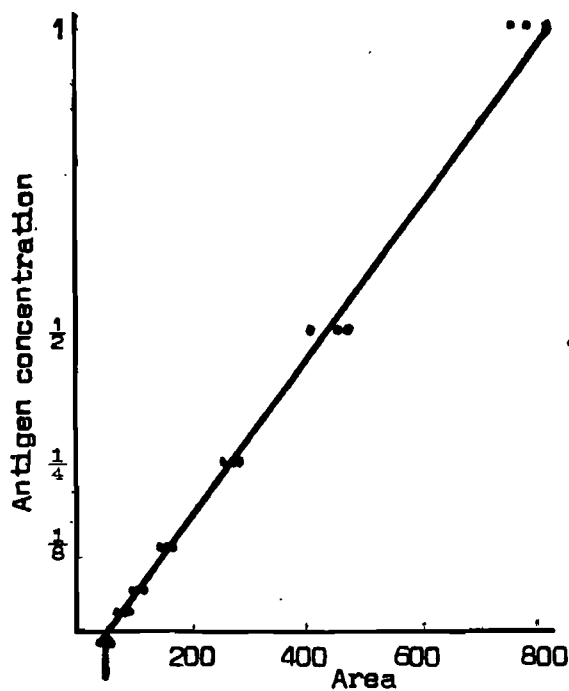


FIG 1. Relation of precipitin area to antigen concentration.

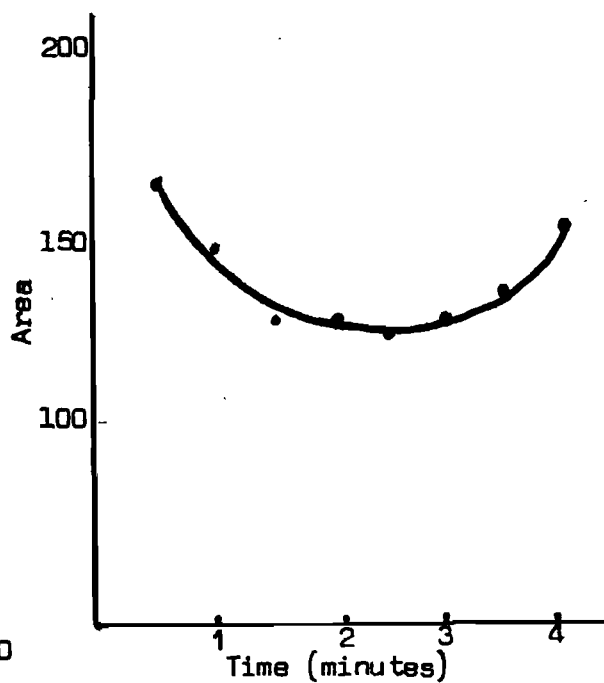


FIG 2. Areas given by serial samples during machine milking.

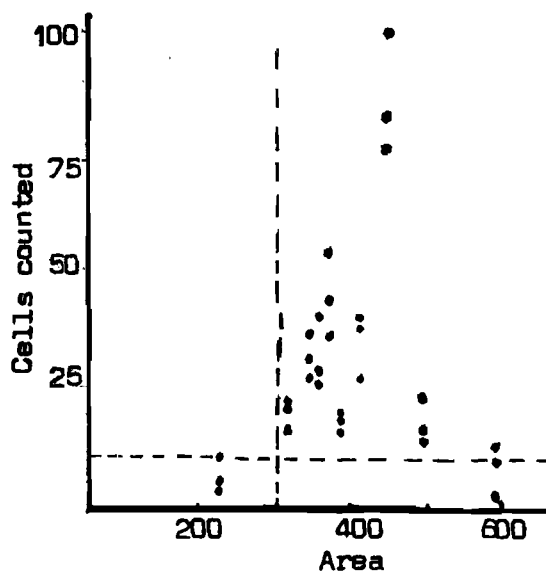


FIG 3. Areas and cell counts given by tanker samples from ten commercial producers.

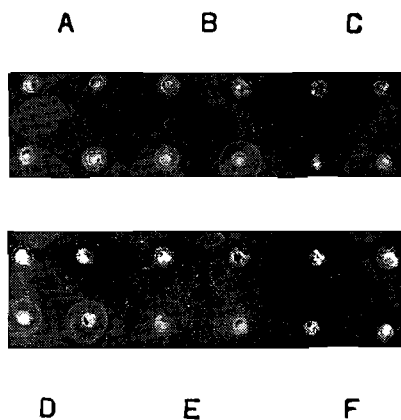


FIG 4. Immunodiffusion tests on quarter samples from six cows.



indicated that it occurred in the cell-free supernatant and that it survived heating to 56°C but not to 100°C. Fluorescent microscopy clearly showed that this antigen was associated with the bovine cells and not with the bacteria. Bovine serum and the bacterial extract were both inactive.

Strongly positive results could be obtained within 2 hours at room temperature by quantitative diffusions, using serial dilutions of whole milk. After 24 hours a direct linear relationship was reached between the area enclosed by the precipitin ring and the antigen concentration, as shown in Fig. 1, where the arrow indicates the area of the well. Each dilution was tested in triplicate. A series of milk samples taken from one quarter at 30 sec. intervals during the milking process gave the results shown in Fig. 2. Cell counts, in triplicate, and precipitin ring areas given by the 10 bulk milk samples are shown in Fig. 3.

Fig. 4 shows two typical slides with quarter samples from six cows. Cow C appears to be completely clear while cows B and D have given clearly positive reactions. Cows A, E and F each show slight activity.

A wide range of precipitin areas was found amongst the 25 cows. Rings of essentially the same size as the well were seen where the milk showed low cell counts and no bacteria. Very large rings were observed with milk containing high cell counts and known mastitis-causing bacteria. As an example of some of the relationships encountered, Table 1 shows the results with milk from the quarter which gave the largest precipitin ring from each cow. Only cow number 24 in this series suffered from visible mastitis with blood and pus in the milk. The remainder appeared healthy.

The single series of colostrum samples tested showed high cell counts and large rings throughout.

Comparative tests performed with paper gave results similar to those obtained using wells.

#### DISCUSSION

A serious shortcoming in mastitis control programmes is the difficulty of identifying precisely the extent, nature and location of inapparent mastitis infections in a herd. The cost and work involved in obtaining accurate details of cell counts and bacteria in the milk of every cow in a herd would usually be prohibitive; nevertheless, specific treatment must be applied to specific cows

if effective therapy is to be achieved and bacterial resistance is to be avoided. The results presented suggest a novel and practical approach to the selection of individual cows for detailed bacteriological attention.

The electrophoretic mobility of the major antigenic component of mastitis milk and the position of the band relative to the wells in gel diffusion, suggest that it may be an immunoglobulin. The fact that this component is found free in the milk, and that immunofluorescence indicated that it is a product of the leucocytes and not the bacteria, supports this contention. Bovine serum did not react with the antiserum, so the active factor is possibly a secretory antibody produced in the udder in response to infection. This speculation will require biochemical confirmation.

The use of agarose gels rather than agar is considered an important factor in quantitative immunodiffusion tests involving complex protein solutions, such as milk and serum, as non-specific precipitations must be avoided.

Fig. 1 shows that the repeatability of the test over the whole range of antigen concentrations is excellent. This is not true of cell counts, where the actual number of cells counted influences repeatability and hence the fewer actually counted, the more variable is the result.

Fig. 2 shows less variation through the milking process than is often found with cell counts. This is no doubt due to the fact that the antigen is a soluble protein which can diffuse away from the inflamed foci where it is produced. The result is that an assessment of the extent of immunological activity in the udder is obtained rather than a random sampling of the foci themselves, which is obtained from the corresponding cell counts.

Fig. 3 shows some correlation between precipitin ring areas and cell counts. The lack of statistical reliability in the data presented makes a rigorous relationship impossible. It must be stressed, however, that this immunodiffusion test gives a reflection of cellular *activity* rather than cell numbers and that old cells and dead cells, which would be counted, would not contribute to the ring size. In many cases the antigenic activity observed in the milk was lower than the numbers of cells counted would suggest.

Fig. 4 suggests that samples should be taken from cows B and D for bacteriological



Table 1: DIAGNOSIS OF BOVINE MASTITIS BY A RADIAL IMMODIFFUSION TEST

Cow	Area	Cells	Bacteria
1	50	nil	nil
2	50	nil	Strep.
3	80	0.02	E. coli
4	80	0.08	Staph. (c.p.)
5	110	1.06*	Staph.
6	187	0.12	Staph.
7	222	0.06	Pseudomonas
8	244	0.08	E. coli
9	248	0.26	Micrococcus
10	250	0.64*	Staph. (c.p.)
11	271	0.26	Staph. (c.p.)
12	287	0.28	nil
13	310	9.74*	Pseudomonas
14	364	2.66*	Staph. (c.p.)
15	367	0.46	Staph. (c.p.)
16	472	0.38	Staph. (c.p.)
17	472	2.00*	Staph. (c.p.)
18	475	0.70	nil
19	486	0.44	E. coli
20	640	1.06	Staph. (c.p.)
21	690	0.50	Staph. (c.p.)
22	705	2.04	Staph. (c.p.)
23	710	1.00	E. coli
24	1545	4.80	Staph. (c.p.)
25	1800	9.60	Staph. (c.p.)

AREA = Total area bounded by precipitin ring

CELLS = Cell count in millions per ml

(c.p.) = Coagulase positive

\* = Cell counts higher than would be expected from corresponding areas

investigation to determine the cause of the inflammation. Antibiograms may then indicate the drug of choice. For practical purposes positive reactions can be read without making precise measurements.

Table 1 shows the extent of unseen mastitis to be found in a well managed productive herd. Fifteen of the 25 cows were harbouring potentially pathogenic bacteria which presumably affected the over-all yield of the herd, but only cow number 25 had visible mastitis. Cows 3, 8 and 19 are interesting in that they all harboured *E. coli*, which may or may not be pathogenic. In cow 3, the other tests suggest that it was probably only present as a contaminant whereas in cow 19 there appears to be some inflammation and further bacteriology is indicated to determine whether *E. coli* was

in fact responsible for the observed reaction or not. Cow 4, on the other hand, harbours a definite mastitis-causing organism but has not reacted with an inflammatory process. In cows 12 and 18 the bacteriological results are inconclusive and further samples should be taken before deciding that these are in fact cases of aseptic mastitis.

The efficiency of this test in detecting mastitis in bulk milk makes it of value in the quality control of fresh milk supplies in the field of public health. The information gathered in this brief study suggests that an alarming level of inapparent mastitis is to be found in our commercial dairy herds.

These results show clearly the need for a rational approach to mastitis control and an acknowledgement of the limitations of the diagnostic techniques available. No single test can give all the information required, and negative bacteriological findings are certainly not conclusive.

The proposed immunodiffusion test has several inherent advantages for practical application. It is extremely simple and can be performed in the dairy as the cows are being milked if paper discs are used instead of wells: the results may be read merely by holding the slide up to a lighted window. Bacteriological findings in this limited study support the immunological diagnoses over the whole range encountered and the results appear to be more repeatable than cell counts. Further work is obviously necessary to confirm and extend this preliminary study.

#### ACKNOWLEDGEMENT

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# IMMUNITY IN *GAIGERIA PACHYSCELIS* INFESTATION

I. G. HORAK\*

## SUMMARY

Experiments are described in which single infestations with larvae of *Gaigeria pachyscelis* are used to produce immunity to subsequent infestations.

This immunity is effective once the larvae from the initial infestation have reached the lungs. It is still effective 31 days after the anthelmintic removal of the worms responsible for producing the immunity.

A previous *Dictyocaulus filaria* infestation can interfere with the establishment of a subsequent *G. pachyscelis* infestation, but the converse is not true.

## INTRODUCTION

The Sandveld hookworm, *Gaigeria pachyscelis*, and its canine adapted counterpart, *Ancylostoma caninum*, are both avid bloodsuckers<sup>1,2</sup> and consequently highly pathogenic. As few as 43 adult *G. pachyscelis* are capable of killing a sheep<sup>3</sup>, while the equivalent number of *A. caninum* is 600 to 800 worms<sup>4</sup>. It is therefore not surprising that in both these infestations immunity to reinfection plays a role, thus ensuring the survival of both host and nematode.

Considerable research has been devoted to investigating the immunity in *A. caninum* infestation<sup>4,5,6,7</sup>. But in the case of *G. pachyscelis*, although it has been stated that this parasite does not lend itself to repeated infestation as only the first larval dose is viable<sup>8</sup>, no experimental proof has been forthcoming.

The following experiments were conducted to investigate the immunity in *G. pachyscelis* infestation and to determine the effect of anthelmintic treatment on this immunity. In addition, a possible cross-immunity with *Dictyocaulus filaria* was investigated.

## MATERIALS AND METHODS

### Experiment 1

Sixteen Merino sheep, consisting of eight ewes and eight wethers, previously either naturally or artificially infested with nematodes, excluding *G. pachyscelis*, were treated with thiabendazole\*\* at 88 mg/kg liveweight. Thereafter they were confined in brick-floored pens under worm-free conditions.

The sheep were divided into four groups, each group consisting of two ewes and two wethers. They were infested, treated with thiabendazole at 200 mg/kg liveweight, reinfested and slaughtered according to the experimental design summarized in Table 1.

The initial infestation was applied to the skin in the right axillary region with the sheep lying on its side; the challenge infestation was similarly applied to the left side.

At slaughter the lungs were opened by cutting along the trachea and bronchi as far as possible. The lungs were then cut into cubes approximately one centimetre square and processed as for *D. filaria* recovery in a water bath<sup>9</sup>. The contents of the small intestine were also processed in the water bath, using slightly modified techniques to those already described<sup>10,11</sup>, while the contents of the large intestine were washed on a sieve with 150  $\mu$ m apertures.

Total microscopic worm counts were done on the lung and small intestinal filtrates, while one to four 1/10th aliquots of the small intestinal residue and large intestinal ingesta were examined microscopically. The remainder was examined macroscopically on a white background, after staining the intestinal contents with a concentrated iodine solution.

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\*\*THIBENZOLE, Reg. trade mark of MSD (PTY) LTD, Merck Sharp & Dohme International, Division of Merck & Co., Inc., Rahway, N.J., U.S.A.



Table 1: EXPERIMENTAL DESIGNS  
Groups of Sheep, Infestation, Treatment and Slaughter

#### Experiment 1

Day	Control I	Control II	Immunized I	Immunized II
0	250 larvae	—	250 larvae	250 larvae
28	—	—	—	Anthelmintic
59	—	540 larvae	540 larvae	540 larvae
77—94	Slaughter	Slaughter	Slaughter	Slaughter

#### Experiment 2

Day	Control I	Control II	Immunized I	Immunized II	Immunized III
0	400 larvae	—	400 larvae	400 larvae	400 larvae
2	—	—	Anthelmintic	—	—
7	—	—	—	Anthelmintic	—
14	—	—	—	—	Anthelmintic
28	—	380 larvae	380 larvae	380 larvae	380 larvae
65	Slaughter	Slaughter	Slaughter	Slaughter	Slaughter

#### Experiment 2

Fifteen Merino ewes naturally infested with nematodes other than *G. pachyscelis* were each treated with thiabendazole at 88 mg/kg liveweight and confined to brick-floored pens under worm-free conditions.

The sheep were divided into five numerically equal groups, infested, treated with thiabendazole at 200 mg/kg liveweight, reinfested and slaughtered as summarized in the experimental design in Table 1.

The initial infestation was applied to a shaved, moistened patch between the shoulder blades and the subsequent infestation to a similar patch on the rump.

At slaughter the small intestinal ingesta were processed in a water bath and the large intestinal ingesta washed on a sieve with 150  $\mu$ m apertures. Small intestinal filtrates were examined microscopically *in toto*, while two 1/10th or 1/20th aliquots of the small intestinal residue and large intestinal contents were examined microscopically and the remainder macroscopically.

#### Experiment 3

This experiment was conducted to verify the results obtained in one sheep in Experiment 1 in which it appeared that a previous infestation with *D. filaria* had interfered with the subsequent establishment of the *G. pachyscelis* infestation.

Two sheep previously infested with *D. filaria* and two sheep previously infested

with *Nematodirus spathiger* were used in this experiment. The experimental design is summarized in Table 1.

At slaughter the lungs were processed in a water bath and the small intestinal ingesta washed on a sieve with 150  $\mu$ m apertures. The lung filtrates and one 1/10th aliquot of the small intestinal ingesta were examined microscopically and the remainder of the ingesta macroscopically.

#### Experiment 3

Day	Group I	Group II
0	16 000 <i>N. spathiger</i> larvae	—
35	—	900 <i>D. filaria</i> larvae
174	525 <i>G. pachyscelis</i> larvae	525 <i>G. pachyscelis</i> larvae
208	Slaughter	Slaughter

#### Experiment 4

Day	Group I	Group II
15—39	450 <i>G. pachyscelis</i> larvae	85 to 105 <i>D. filaria</i> every third day
22—29	85 to 110 <i>D. filaria</i> every day	—
51—56	Slaughter	Slaughter



#### Experiment 4

Then Dorper sheep, approximately eight months old, were each infested with 450 larvae of *G. pachyscelis*; 22 days later they were each infested with 85 to 110 *D. filaria* larvae on eight consecutive days. Ten Dorper sheep of similar age were each infested with 85 to 105 *D. filaria* larvae at three day intervals on nine occasions. The experimental design is summarized in Table 1.

Infestation with *G. pachyscelis* was achieved by placing the sheep on its back and swabbing the right axillary region with moistened cotton wool before applying the larvae in 0.5 ml of water to the axillary fossa. *Dictyocaulus filaria* larvae were administered on filter paper in gelatine capsules and larvae from the same batch were administered to both groups of sheep, Group I receiving a total of 785 larvae and Group II 870 larvae.

At slaughter the lungs and the intestinal and thoracic lymph nodes were processed in a water bath and the filtrates sieved on a sieve with 37  $\mu$ m apertures. The abomasal, small and large intestinal ingesta of those sheep infested with *G. pachyscelis* were washed separately on a sieve with 150  $\mu$ m apertures. The lung filtrates were examined microscopically and one 1/5th aliquot of the abomasal and large intestinal ingesta and the total small intestinal ingesta macroscopically.

#### RESULTS

##### Experiment 1

The results are summarized in Table 2.

At slaughter, the worms resulting from the initial infestation were adult and could thus be differentiated easily from the fourth or early fifth stage worms originating from the challenge infestation.

The sheep which received only the immunizing infestation had burdens varying between six and 47 worms. Those which received only the challenge infestation had 10 to 132 worms. The sheep in the latter group which had only 10 worms also harboured a *D. filaria* infestation of unknown origin. As this might have interfered with the establishment of the *G. pachyscelis* infestation, Experiment 3 was subsequently devised.

Burdens in the sheep which were immunized and then challenged, varied between 20 and 61 worms for the initial in-

Table 2: Experiment 1. WORM BURDENS

Sheep No.	Worms recovered from immunizing infestation	Worms recovered from challenge infestation
<b>Controls of immunization</b>		
1	47	—
2	39	—
3	6	—
4	23	—
<b>Controls of challenge</b>		
5*	—	10
6	—	70
7	—	132
8	—	48
<b>Immunization and challenge</b>		
9	61	1
10	20	0
11	58	1
12	22	0
<b>Immunization, treatment and challenge</b>		
13	0	3
14	0	0
15	0	0
16	0	1

\*Infested with *D. filaria*.

festation and nought and one worm for the challenge infestation.

In the sheep treated with thiabendazole after the initial infestation, no worms from this infestation were recovered, while the challenge infestation 31 days after treatment resulted in burdens varying between nought and three worms. The one worm in Sheep 16 was a third stage larvae recovered from the lungs.

##### Experiment 2

The results are summarized in Table 3.

Burdens in the sheep receiving only the immunizing infestation, varied between 22 and 38 worms, and those in the sheep receiving only the challenge infestation varied between 25 and 51 worms.

No worms from the immunizing infestation were recovered from the sheep that had been treated.

The challenge burdens in the three sheep treated two days after immunization were nought, three and 41 worms. In those sheep treated after seven days, two sheep



Table 3: Experiment 2. WORM BURDENS

Sheep No.	Worms recovered from immunizing infestation	Worms recovered from challenge infestation
<b>Controls of immunization</b>		
17	34	—
18	22	—
19	38	—
<b>Controls of challenge</b>		
20	—	37
21	—	25
22	—	51
<b>Immunized and treated after two days</b>		
23	0	0
24	0	3
25	0	41
<b>Immunized and treated after seven days</b>		
26	0	11
27	0	0
28	0	0
<b>Immunized and treated after fourteen days</b>		
29	0	0
30	0	0
31	0	3

had no worms and one 11 worms, while amongst the sheep treated after 14 days two sheep had no worms and one had three worms resulting from the challenge infestation.

#### Experiment 3

The results are summarized in Table 4.

Table 4: Experiment 3. WORM BURDENS

Sheep No.	Worms recovered from the initial infestations		Number of <i>G. pachyscelis</i> recovered
32	0	<i>D. filaria</i>	17
33	0	<i>D. filaria</i>	0
34	0	<i>N. spathiger</i>	107
35	42	<i>N. spathiger</i>	53

The sheep which had previously been infested with *D. filaria* harboured no and 17 *G. pachyscelis*. Those that had been infested with *N. spathiger* had 53 and 107 *G. pachyscelis*.

#### Experiment 4

The worm burdens are given in Table 5.

Table 5: Experiment 4. WORM BURDENS

GROUP I			GROUP II	
Sheep No.	No. of <i>G. pachyscelis</i> recovered	No. of <i>D. filaria</i> recovered	Sheep No.	No. of <i>D. filaria</i> recovered
36	148	202	46	64
37	183	218	47	83
38	187	273	48	125
39	142	274	49	142
40	150	281	50	188
41	132	291	51	197
42	174	302	52	263
43	105	311	53	290
44	129	316	54	324
45	196	695	55	338

The sheep infested with *G. pachyscelis* followed by *D. filaria* harboured between 105 and 196 of the former and 202 to 695 worms of the latter species. Those only infested with *D. filaria* had burdens ranging between 64 and 338 worms.

#### DISCUSSION

An initial infestation with *G. pachyscelis* results in an immune response which is capable of preventing the subsequent establishment of worms of this species. Anthelmintic removal of the worms resulting from the initial infestation does not interfere with this immunity, provided that this treatment is instituted only after the worms have reached the lungs or small intestine.

Within the limits of the present experiments, this immunity was effective between 28 and 59 days after initial infestation and for at least 31 days after the anthelmintic removal of the worms responsible for the development of immunity.

The treatment intervals in Experiment 2 are based on the various phases of the life cycle of *G. pachyscelis* as described by Ortlepp<sup>12</sup>. After percutaneous infestation the worms reach the lungs, probably via the bloodstream, on the fourth day; they undergo the third moult and then migrate via the trachea to the pharynx and are swallowed and reach the small intestine on the 13th or 14th day after infestation. Treatment at two days halted infestation just after the cutaneous phase, at seven days during the



lung phase and at fourteen days just after the commencement of the intestinal phase of the life cycle. The results in this experiment indicate that the longer the immunizing infestation is allowed to persist in the host, the more complete is the immunity to reinfestation.

When the pulmonary tissues have been sensitized by an earlier infestation with *D. filaria*, an immunity, presumably to the pulmonary phase of a subsequent infestation with *G. pachyscelis*, develops. Although this immunity is not as effective as that produced by a previous infestation with *G. pachyscelis*, it is nevertheless capable of greatly reducing the number of worms that eventually reach the small intestine.

Peculiarly enough, the converse is not true, as evidenced in Experiment 4 where the burdens of *D. filaria* in the sheep previously infested with *G. pachyscelis* were actually greater than in those sheep not so infested. The worm burdens in the two groups of sheep in this experiment are not strictly comparable because of the differing periods of infestation, but the results show clearly that no immunity to the establishment of *D. filaria* developed after infestation with 450 larvae of *G. pachyscelis*.

The lower takes of *D. filaria* in the group infested only with this species are possibly due to a resistance to repeated reinfestation developing during the more prolonged period

of infestation when compared with the other group.

In South Africa it is most unlikely that *G. pachyscelis* and *D. filaria* would occur naturally in the same sheep. The former species is confined to the arid and semi-arid western half of the country<sup>12</sup>, whereas the latter occurs along the southern and eastern coast spreading into the eastern interior<sup>9</sup>. Thus in this country the cross-immunity between these worms is of little epizootiological significance.

The poor takes of *G. pachyscelis* in the first three experiments were due to a faulty infestation technique, this was eliminated in the fourth experiment and resulted in markedly improved takes.

The results indicate that multiple infestations with *G. pachyscelis*, or mixed infestations in which *D. filaria* is given first, cannot be employed in anthelmintic tests or lifecycle studies in which the number of larvae used for infestation and the number of worms recovered subsequently are of importance.

The efficacy of the immunity and the rapidity with which it develops are probably of considerable importance in ensuring the continuance of the host-parasite relationship between *G. pachyscelis* and its ovine host.

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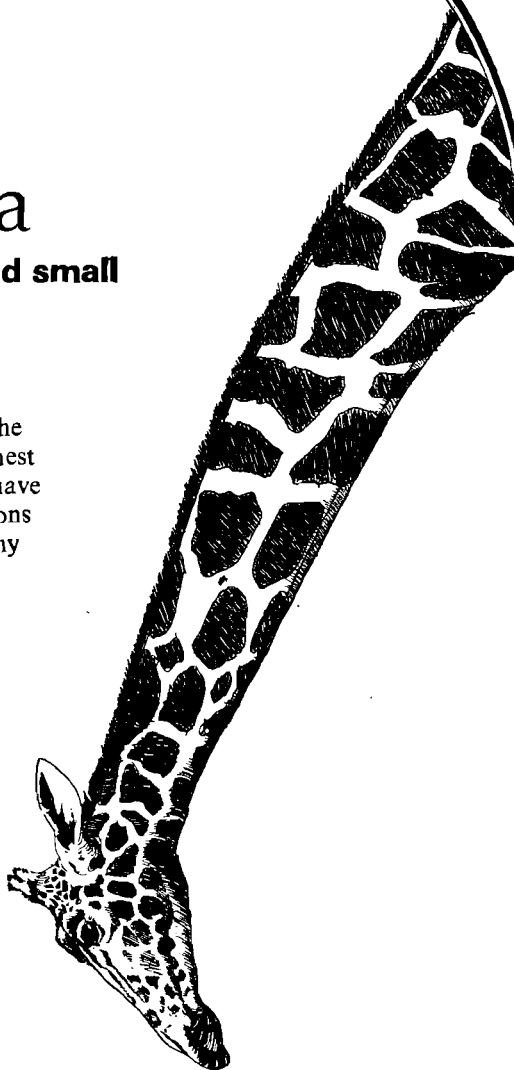


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# LOW LEVEL THIABENDAZOLE ADMINISTRATION TO SHEEP

## 1. SUSCEPTIBILITY OF MEDICATED SHEEP TO NATURAL INFESTATIONS AT OUTENIQUA

A. J. SNIJDERS\*, J. H. STAPELBERG\*\* AND G. L. MULLER\*\*\*

### SUMMARY

Sheep were removed periodically from a flock receiving continuous low level treatment with thiabendazole. This treatment was withheld and they were exposed to natural infestation with sheep which received anthelmintic treatment only when they exhibited clinical signs. The worm burdens in these two groups of sheep were compared at various intervals.

The worm burdens in the sheep previously removed from low level treatment generally exceeded those of the other sheep. This increased susceptibility was outweighed by the increase in productivity, in terms of weight gain, during the period of such treatment.

### INTRODUCTION

Treatment of sheep by drenching with anthelmintics to control worms is used throughout the world. If the treated sheep are then placed on pastures that have been rested for three to four months, the chances of reinfestation are reduced but not eliminated. The infestation can be further reduced by allowing the flock free access to low level treatment with suitable anthelmintics after a full therapeutic dose.

The main effects of licks containing phenothiazine are the reduction of pasture contamination which follows a decrease of the faecal egg count of treated sheep and the inhibition of larval development in the faeces. The use of a phenothiazine medicated mineral lick is particularly successful in the control of *Haemonchus contortus* but is not as successful in the control of other species<sup>1,2,3,4</sup>. With the exception of *Trichuris* spp., thiabendazole†, either in a feed sup-

plement or mineral lick, is highly successful in keeping infestation by all gastro-intestinal nematodes at a very low level<sup>3,4,5,6,7,8</sup>. We have shown that the worm burdens in sheep on low level thiabendazole are markedly reduced when compared with sheep on identical grazing, which are either on low level phenothiazine or treated with thiabendazole at six-weekly intervals<sup>4</sup>.

It has been suggested that immunity to nematodes is dependent on continual exposure to infestation<sup>9,10,11,12</sup>. It was expected that this mechanism would be interfered with by low level treatment, as the end result would be the reduction of infestation to such a degree that sheep would be practically worm free.

The results of an investigation on the susceptibility to nematode infestation of lambs with free access to mineral licks medicated with thiabendazole, are reported. Simultaneously, the effect of treatment, as necessitated by clinical signs of nematode infestation, on lambs with access to non-medicated licks, is given. The latter group was regarded as a control.

### MATERIALS AND METHODS

#### Location and Climate

The experiment was carried out at the Outeniqua Experimental Station (latitude 22° 25', longitude 34° 0') near George, in the Cape Province. The rainfall is non-seasonal and averages 820 mm *per annum*. June and July are usually the driest months. The climate is temperate and frosts are rare.

Rainfall and temperatures were recorded throughout the experiment.

#### Sheep

Eighty adult Merino ewes from an

\*MSD (PTY) LTD, P.O. Box 7748, Johannesburg.

\*\*Elsenburg Experimental Farm, Stellenbosch, P.O. Box 3010, Stellenbosch.

†THIBENZOLE, Reg. trade mark of MSD (PTY.) LTD, Merck Sharp & Dohme International, Division of Merck & Co. Inc., Rahway, N.J., U.S.A.



adjacent district were introduced and, together with ten German Merino ewes, were indentified by numbered eartags and divided at random into two comparable groups, each consisting of forty Merino and five German Merino ewes, on 2 November, 1964.

The sheep in one group (T) were each treated with 2.8 g thiabendazole, while those in the other group (C) each received 21 g of micronized phenothiazine.

#### Mineral Licks

Group T had free access to the following medicated lick:

- 40 lb Salt
- 40 lb Bonemeal
- 10 lb Slaked lime
- 5 lb Flowers of Sulphur
- 4 oz Magnesium sulphate
- 1 oz Cobalt chloride
- 8 oz Copper sulphate
- 2 oz Manganese sulphate
- 2 lb Thiabendazole
- 2 lb Calcium phosphate (as carrier for the thiabendazole in a "premix")

assaying at 20 mg thiabendazole per gram of lick.

The lick supplied to Group C was identical to the one above but did not contain the "premix", i.e. neither thiabendazole nor calcium phosphate. The total amount of lick consumed by the two groups was recorded during the experiment.

The sheep in Group C were each treated with 21 g of micronized phenothiazine on 11 March and 11 June, 1965.

#### Husbandry

The groups were separated and penned at night to protect them from marauding dogs. On 7 December, 1964, thirty-five days after commencement of the experiment, the two groups, which had grazed together during the day, were separated, placed in different paddocks and one Merino ram was placed with each group. The rams were alternated between the two groups until their removal 32 days later, on 8 January, 1965. The ram running with Group T had access to the medicated lick at night. From 8 January until 15 February, 1965, the ewes grazed together during the day. Thereafter they were completely separated.

Lambing commenced on 3 May, i.e. 147 days after introduction of the rams, and

continued until 29 May, i.e. 141 days after removal of the rams. The birth weights of the lambs were recorded and the lambs were identified according to birth sequence and their dams' group (Group T<sub>1</sub> and C<sub>1</sub>). The lambs were weighed periodically and weaned on 23 August, 1965, when they were on an average 100 days old.

Both groups were treated at regular intervals with niclosamide\* for tapeworms and hexachlorophenol† for liver fluke, and received routine inoculations.

#### Grazing

**Group T and T<sub>1</sub>:** Separate paddocks were provided for the exclusive use of this group (ewes and their lambs) from the 105th day of commencement of the experiment, namely from 15 February, 1965. The grazing consisted of oats (*Avena sativa*), lucerne (*Medicago sativa*), rye-grass and clover (*Lolium perennae* and *Trifolium repens*) and Kikuyu grass (*Pennisetum clandestinum*). At weaning the ewes were removed, while the lambs remained on these paddocks. The stocking rate did not exceed 7–8 sheep per ha.

**Group C and C<sub>1</sub>:** The ewes and lambs grazed with the farm flock on teff (*Eragrostis teff*), *Setaria* spp., cocksfoot (*Dactylis glomerata*) and clover, Kikuyu and babala (*Pennisetum* sp.)

The ewes were removed at weaning on 23 August, 1965, and the lambs grazed with other lambs on the farm at a stocking rate of 3½ to 5 sheep per ha.

#### Experimental Procedure

From seven days of birth of the first lamb in Group T<sub>1</sub>, each lamb in this group was dosed daily for 53 days with 5 g of medicated lick (100 mg thiabendazole). Five lambs were selected at random every eight weeks and exposed to natural infestation by transfer to the camp grazed by lambs of Group C<sub>1</sub>. After transfer they received no medicated licks. These particular animals were slaughtered 12 weeks later as follows:

Selection and Exposure Date	Slaughter Date
1 September, 1965	24 November, 1965
27 October, 1965	19 January, 1966
22 December, 1965	14 March, 1966
16 February, 1966	16 May, 1966
13 April, 1966	5 July, 1966
8 June, 1966	31 August, 1966

\*LINTEX, Reg. trade mark of Bayer Agro-Chem (Pty.) Ltd., P.O. Box 1366, Johannesburg.

†BIS 356 Reg. trade mark of Cooper & Nephews S.A. (Pty.) Ltd., P.O. Box 2963, Johannesburg.



On the selection dates, groups of five lambs from Group C<sub>1</sub> were identified and slaughtered with the five lambs from T<sub>1</sub>.

On 10 October, 1965, the lambs in Group C<sub>1</sub> were each treated with 10 g phenothiazine. On 4 March, 1966, the remaining lambs in this group as well as the ten T<sub>1</sub> lambs running with them were each treated with 2 g thiabendazole.

#### *Infested Merino Lambs (Group M)*

A group of 25 naturally infested weaned Merino lambs were acquired and grazed with control animals (Group C<sub>1</sub> and exposed lambs from Group T<sub>1</sub>) from 7 February, 1966 until the conclusion of the trial. These lambs were used to seed the pastures with nematode eggs and, because of clinical signs of helminthiasis, had of necessity to be treated with 10 g and 12 g of micronized phenothiazine on 16 March and 14 July, 1966, respectively.

#### *Faecal Worm Egg Counts*

Faecal worm egg counts, using a modified McMaster technique, were made each month as follows:

Groups T and C from November, 1964 to August, 1965;

Groups T<sub>1</sub> and C<sub>1</sub> from September, 1965 to August, 1966, and

Group M from April to August, 1966.

#### *Autopsy Procedures*

Before slaughter, sheep were starved overnight and autopsies and worm counts carried out as described<sup>13,14</sup>. Only the abomasum and small intestine were digested and the digestion was completed in 8 hours at 45°C instead of 12 hours at 37.5°C.

#### *Parasite Identification*

Species differentiation was carried out on at least 100 adult male worms per genus, or on all males, if present in moderate numbers only, and classified according to Yamaguti<sup>15</sup>. Larvae were identified as described by various workers correlated in a laboratory manual<sup>16</sup> and each moult classified with the preceding stage. Immature adult worms were classified as fifth stage worms.

#### RESULTS

##### *Rainfall and Temperature (Table 1)*

These data are summarized in Table 1 and presented graphically in Figs 2 to 4. From May, 1965, to 31 August, 1966, 789 mm of rainfall was recorded, compared to an average of 901 mm for the previous ten years. Seven out of 16 months were drier than average, while average rainfall was recorded for six months only. The two driest months were June (6 mm) and July, 1966 (8 mm) and the coldest months were from June to August in both years.

Table 1: RAINFALL AND TEMPERATURES

Period	Rainfall mm	No. of days on which rain fell	Mean rainfall (1957-1966) mm	Mean max. temp. °C	Mean min. temp. °C	Mean monthly temp. °C
November	40	4	54	—	—	—
December	16	2	64	—	—	—
1965						
January	25	3	61	—	—	—
February	57	3	45	—	—	—
March	18	7	114	—	—	—
April	37	6	50	—	—	—
May	76	5	59	21	11	16
June	20	3	32	19	8	13.5
July	34	3	29	19	11	15
August	13	3	69	21	10	15.5
September	21	4	53	22	11	16.5
October	152	8	81	22	12	17
November	122	8	54	23	13	18
December	74	3	64	24	14	19
1966						
January	52	5	61	26	17	21.5
February	41	8	45	25	15	20
March	13	2	114	27	16	21.5
April	31	7	50	24	14	19
May	54	6	59	21	12	16.5
June	6	2	32	21	9	15
July	8	2	29	19	9	14
August	72	8	69	19	9	14



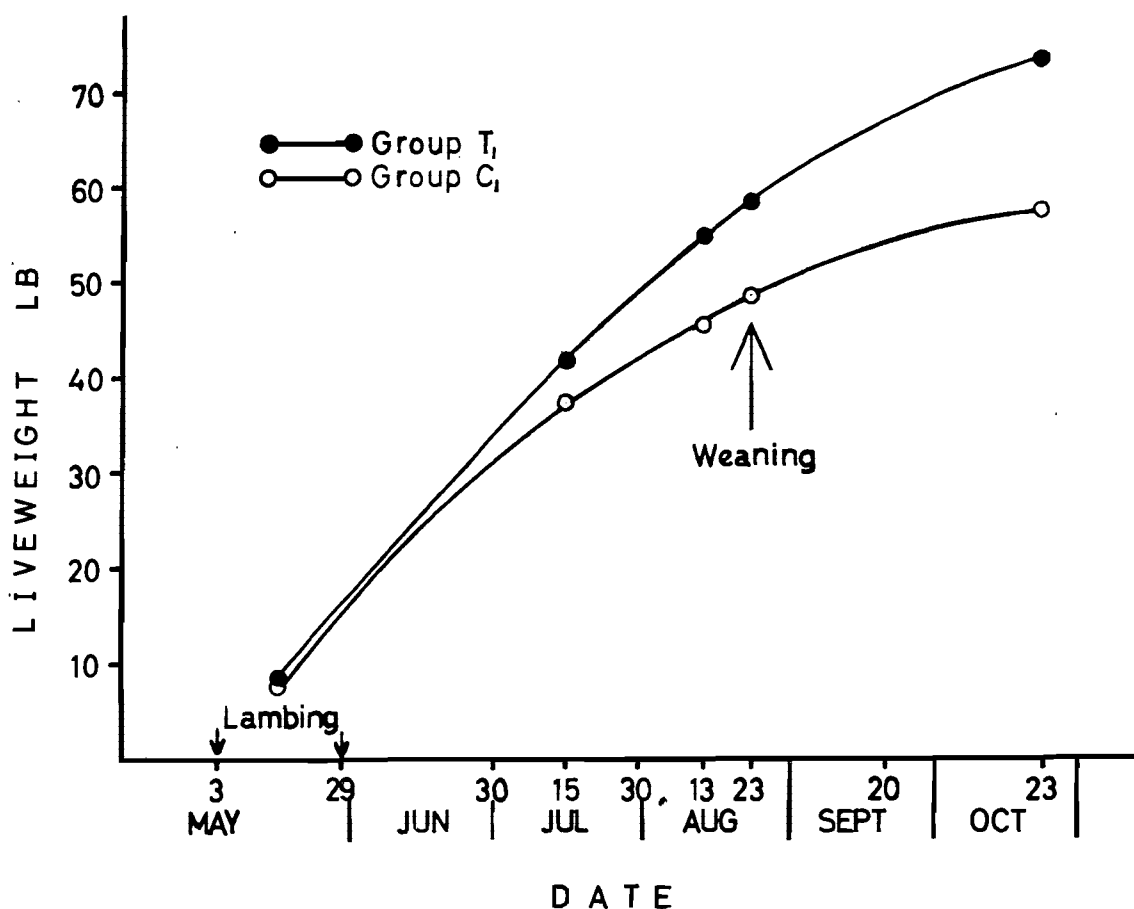


FIG. 1. Liveweights from birth to post-weaning of treated ( $T_1$ ) and control ( $C_1$ ) lambs.

#### Lambing Percentage (Table 1)

**Group T:** Of 45 ewes mated, 30 lambed with three twins, yielding 32 live lambs, i.e. an overall lambing percentage of 71%. Thirty one lambs were weaned.

**Group C:** Of 45 ewes mated, 33 lambed, producing 35 lambs (one dead) with two twins, i.e. a lambing percentage of 78%.

Thirty two lambs were weaned.

#### Quantity of Mineral Lick Consumed (Table 2)

The consumption was determined by weighing the amount of residual lick when it was replenished from time to time. It was calculated that each lamb in Group  $T_1$

Table 2: LICK CONSUMED BY EXPERIMENTAL ANIMALS

Group	Period	Lick in kg	Thiabendazole in kg
T	2 November 1964 to 3 May 1965 183 days	150	3,0
C		147,2	—
T+T <sub>1</sub>	4 May 1965 to 23 August 1965 113 days	131,8 + 9,3*	2,636 + 0,186*
C+C <sub>1</sub>		134,1	—
T <sub>1</sub>	24 August 1965 to 18 June 1966 299 days	138,6	2,772
C <sub>1</sub> **		169,1	—

\*Amount administered orally 14 May—2 July, 1965

\*\*Including exposed Group T<sub>1</sub> and Group M—From 7 February, 1966



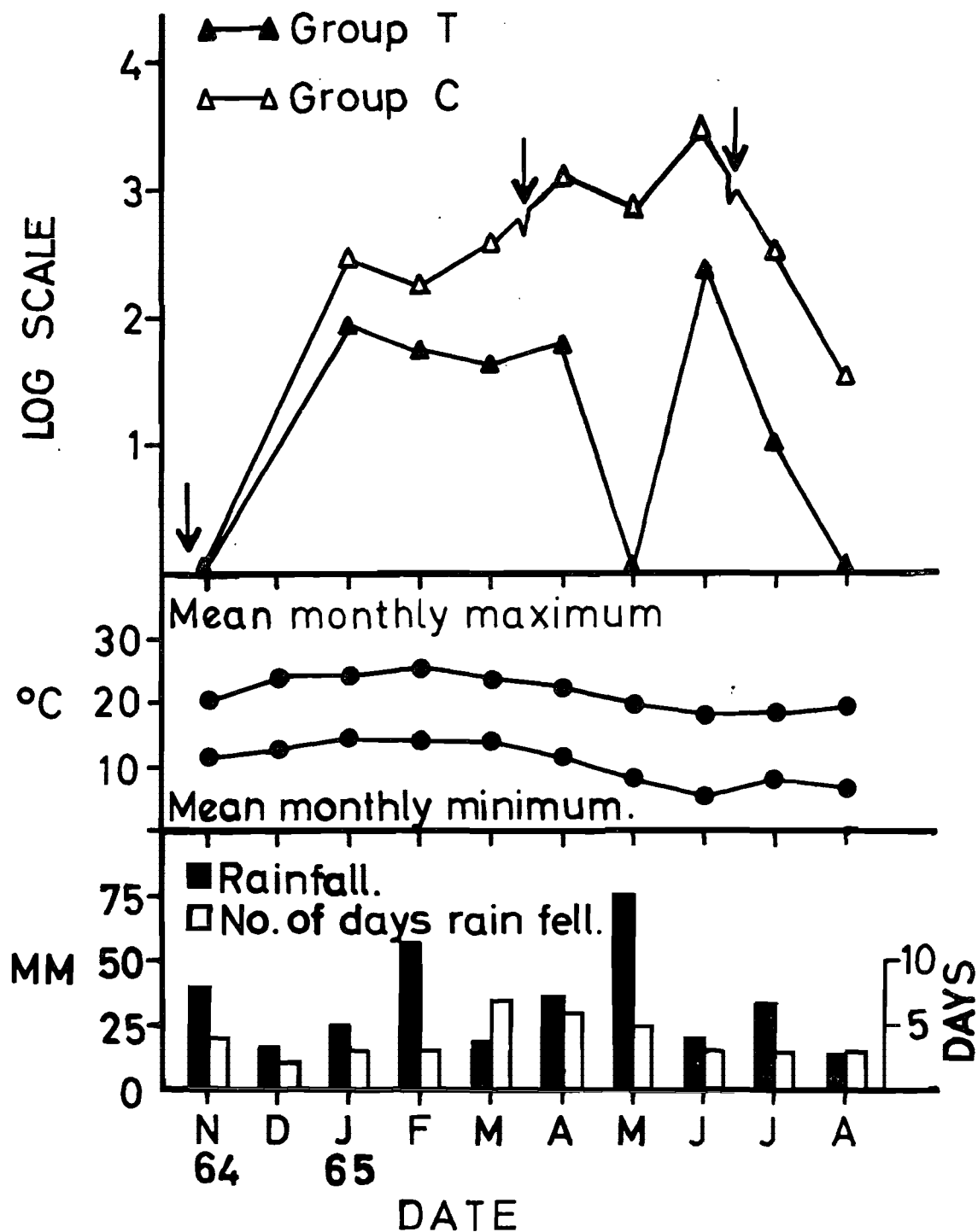


FIG. 2. The faecal worm egg counts of treated (T) and control (C) parent stock. Vertical arrows indicate dates of anthelmintic treatment. First arrow: thiabendazole for both groups. Second and third arrows: Phenothiazine to control groups only.



from weaning (23 August, 1965) until 18 June, 1966, consumed a daily average of 28 g lick containing 650 mg thiabendazole.

#### Faecal Worm Egg Counts (Figs 2 and 3)

**Group T and C:** After treatment of both groups on 2 November, 1964, the ewes had low worm egg counts in December. Egg counts started rising in Group C in February, 1965, as shown by the March examination, yet despite treatment with phenothiazine on 11 March, rose to a peak later in March followed by a drop in April and a steep rise early in June. Treatment with phenothiazine on 11 June was followed by a sharp drop in July and August after which no further counts were carried out.

During this period egg counts were markedly lower in Group T, although the same pattern was followed. Only four of the 45 ewes had positive counts.

**Groups T<sub>1</sub>, C<sub>1</sub> and M:** Egg counts were negative in Group T<sub>1</sub> throughout the experiment, with the exception of one lamb with a count of 33 eggs per gram (epg). The average egg counts in lambs of Group T<sub>1</sub> after exposure to infestation together with Group C<sub>1</sub> are not reflected in the graph, because the group was comparatively small; their egg counts, however, never exceeded those of Group C<sub>1</sub>.

Group C<sub>1</sub> was treated with phenothiazine on 10 August, 1965, before worm egg counts were carried out.

Moderate egg counts were recorded until late February, 1966 when all lambs had positive counts ranging from 166 to 2666 epg, averaging 881 epg. Treatment with thiabendazole on 4 March was followed by a negative egg count in April, 1966, followed by very low counts until July, rising in August.

The faecal worm egg counts of Group M rose from April to June, 1966, followed by a decline until August. These lambs were treated with phenothiazine on 16 March and 14 July, 1966.

#### Worm Counts at Autopsy

The worm counts of numerically dominant genera are summarized in Table 3 and illustrated in Fig 4. Six groups of lambs were slaughtered during the experimental period. The highest total worm counts were recorded from lambs slaughtered on 29 November, 1965 and 19 January, 1966 (summer).

This was followed by markedly lower counts on 14 March and 16 May to increase again on 5 July and 30 August (winter).

The following species were identified:

*Ostertagia circumcincta* (Stadelmann, 1894)  
*Ostertagia trifurcata* Ransom, 1907  
*Haemonchus contortus* (Rudolphi, 1803)  
*Marshallagia marshalli* Orloff, 1933  
*Trichostrongylus axei* (Cobbold, 1879)  
*Trichostrongylus colubriformis* (Giles, 1892)  
*Trichostrongylus pieterseii* le Roux, 1932  
*Trichostrongylus vitrinus* Looss, 1905  
*Trichostrongylus falculatus* Ransom, 1911  
*Nematodirus filicollis* (Rudolphi, 1802)  
*Nematodirus spathiger* (Railliet, 1896)  
*Cooperia curticei* (Railliet, 1893)  
*Chabertia ovina* (Gmelin, 1790)  
*Oesophagostomum venulosum* (Rudolphi, 1809)  
*Oesophagostomum columbianum* (Curtice, 1890)  
*Dictyocaulus filaria* (Rudolphi, 1809)  
*Muellerius capillaris* (Müller, 1889)

The species of the genus *Trichuris* were not identified.

During the experiment, *T. colubriformis*, *O. circumcincta* and *N. filicollis* were most numerous.

#### Variations in Worm Burdens

Lambs were heavily infested in both groups as reflected by the worm burdens of those slaughtered in November and January (Table 3).

The clinical condition of the remaining lambs of Group C<sub>1</sub> had deteriorated to such an extent that treatment was essential to prevent mortalities. Accordingly on 4 March, 1966, both the lambs in Group C<sub>1</sub> and the two groups of lambs from Group T<sub>1</sub> grazing together with them were treated with thiabendazole. Lambs from Group M however, were not treated, as their function was to maintain stocking density and contaminate the pasture. Some of the latter lambs died but no autopsies were carried out.

The treatment with thiabendazole (Group C<sub>1</sub> and T<sub>1</sub>) combined with unseasonal drought (Table 1) reduced the number of infective larvae available on the grazing. This was reflected in the small worm burdens of the sheep slaughtered from March until July.



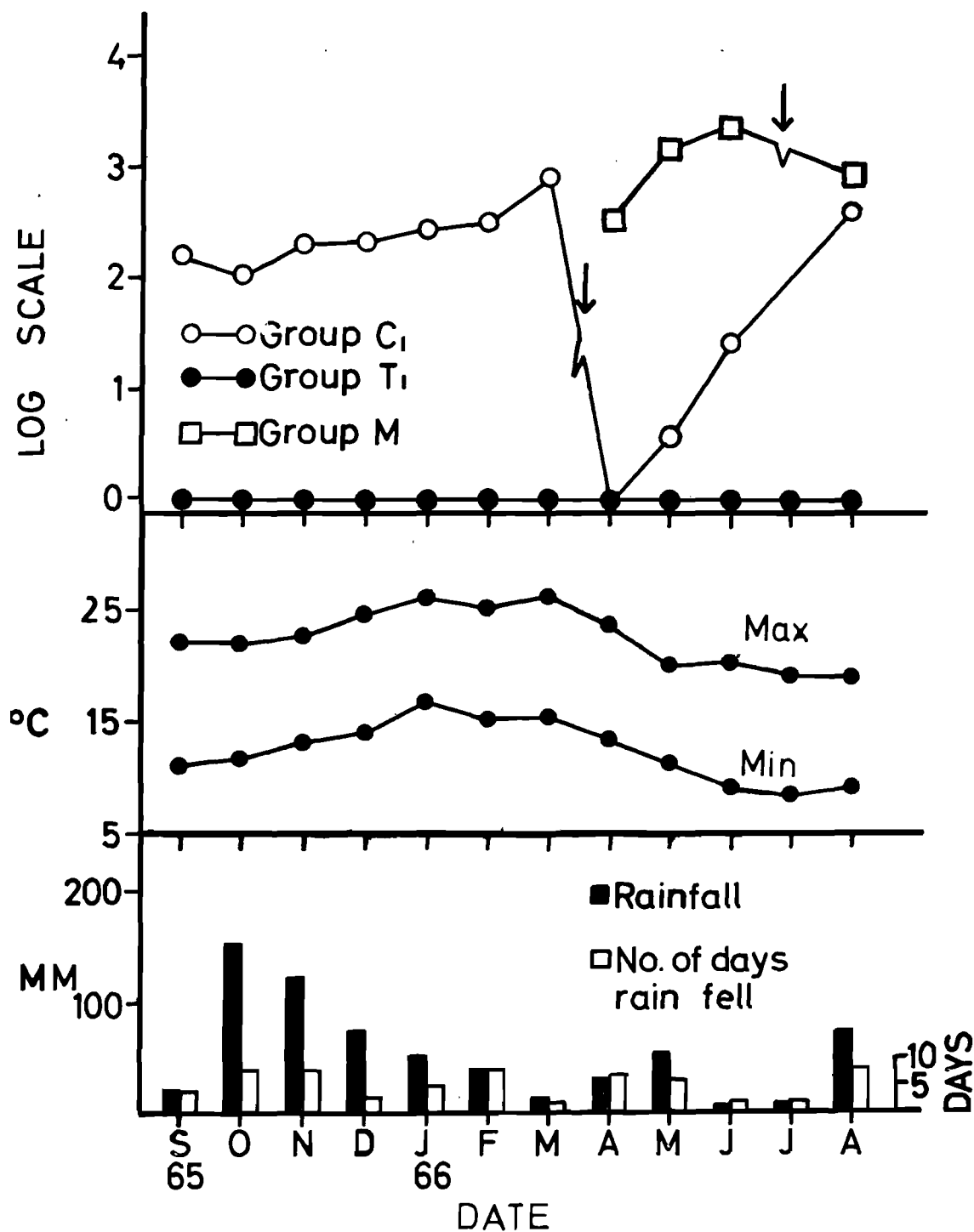


FIG. 3. The faecal worm egg counts of treated (T<sub>1</sub>), control (C<sub>1</sub>) and seeder (M) lambs. Vertical arrows indicate dates of anthelmintic treatment. First arrow: thiabendazole administered to Group C. Second arrow: phenothiazine to Group M.



Table 3: WORMS RECOVERED POST MORTEM

Slaughter dates	Group and Number	Ostertagia spp		Trichostrongylus spp		Nematodirus spp		Cooperia spp		Marshallagia spp		C. ovina		Trichuris spp		O. venulosum		Haemonchus spp	
		4th	Adult	4th	Adult	4th	Adult	4th	Adult	4th	Adult	4th	Adult	4th	Adult	4th	Adult	4th	Adult
24/11/65	T2	6 105	580	153	3 691	1 916	480	—	—	—	—	—	6	—	14	51	42	—	—
	T14	73 560	222	133	10 935	6 110	498	133	—	—	—	—	6	—	19	—	95	—	—
	T15*	11 560	6 254	179	3 620	—	1 400	—	—	—	—	—	—	—	—	—	410	172	72
	T20	66 903	980	172	5 703	6 707	960	—	390	—	—	—	7	—	12	—	57	—	—
	T28	36 400	1 159	411	3 140	1 890	203	—	66	—	14	—	4	—	23	—	25	1 077	—
	Mean	38 906	1 845	210	5 418	3 325	708	26	91	—	3	—	5	—	14	10	126	250	14
	C2	22 504	1 557	—	3 900	2 395	20	—	20	—	—	—	8	—	7	—	25	—	—
	C5	8 587	1 742	42	2 983	1 381	1 304	42	21	—	—	—	2	—	23	—	9	—	—
	C20	1 860	100	—	4 757	485	—	—	—	—	—	—	3	—	11	—	98	—	—
	C25	23 689	6 333	273	7 438	3 785	1 367	—	320	—	—	—	13	—	—	—	55	—	—
19/1/66	C35	32 670	3 155	110	7 427	1 853	2 185	—	320	—	338	43	3	—	27	—	37	—	—
	Mean	17 862	2 362	85	5 301	1 980	975	8	72	—	68	9	6	—	13	—	45	—	—
	T3	10 204	2 569	—	10 820	230	762	—	120	—	—	—	—	—	4	—	212	—	—
	T4	6 379	2 312	8	8 335	247	540	—	—	—	—	—	4	—	7	—	20	—	154
	T10	(240)	—	—	6 356	10	393	30	100	—	—	—	3	—	—	—	76	—	43
	T12	(15)	—	—	5 853	40	577	—	15	—	—	—	5	—	—	—	109	—	—
	T27	482	133	—	6 429	360	130	—	—	—	—	—	—	—	4	—	92	—	—
	Mean	3 416	805	—	7 559	177	481	6	47	—	—	—	2	—	6	—	102	—	39
	C3	6 610	3 380	—	6 536	60	750	—	254	—	—	—	6	—	31	—	3	—	—
	C4	1 702	163	—	40	20	530	—	—	—	—	—	—	—	4	—	2	—	—
14/3/66	C12	435	740	—	5 270	1 840	530	—	—	—	—	—	4	—	21	—	2	—	20
	C22	2 625	1 265	—	9 650	580	1 320	—	—	—	—	—	—	—	13	—	—	—	—
	C24**	7 564	6 963	—	5 790	180	1 430	—	—	—	—	—	3	—	5	—	40	—	—
	C27	2 484	2 052	—	8 687	490	3 482	—	—	—	—	—	8	—	34	—	158	—	100
	Mean	3 570	2 443	—	5 996	528	1 286	—	42	—	—	—	4	—	18	—	34	—	20
	T6	—	—	—	—	10	—	—	—	—	—	—	—	—	—	—	—	—	—
	T23	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	T24	10	210	—	1 080	10	9	—	—	—	—	—	—	—	—	—	10	—	—
	T26	10	130	—	40	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	T31	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—
16/5/66	Mean	4	48	—	224	4	18	—	—	—	—	—	—	—	2	—	2	—	—
	C6	—	—	—	10	—	—	—	—	—	—	—	—	—	18	—	—	—	—
	C15	—	190	—	—	—	—	—	—	—	—	—	—	—	5	—	—	—	—
	C26	—	—	—	—	—	—	—	—	—	—	—	—	—	18	—	—	—	—
	C31	—	10	—	—	20	—	—	—	—	—	—	—	—	31	—	1	—	—
	Mean	—	49	—	2	5	—	—	—	—	—	—	—	—	18	—	<1	—	—
	O. columbianum																		
		4th		Adult															
	T1	—	47	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	94
	T5	—	—	—	—	—	14	—	—	—	—	—	—	—	—	—	—	10	—
	T17	—	523	—	498	—	—	—	—	—	—	—	15	—	—	23	23	—	550
	T19	—	108	—	—	—	10	—	—	—	—	—	—	—	—	—	—	142	—
	T29	—	136	—	324	—	216	—	—	—	—	—	—	—	13	3	85	—	89
5/7/66	Mean	—	163	—	164	—	48	—	—	—	—	—	3	—	3	5	22	—	177
	C1	—	73	23	111	48	23	—	—	—	—	—	—	—	—	—	—	72	—
	C7	—	—	—	15	64	152	—	—	—	—	—	—	—	—	—	10	—	20
	C17	16	53	—	10	—	10	—	—	—	—	—	—	—	6	—	—	96	—
	C19	—	110	—	66	—	44	—	—	—	—	—	—	—	23	—	10	—	85
	C29	—	5	—	—	20	—	—	—	—	—	—	—	—	3	—	1	—	20
	Mean	3	49	5	41	22	49	—	—	—	2	—	—	—	7	—	4	—	59
	T8	63	211	—	—	—	—	—	—	—	—	—	—	—	2	—	—	21	85
	T9	21	145	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	6
	T11	64	293	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	43
30/8/66	T18	59	310	—	15	—	3	—	—	—	—	—	—	—	—	—	1	89	89
	T22	—	70	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Mean	41	206	—	3	—	6	—	—	—	1	—	—	—	<1	—	<1	22	42
	C9	68	296	—	—	40	300	—	—	—	—	—	—	—	5	—	—	330	550
	C16	58	79	—	—	—	—	—	—	10	12	—	4	—	4	—	—	14	20
	C18	130	555	—	—	2	37	—	—	—	—	—	—	—	—	—	—	—	25
	C23	—	—	—	—	10	—	—	—	—	—	—	—	—	8	—	—	29	70
	C30	15	21	—	—	—	—	—	—	—	1	—	1	—	12	—	—	29	96
	Mean	54	190	—	—	10	68	—	—	2	3	—	1	—	6	—	—	75	152
	T7	210	2 058	42	826	70	70	—	10	—	—	—	—	—	—	—	9	89	1 214
	T13	765	1 418	50	656	100	—	—	18	—	—	—	—	—	—	—	2	375	873
30/8/66	(30)	—	—	(10)	—	—	—	—	—	—	—	—	—	—	—	—	—	(60)	—
	T16	666	1 251	—	595	197	178	—	—	—	—	—	—	—	4	—	10	114	105
	T21	285	1 237	58	214	40	58	—	—	—	—	—	—	—	—	—	4	38	570
	T25	968	1 410	—	403	66	—	—	—	—	—	—	—	—	14	—	52	148	1 016
	(74)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	T32	1 259	1 689	15	500	106	34	—	—	—	—	—	—	—	—	—	4	140	292
	Mean	692	1 511	28	533	97	57	—	5	—	—	—	—	—	3	—	14	151	772
	(17)	—	—	(2)	—	—	—	—	—	—	—	—	—	—	—	—	—	(13)	—
	C10	60	827	73	751	73	72	—	—	—	—	—	—	—	—	—	6	186	326
	(20)	—	—	(20)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	C13	445	219	—	1 907	70	80	—	90	—	—	—	1	—	—	—	4	37	911
30/8/66	(74)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	C21	200	568	—	340	58	—	—	—	—	—	—	—	—	—	—	3	256	269
	C28	86	351	—	470	80	175	—	—	—	—	—	—	—	—	—	5	—	531
	C32	140	225	30	598	83	110	—	—	—	—	—	—	—	1	—	10	—	101
	C33	200	1 096	—	912	92	260	—	10	—	—	—	—	—	—	—	21	—	958
	(20)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Mean	209	565	15	793	79	106	—	14	—	—	—	<1	—	1	<1	8	86	528
	(25)	—	—	(5)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

\*Died  
 \*\*Emergency slaughter—Pericarditis

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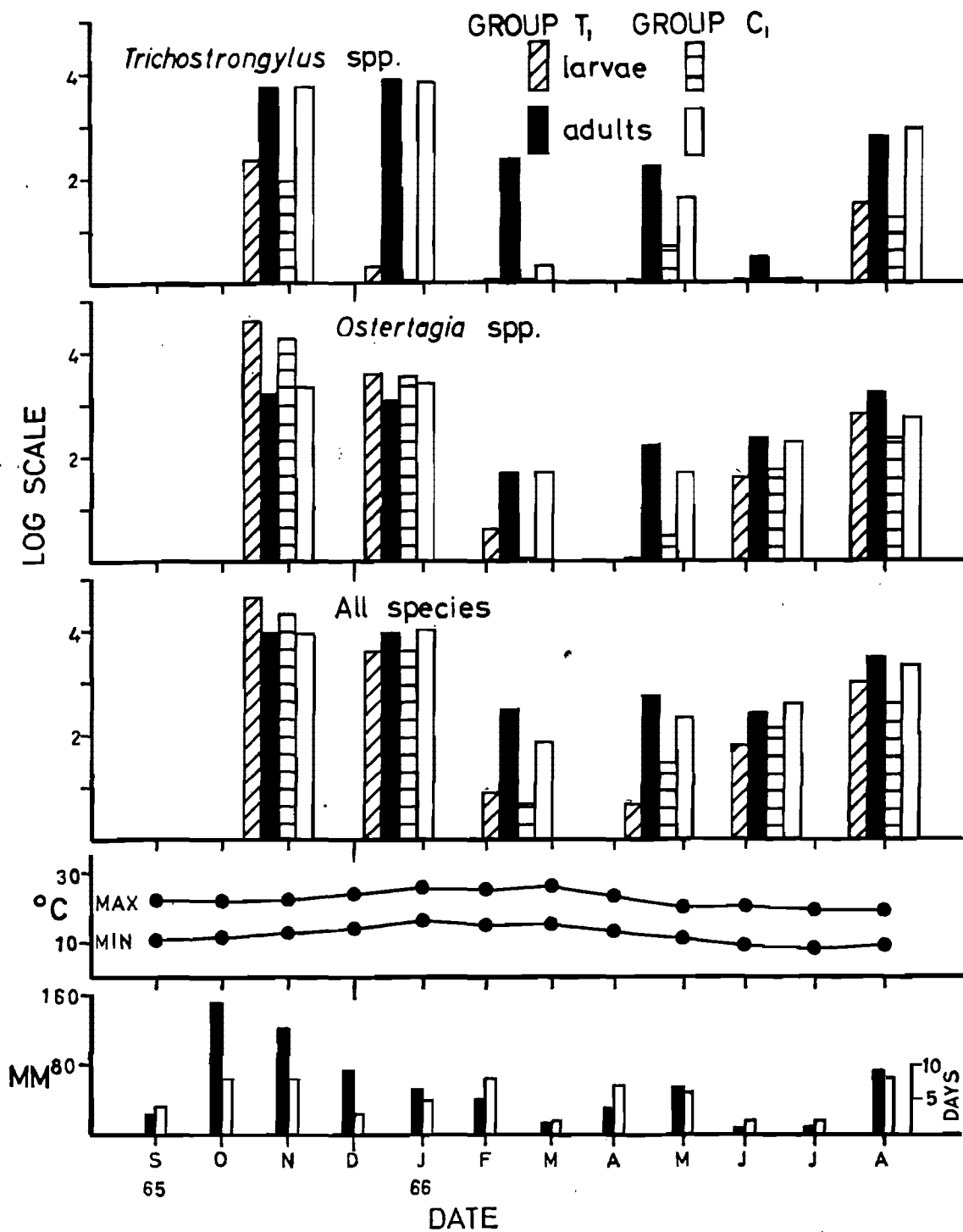


FIG. 4: Periodical variations in immature and adult worm burdens of treated (T<sub>1</sub>) and control (C<sub>1</sub>) lambs.



Normal rainfall in August resulted in a marked increase in worm burdens including the recovery of third and fourth stage larvae of the predominant genera. This indicates that environmental conditions prevailing from March to July were unfavourable for the development and survival of infective larvae in spite of pasture contamination by lambs of Group M (Fig 3).

*Trichostrongylus* spp.: This genus was present in large numbers from November to January (Table 3), decreasing markedly until August. Fifth stages and adults always exceeded fourth stage larvae.

*Ostertagia* spp.: The seasonal trends were similar to those found for *Trichostrongylus* spp. In those animals slaughtered during November and January, fourth stage larvae exceeded fifth stage and adult worms to a marked extent (Table 3). This relationship was reversed from March to August.

*Nematodirus* spp.: Peak worm burdens were recorded in November and decreased in January, to remain at a low level thereafter. Fourth stage larvae exceeded worms in the fifth and adult stages in November but this proportion was reversed from January onwards. While *N. filicollis* was dominant in most of the sheep slaughtered in November and January, *N. spathiger* increased proportionately thereafter.

*Oesophagostomum* spp.: With one exception (Lamb C22), *O. venulosum* was recovered in moderate numbers from all lambs slaughtered in November and January. Its appearance was erratic until August, when all sheep were positive. A few *O. columbianum* were recovered from some sheep slaughtered in May and July. Their presence was probably due to the introduction of the sheep in February (Group M), but their disappearance from sheep slaughtered in August seems to confirm that this species is not adapted to conditions at Outeniqua<sup>17</sup>.

*Haemonchus contortus*: With the exception of Lamb T22, this species was consistently present from May to August.

*Trichuris* spp.: This genus was present in moderate numbers in most of the sheep slaughtered.

*Chabertia ovina* and *Cooperia curticei*: While *C. ovina* was present in small numbers until January, it was recovered from only seven out of 32 sheep slaughtered from March onwards. *C. curticei* was present in ten out of 21 animals slaughtered in November and January and then absent, with the

exception of four out of twelve sheep slaughtered in August.

*Muellerius capillaris* and *Dictyocaulus filaria*: *M. capillaris* were not counted but their presence noted by lesions. These were present in ten out of 42 sheep slaughtered from January to August. *D. filaria* was recovered from the bronchuli of one sheep.

#### Medicated and Control Lambs

Most of the lambs in the medicated group (Group T<sub>1</sub>) exposed to challenge for 12 weeks acquired heavier infestations than their non-medicated counterparts (Group C<sub>1</sub>). There were marked variations between individual animals in each group, the range of worm burdens in the controls falling within the range of those in the medicated group (Table 3). When the average worm counts were expressed logarithmically (Fig 4), the differences are not as marked, particularly in November and January, when lambs were heavily infested.

## DISCUSSION

### Productivity

The above experiment was designed to investigate the susceptibility of lambs, raised "worm-free", to subsequent exposure to natural challenge. During the course of the experiment, however, certain results were obtained illustrating the effects of helminth infestation on the liveweights of lambs.

It is interesting to note that the lambs born to ewes receiving the mineral lick containing thiabendazole had a birthweight advantage of 0.9 lb over the lambs born to control ewes. The treated lambs at weaning weighed 9.6 lb (19.7%) more than the control lambs and the subsequent liveweight response showed a progressive increase<sup>18</sup>, so that at nine months of age treated lambs weighed 28 lb (40%) more than controls.

### Susceptibility to Infestation

This experiment showed that when medicated sheep were exposed to infestation for a period of twelve weeks after withdrawal of the medicated lick, they became more heavily infested than the control animals grazing the same pastures.

The trial period can be divided into three phases.

1. *A period of severe challenge* (September to February): Worm counts at autopsy in November and January and the deterioration in condition of the control lambs until



after treatment in March, indicated that pastures were heavily infested. The difference in worm burdens between the medicated and control lambs was not very great: both groups had average burdens exceeding 20 000 and 10 000 worms in November and January respectively (Table 3; Fig 4).

It had been expected that medicated lambs subsequently exposed to severe challenge would either die, or that survivors should have had acquired materially higher worm burdens than lambs continuously exposed to infestation from birth<sup>19</sup>.

The cumulative effects of this massive infestation, however, necessitated treatment in March to prevent mortalities.

2. *Low challenge* (March to July): Treatment of the control and medicated sheep grazing on the same pastures, combined with the low rainfall from March to July, resulted in low average burdens of less than 600 worms, despite contamination by lambs from Group M (Fig 3). No conclusions can be drawn regarding the respective immunological reactions of the two groups.

3. *Moderate challenge* (August): In view of the preceding statements, the increased worm burdens can only be ascribed to improved ecological conditions due to 72 mm of rain during this month. Although worm burdens increased, differences between the two groups were not as marked as during the preceding dry period.

#### Immunity

If the degree of immunity of the host to parasites is indicated by the number of worms established in the medicated groups as compared with unmedicated controls, then this experiment confirms the increased susceptibility of treated sheep (Group T<sub>1</sub>) to challenge<sup>20, 21, 22, 23</sup>. Despite severe challenge, lambs from Group C<sub>1</sub> did not develop a solid immunity, confirming Muller's<sup>24, 25</sup> observations on the same farm that lambs had to be treated in March to prevent deaths. Although non-medicated control lambs may develop a host-parasite relationship in which less worms are present than those in medicated sheep, the effects of these worms are cumulative and may cause the death of the host.

The lack of solid immunity in young lambs is probably due to immunological immaturity<sup>26, 27</sup>. Lambs under ten months of age had little immunity. This was confirmed

by the high worm burdens until January, as well as the deterioration in condition of control lambs in February.

Therapy should not be delayed to the point where it is merely used to prevent deaths. In young lambs particularly, the beneficial effects of anthelmintics on growth and production have been proved<sup>4, 18</sup>. Furthermore, until the immune status of the host is capable of suppressing the worm burden to a sub-clinical level, the host should be treated.

#### Specific worm burdens

Since *Trichostrongylus* spp. and *O. circumcincta* predominated, the following remarks are relevant.

*Trichostrongylus* spp.: Infestation by worms of this genus reached a peak in January, in contradistinction to Muller's findings<sup>17</sup> of peak burdens in August. The total rainfall and its distribution, however, were markedly different and the physical (lush) nature of the pasture probably contributed to the availability of infective larvae<sup>11</sup>. The relationship between fourth stage and fifth stage larvae, plus adult worms, confirmed his and other observations<sup>17, 28, 29, 30</sup>, namely that the latter stages always exceeded the former.

It would appear that immunity against trichostrongyle infestation is not reflected by retarded development in the fourth stage but rather a decrease in total worm burdens.

*Ostertagia circumcincta*: This species also reached a peak in November and January, when fourth stage larvae predominated. This is due to population pressure<sup>31</sup>, since the highest counts of fourth stage larvae were encountered when both fifth stage and adult worms were present in their largest numbers. When the latter decreased proportionately, fewer fourth stage larvae were recovered, i.e. fifth stage and adult worms predominated. These observations did not confirm the over-wintering hypothesis<sup>17</sup> in sheep nor do they resemble Type 2 ostertagiasis in cattle, which is seasonal (autumn to spring)<sup>32</sup>, but they do agree with other findings on the population distribution of this genus<sup>33, 34, 35</sup>.

#### CONCLUSION

Continuous low level anthelmintic (thiabendazole) therapy is conducive to high



productivity under conditions of severe parasitism. Sheep subjected to such treatment, however, are more susceptible to re-infestation when treatment is ceased and they are run with a control flock receiving periodic treatment only when clinical signs

necessitate such a procedure; they generally carry heavier worm burdens than the controls.

#### ACKNOWLEDGEMENT

The technical assistance of Mr. J. P. Louw is gratefully acknowledged.

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## STUDIES ON SCHISTOSOMIASIS

### 1. AN IMPROVED METHOD OF CONCENTRATING MIRACIDIA

J. A. VAN WYK\*

#### SUMMARY

A method is described of concentrating *S. mattheei* miracidia in water that is sufficiently free of faecal particles for the miracidia to be counted by means of an electronic particle counter.

#### INTRODUCTION

Whether schistosome miracidia in ruminant faeces are hatched by the method of Kruger & Heitmann<sup>1</sup> or by the method of McMullen & Beaver<sup>2</sup>, both the miracidia and the faecal particles remain in suspension. Because no distinction can be made between different types of particles of the same volume and/or similar shape by the existing mechanical counting methods, the miracidia have to be counted manually. This process is both tedious and inaccurate when large numbers of miracidia hatch from heavily infested faecal specimens.

Miracidia concentrated by means of the apparatus described in this paper are suspended in water which is free of faecal particles. It is then possible to count them in an electronic particle counter.

#### METHOD

Five to 10 g of faecal pellets from sheep infested with *Schistosoma mattheei* are broken up in a small quantity of physiological saline at 4°C and transferred to a measuring cylinder containing 2 l of the same solution. The specimen is allowed to sediment for 30 minutes in the dark, the supernatant is aspirated and the sediment washed with water at room temperature on a 37 µm aperture sieve. The residue is suspended in 350 ml water and placed in bright cold (fluorescent) light for 2 hours. During this time the faecal suspension is stirred mechanically.

Thereafter the miracidia are concentrated in an apparatus which is a modification of that described by Kruger & Heitmann<sup>1</sup>. It consists of a side arm flask coated inside with pitch, which, being matt black, does not reflect light entering the flask via the side arm. The side arm is connected to a bottle which is sealed off with stoppers at the top and bottom. A stereoscopic microscope lamp (Wild) is placed 40 cm from the apparatus so that the light shines directly down the side arm into the main flask.

Both the flask (1) and the bottle (2) attached to the side arm (3) are filled with filtered, distilled water which is at the same temperature as the faecal suspension. The side arm is closed and the water in the main flask replaced with the faecal suspension (6). This is allowed to sediment for 1 to 2 minutes before the side arm is reopened. Within two minutes miracidia are seen streaming into the side arm bottle. After fifteen minutes the side arm is closed and the side arm bottle containing the miracidia emptied. The faecal suspension is then removed from the apparatus and the entire process is repeated from the beginning until no further miracidia from this particular faecal specimen enter the side arm bottle. After an hour insignificant numbers of miracidia are obtained from the side arm bottle.

#### DISCUSSION

This apparatus concentrated miracidia more effectively than that of Kruger & Heitmann<sup>1</sup>, which was blackened on the outside. In their apparatus appreciable numbers of miracidia remain in the flask after an hour<sup>3</sup>. The inner glass surface possibly reflects light, thus making it difficult for the miracidia to locate the light source.

\*Section of Helminthology, Veterinary Research Institute, Onderstepoort.



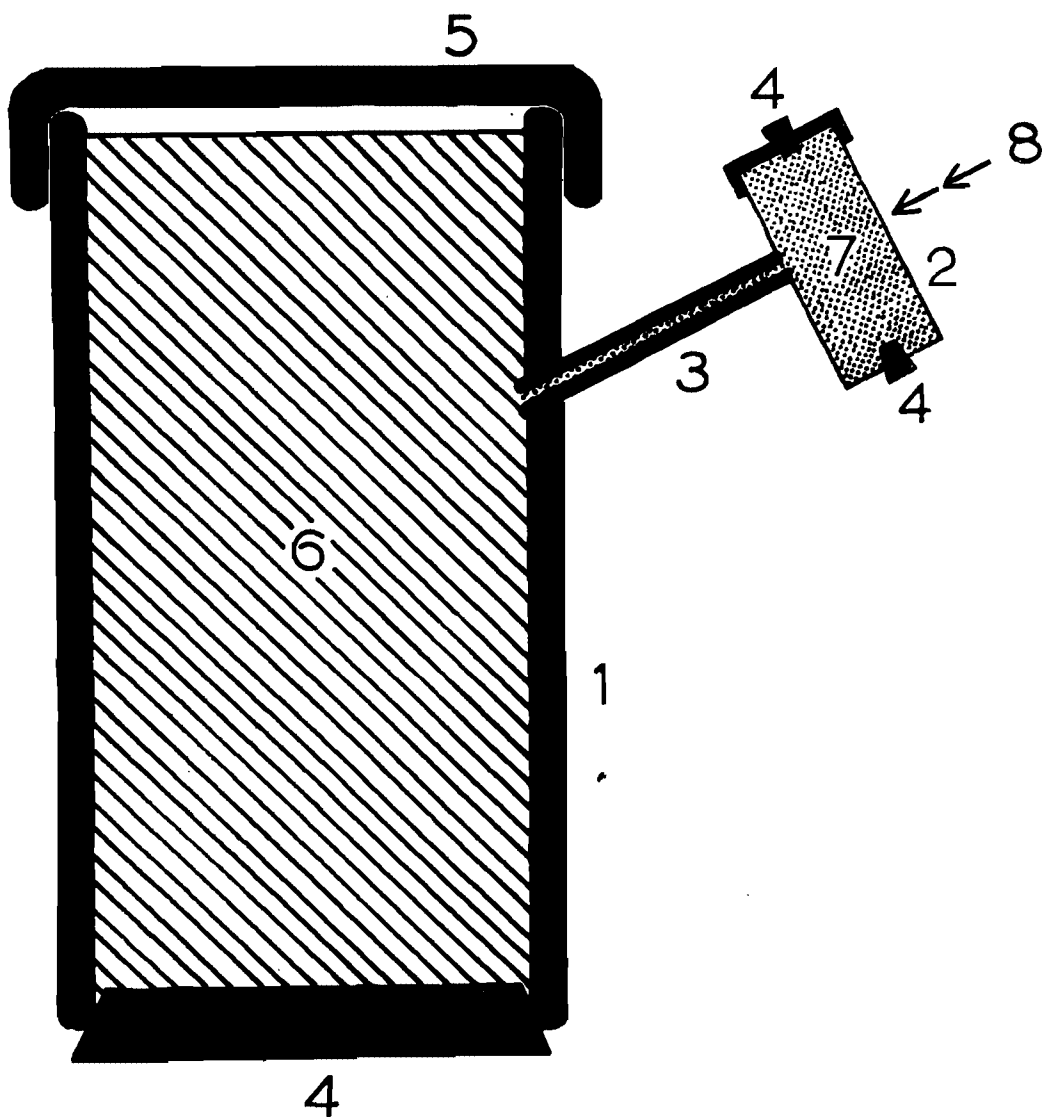


FIG. Apparatus for concentrating miracidia: 1. Main flask (lined with pitch). 2. Side arm bottle (transparent). 3. Side arm (glass, covered with rubber). 4. Rubber stoppers. 5. Lid (loose fitting, blackened). 6. Faecal suspension. 7. Water (filtered, free of particles). 8. Light beam.

#### ACKNOWLEDGEMENTS

I thank Dr. Anna Verster, Miss M.

Collins and my wife for assistance in preparing this paper.

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## STUDIES ON SCHISTOSOMIASIS

### 2. AN IMPROVED TECHNIQUE FOR COUNTING OVA AND CERCARIAE

J. A. VAN WYK\*

#### SUMMARY

A technique has been developed for mechanical sampling and counting of schistosome ova and cercariae with an electronic particle counter.

#### INTRODUCTION

The diagnosis of schistosomiasis either by identifying and counting the ova<sup>1</sup> or by hatching and counting the miracidia<sup>1</sup> is time consuming and inaccurate. Cercarial counting is also a laborious process and to obtain a true estimate of their number, large aliquots must be counted.

An improved method for carrying out the counts by the use of an electronic particle counter is described.

#### APPARATUS

An electronic particle counter (Coulter Electronics Model B particle counter with an industrial sampling stand, an electric agitator and 280  $\mu\text{m}$  and 400  $\mu\text{m}$  aperture tubes), an automatic timer (Coulter Electronics Model EE Timer) and an automatic plotter (Coulter Electronics Model J Plotter) were used to count and plot the size distribution of *Schistosoma mattheei* ova and cercariae and *Schistosoma haematobium* ova.

During the process the ova and cercariae were suspended in an electrolyte solution (0,3% NaCl solution) and were kept in suspension by the electric agitator operated at varying speeds according to the density of the material. The aperture current and amplification settings of the particle counter were estimated from the average height of impulses on the counter screen and the matching switch settings from the electrical resistance determined for each electrolyte and aperture tube used. A size distribution plot at these settings provided the threshold settings. Particles of known size (ragweed pollen) were used to calibrate the particle

counter and plotter for each aperture tube used<sup>2</sup>. The counts and size distribution plots were carried out with the 280  $\mu\text{m}$  aperture tube for ova, and the 400  $\mu\text{m}$  tube for cercariae.

Series of 28,3 second counts were carried out on varying concentrations of the different specimens to determine whether they were randomly distributed or not.

#### PREPARATION OF OVA

The ova of *S. mattheei* were liberated from the tissue of heavily infested sheep by digestion with 10% KOH at 40 to 45°C<sup>3</sup>. Impurities were removed from the specimen by sieving on a 37  $\mu\text{m}$  aperture sieve and removing foreign particles with a pipette. Ova of *S. haematobium* in formalinized urine

Table 1: SERIES OF 28,3 SECOND COUNTS OF *S. MATTHEEI* CERCARIAE

(In each of these periods the cercariae in 9,2 ml suspension were counted).

DILUTIONS	1	1	2	3	4	5
Concentration/ml	69,6	69,6	18,7	6,19	2,0	0,96
Counts 1	593	670	169	57	23	4
2	630	630	169	54	22	9
3	663	631	170	55	17	6
4	676	640	179	54	14	10
5	651	631	162	68	15	14
6	641	618	180	52	19	9
Mean count	642	637	172	57	18,3	8,7
Coefficient of variation (%)	4,5	2,8	4,0	10,2	19,9	39,5

\*Section of Helminthology, Veterinary Research Institute, Onderstepoort.



were subjected to digestion for 2½ to 3 hours to free them from adherent organic particles.

#### PREPARATION OF CERCARIAE

Three hundred thousand cercariae of *S. mattheei* from experimentally infested *Bulinus (Physopsis) globosus* and *Bulinus (Physopsis) africanus* snails were killed by adding the cercarial suspension to formal saline (final concentration 3% formalin and 1% saline). Thereafter the cercariae were washed on a sieve, and suspended in 0.3% saline.

#### RESULTS

The size distribution histograms are reproduced in Figs. 1 and 2. The number of *S. mattheei* ova in each aliquot is summarized in Table 1.

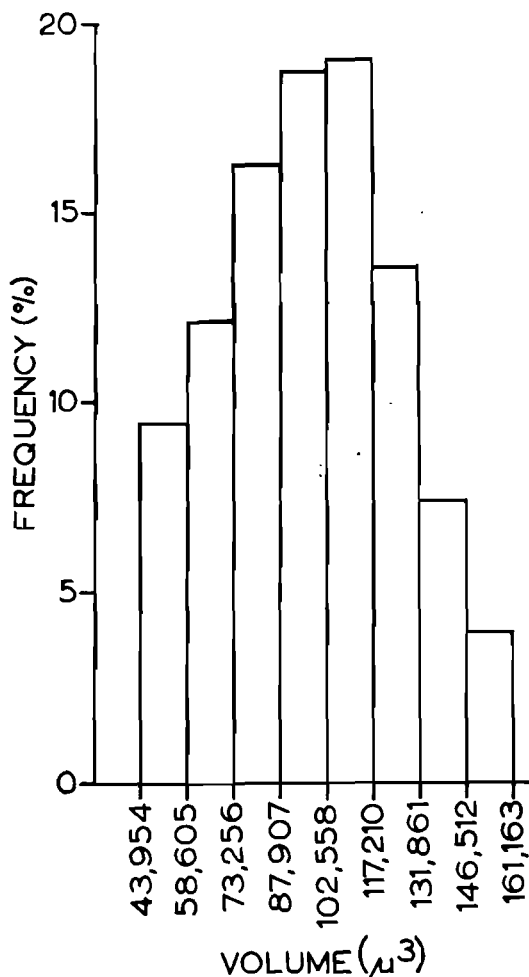


FIG. 1. *S. mattheei* ova: Size distribution plot of ova digested from tissue of infested sheep.

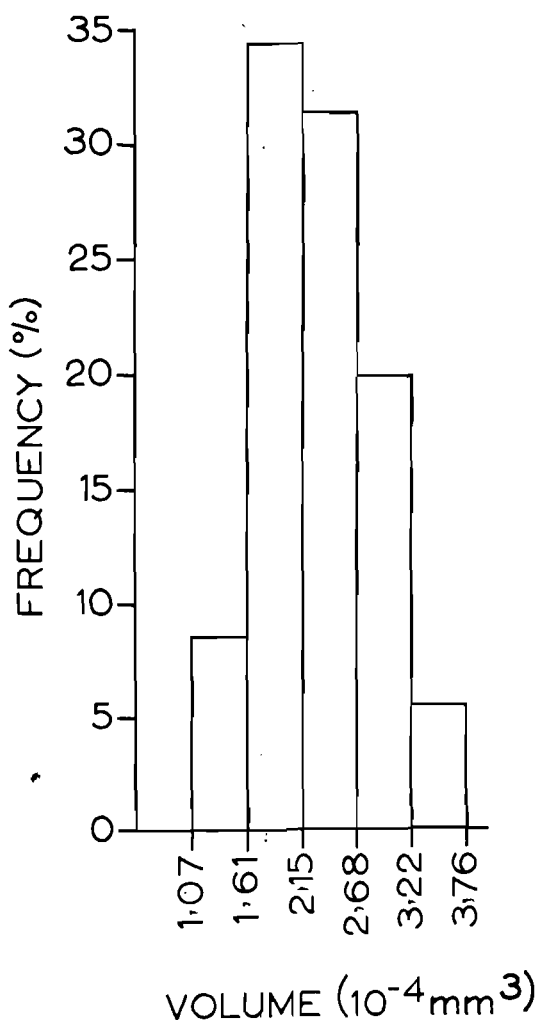


FIG. 2. *S. mattheei* cercariae: Size distribution plot of cercariae killed with formal saline.

The mean volume of *S. mattheei* ova examined was 97 430 μm³ with a range of 43 950 μm³ to 161 160 μm³ (Fig. 1). The volume of *S. haematobium* ova was about 75 000 μm³; as insufficient ova were available, the range of variation could not be determined with accuracy.

The mean volume of *S. mattheei* cercariae was 230 790 μm³ and the volumes varied from 107 340 μm³ to 375 700 μm³ (Fig. 2).

#### DISCUSSION

This method may be used for the routine estimation of the number of schistosome cercariae or ova in tissues and in urine. Small numbers of ova and cercariae could



probably still be counted manually with greater ease.

The disadvantage of the technique is that all foreign particles which are similar in volume to the ova or cercariae respectively must be removed from the specimen to be examined. To a certain extent the difficulty was overcome by subjecting tissues and urine to digestion with KOH.

The volumes of the ova of *S. mattheei* and *S. haematobium* described in this paper were determined on specimens treated as described above. Subsequently it was found that *S. mattheei* ova digested from tissues were cleaned more effectively by application of a method developed by Pitchford<sup>4</sup> for concentrating *Schistosoma mansoni* ova in faeces: the specimens were washed under pressure through a 74  $\mu\text{m}$  aperture sieve, and collected on a 37  $\mu\text{m}$  aperture sieve. In the case of fresh urine digestion for 1 to 2 hours freed the ova of organic matter, while formalin specimens required treatment for

2½ to 3 hours.

The ova used for obtaining the size distribution histograms were probably distorted by the digestion process and the cercariae were contracted as a result of formal saline treatment<sup>5</sup>; the volumes recorded for the ova and cercariae in these experiments cannot therefore be considered representative of those of fresh ova and relaxed cercariae.

It is possible that the technique can be adapted to count schistosome miracidia concentrated in water free of particles<sup>6</sup>.

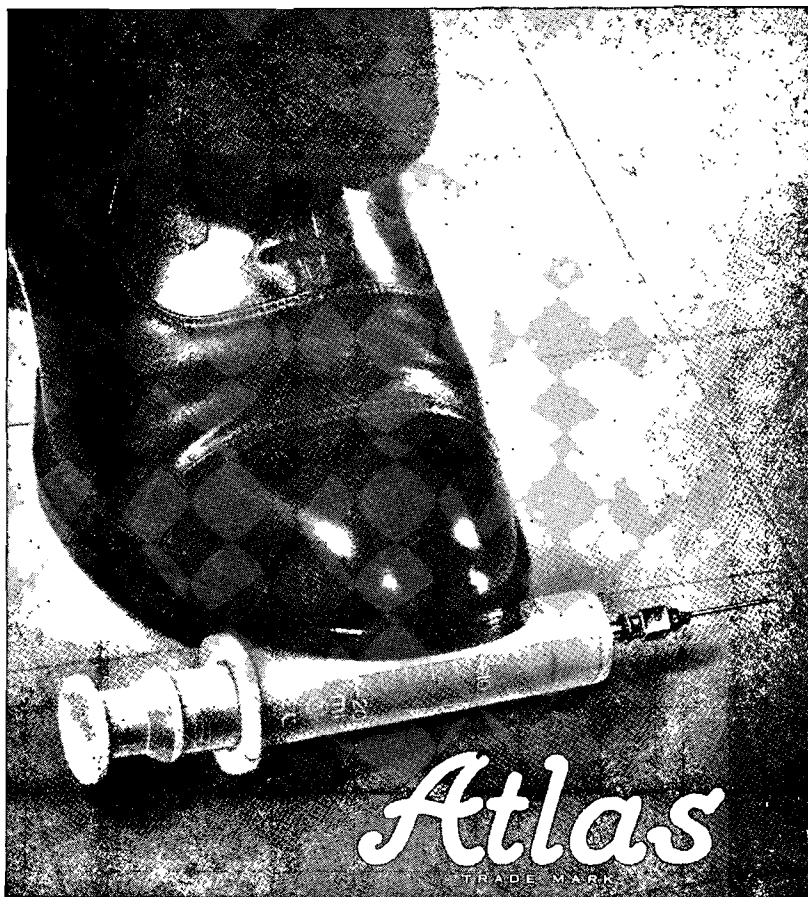
#### ACKNOWLEDGEMENTS

Messrs. Coulter Electronics SA (Pty) Ltd. are thanked for providing the Coulter industrial stand, dual switch and automatic timer, Dr. Anna Verster, Miss M. Collins and my wife for assistance in preparing this paper, and Dr. R. J. Pitchford and Dr. R. Elsdon-Dew for providing the *S. haematobium* ova.

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# ASPERGILLUS FUMIGATUS INFECTION IN THE SCALY WEAVER (*Sporopipes squamifrons*)

O. P. M. PROZESKY\*, S. B. BUYS\*\*, H. N. VAN DER MADE\*\*\*,

P. J. DE WET\*\*\*\* AND J. M. M. BROWN\*\*\*\*

## SUMMARY

An outbreak of *Aspergillus fumigatus* infection amongst the colony of scaly weavers (*Sporopipes squamifrons*), maintained at the Department of Physiology, Faculty of Veterinary Science, Onderstepoort for studies on water metabolism in this species, is described. The epizootiology, symptomatology, mortality rate, pathology and control of the disease, as seen under the particular conditions prevailing in the colony mentioned, are described.

## INTRODUCTION

As recorded previously the scaly weaver (*Sporopipes squamifrons*, Ploceidae) has been the subject of a special study dealing with its water metabolism<sup>1</sup>. These weavers were trapped, for the purposes of this work, in mist nets on various farms in the Zeerust district of the Western Transvaal before being brought to the Department of Physiology, at the Faculty of Veterinary Science, Onderstepoort.

The scaly weaver is a very common gregarious bird of the drier parts of the central and northwestern regions of Southern Africa<sup>2,3</sup>. It normally takes well to captivity and adapts easily and rapidly to its new surroundings. Until the time of outbreak of the infection described here, our colony of these birds had been free from untoward mortality or illness of any kind. On the same day of the introduction of the latest batch of birds, however, heavy mortality occurred and continued at the rate of

four to five birds per day during the course of about a week. About thirty birds were lost before the infection was brought under control.

## EPIZOOTIOLOGY

The scaly weaver is a small ploceid bird occurring in the drier savannas, semi-desert and desert areas of Southern Africa. It generally occurs in small flocks frequenting the acacia thorn bushes typical of these areas, feeding probably exclusively on grass seeds and possibly other seeds as well<sup>3,4</sup>. It is found around farmyards, feeding wherever grain is scattered.

The batch of birds which apparently introduced the infection into our colony was captured on one particular farm at Zeerust by means of mist nets erected in the vicinity of a threshing floor. The farmer had completed threshing his crop of kaffir-corn (*Sorghum caffrorum*, Gramineae) some weeks before and a wide variety of seed-eating birds had been attracted to the floor. The floor residues on which the birds were feeding consisted of grain chaff and copious amounts of a fine, greyish, farinaceous dust derived from the sorghum heads. This dust is highly irritating to the eyes and mucous membranes of the throat and upper respiratory tract of many a human being. The late summer months, during which the threshing had taken place, had been marked by the rather infrequent thunderstorms and very hot, humid conditions usual in this area at this time of the year. Despite these

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conditions, a severe drought was prevailing and a wide variety of seed-eating birds had gathered around homesteads, where food was generally more abundant.

Thirty-five scaly weavers, four yellow-spot sparrows (*Petronia supercilialis*, Ploceidae), four yellow-eyed canaries (*Serinus mozambicus*, Fringillidae) and four masked weavers (*Ploceus velatus*, Ploceidae) were captured at the above-mentioned locality. At the same time twenty scaly weavers were trapped on a homestead located twenty miles north of Pretoria, near fowl runs. All these birds were included in our colony.

Since all the birds were caged at once after capture and transported by car to our colony immediately thereafter, no further contact with any avian species was possible. The birds were maintained in our laboratories in wood and wire-netting cages, measuring  $2 \times 1,3 \times 1$  m, these dimensions allowing at least  $0,08 \text{ m}^3$  of space for each bird in each of the cages, which housed two dozen birds. They were fed on golden millet seed (*Setaria sphacelata*, Gramineae) and received water *ad libitum*. The birds, newly introduced from Zeerust, were placed amongst previous introductions from the same area and those captured north of Pretoria. They were readily distinguished from their cage-mates by their smaller size and poorer condition, attributable to the prevailing drought conditions.

The first dead birds were found in the cages within a few hours after the introduction of the birds from Zeerust. No autopsies were performed on these birds, nor on the few found dead during the next day or so, since it was thought that these newly introduced birds might have been injured in some way or another during capture or transport to Onderstepoort. The mortality continued and, by the fourth day after introduction of the new birds, two of the yellow-eyed canaries and one of the yellow-spot sparrows, had also succumbed. The birds latest dead were then submitted to the Department of Poultry Diseases for examination.

The scaly weaver is a very sociable and confiding bird. Their habit of sleeping very closely huddled together is possibly of importance with regard to the spread of disease amongst them. Even when maintained in aviaries allowing at least  $0,3 \text{ m}^3$  of space

per bird and provided with the conventional square budgerigar nesting boxes they will roost in such a fashion, in the temperate climate of Pretoria at least, that five to six birds are huddled together on top of one another in the entrance apertures of these boxes, rather than roost on perches or shelves where space is decidedly more plentiful. Similar behaviour prevails in their natural roosting places.

#### SYMPTOMATOLOGY

The scaly weaver in captivity is a most lively and rather inquisitive bird, which utters a continuous chattering note whilst feeding intermittently throughout the day<sup>1</sup>. One of the first indications that something was seriously amiss in our bird colony, besides the presence of dead birds, was the noteworthy diminution in the volume of sound coming from the birds. The disease was characterized by an apparently rapid onset and an equally rapid course. On cursory examination affected birds appeared at first to be merely inactive, not feeding, silent and sitting with their feathers puffed out. Closer examination showed that they were noticeably light during the "puffed-up" stage of the illness, marked atrophy of the keel muscles being a very prominent sign. No obvious nasal or ocular discharges were present in any of the affected birds and at no time did we ever notice any coughing or spluttering amongst the members of the colony. Dyspnoea and hyperpnoea best describe the signs observed. Within twelve to twenty-four hours these birds would be found lying on their sides, *in extremis*, with marked signs of respiratory distress. Death generally supervened within a few hours thereafter.

#### GROSS PATHOLOGY

Emaciation was the first striking autopsy feature in affected birds. Lung lesions included either focal disseminated or fairly diffuse patches present on the surface of one or both lungs, varying in size from a few millimeters in diameter to large patches involving the entire distal third of the lung. Smaller lesions were white, powdery and demarcated by a dull red boundary. Large lesions consisted largely of a dull, dark red area appearing more solid on section than the rest of the lung. No lesions of particular note were observed in any of the other viscera.



#### MYCOLOGY

Specimens were taken from the affected lung tissues under aseptic conditions. Cultures were made on Sabouraud's agar and after two days fungal growth was evident. The organism concerned was identified for us by mycologists of the Plant Protection Research Institute as *Aspergillus fumigatus*, growing in virtually pure culture in all cases.

#### HISTOPATHOLOGY

Specimens were collected at autopsy and fixed in 10% formal saline for this purpose. The only lesions of note were those involving the lungs. The epithelium of some of the bronchi of affected lungs was found to have sloughed away and was replaced by a dense network of proliferating hyphae, which had invaded the walls of these bronchi and the adjacent tissues. These tissues were generally necrotic, haemorrhagic and infiltrated by polymorphonuclear cells. Some of the affected areas contained long hyphae with only a very mild attendant cell reaction, congestion and oedema.

The most prominent lesions consisted of localized areas of unencapsulated, caseating, necrotic tissue, which contained the fungus, surrounded by a haemorrhagic, oedematous zone infiltrated by varying numbers of polymorphonuclear cells and macrophages. Most of the walls of the larger blood vessels present in the affected areas were invaded by hyphae and thrombosed.

The nature of the lesions described above indicated an early progressive type of infection<sup>4</sup>.

#### THERAPEUTIC AND CONTROL MEASURES

The moment that the presence of aspergillosis amongst the colony was suspected, following the first few autopsies, the birds were moved to unused cages. The infected cages were thereupon scrubbed down with Halamid-Vet\* using a concentration of 50 g per 10 litres of water. They were then allowed to dry for two hours in the sun, before the birds were replaced.

The birds, at this stage weighing 10 g on an average, were treated daily for two weeks with mycostatin\*\*, at a dosage rate of 6.5 units per gram body weight administered in the drinking water, which was replenished daily. Medication of the feed was considered but thought less practicable.

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After a week of mycostatin treatment, a daily dose of Vitmin B Complex\*\*\* was included at the dosage rate of 1 ml of the injectable complex for every litre of water supplied. At this time a marked decrease in the mortality rate was already evident and by the end of the third week of treatment the mortality had ceased altogether.

#### DISCUSSION

*Aspergillus fumigatus* infection of the respiratory tract of man and birds needs no introduction to veterinary pathologists. It is probably best known in the form of brooder-house pneumonia in large commercial poultry concerns. We believe that this is the first report of its occurrence in the scaly weaver and possibly even of its occurrence amongst Ploceid birds normally resident in South Africa.

The history of the outbreak indicates that the infection must have been rife amongst the scaly weavers on the farm at Zeerust at the time they were trapped. The environmental conditions then were certainly conducive to luxuriant fungal growth in the threshing residues on which the birds were feeding. Although no certainty exists on this point, the only birds which appeared to contract and succumb to the infection were those introduced from this farm. Their poor state of nutrition, together with their peculiar roosting habits, undoubtedly contributed to the rapid spread of the infection amongst them. Although the yellow-spot sparrows and yellow-eyed canaries normally feed together with a wide variety of seed-eaters, they generally do not roost in the same sites or in the same manner as the

scaly weavers. The condition of the birds of these two species which succumbed to the disease was also very poor. We can only assume from the history of the outbreak that they contracted the disease during their intimate confinement with the weavers whilst in transit to our colony at Onderstepoort. The time which elapsed between this close contact and the death of these birds was between four to five days. This period, which includes both the incubation and active stages of the disease, is indicative of the virulence of the condition in undernourished birds.

The therapeutic and control measures adopted certainly appeared to put a dramatic end to the morbidity and mortality amongst the survivors in the colony: no further deaths occurred later than three weeks after institution of these measures. Naturally one cannot be sure that the infection did not clear up spontaneously. Ten months have now elapsed without further illness or mortality in the colony: none of the birds subsequently sacrificed for studies on their water metabolism suffered any sign of having contracted the disease at any time.

#### ACKNOWLEDGEMENTS

We are most grateful to Dr. L. Abrams, Private Consultant in Avian Pathology, Johannesburg and to Dr. L. Coetzee, Head of the Department of Poultry Diseases, Veterinary Research Institute, Onderstepoort for their advice and assistance in bringing the outbreak of the disease so speedily under control.

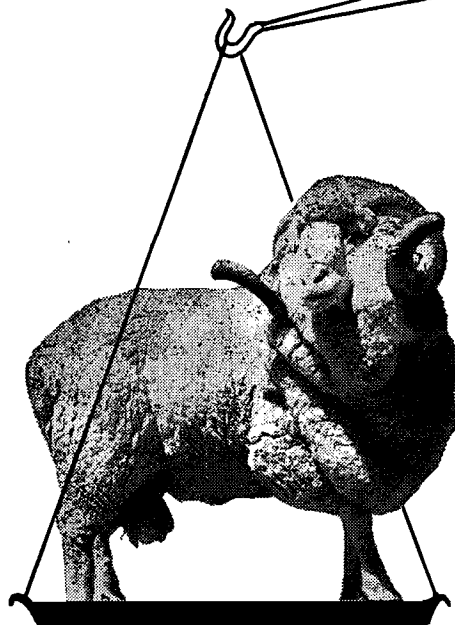
\*\*\* Farmbeco", Fabrus, Bethlehem, O.F.S.

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# RESEARCH NOTE

# NAVORSINGSAANTEKENING

## TRANSMISSION OF OVINE JAAGSIEKTE USING NEOPLASTIC CELLS GROWN IN TISSUE CULTURE

R. C. TUSTIN\* AND SOPHIA M. GEYER\*\*

### SUMMARY

The successful transmission of jaagsiekte using the tumour cells grown for 22 days in tissue culture as inoculum is described. One of the five sheep used manifested typical, but not advanced, lesions of the disease when slaughtered 1070 days after inoculation. A transmission attempt in which the cell-free medium used for growing these cells was inoculated into five sheep, failed.

This result suggests that the infectious agent of jaagsiekte may be cell-bound.

### INTRODUCTION

Tustin<sup>1</sup> reported the successful transmission of ovine jaagsiekte to two sheep following the intravenous inoculation of the tumour cells which had been grown on tissue culture for 10 days during which time the medium had been changed twice. One of the sheep died of jaagsiekte after 249 days and the other had advanced lesions of the disease when it was killed 253 days after inoculation. Attempts to repeat this experiment in 10 sheep with cells grown for 21 days and after three changes of medium, failed.

As it was considered that the aetiological agent of jaagsiekte might be cell-associated the experiment described below was undertaken.

### MATERIALS AND METHODS

A sheep thought to be suffering from jaagsiekte was electrocuted. Immediately after death the thorax was opened and the lungs were examined grossly. Typical lesions of the disease were present. Material from affected parts of the lung were taken for tissue culture and histological examination.

The material for tissue culture was processed as follows: It was cut into small pieces which were placed for one hour in Hank's solution containing the following antibiotics per millilitre: streptomycin 200 µg, penicillin 200 units, mycostatin 100 units, fungizone 5 µg and neomycin 100 µg. This was followed by trypsinization according to routine procedures and the resultant individual cells were dispensed into Roux flasks at a concentration of  $2.5 \times 10^6$  cells per millilitre of medium. Eagle's medium, containing twice the recommended amount of vitamins and amino acids, and supplemented with 5% bovine serum, was used. The flasks were incubated at 37°C and after 22 days the medium was removed from one flask and centrifuged at 800 r.p.m. for 10 minutes. Five millilitres of the supernatant fluid were inoculated by the intravenous route and by the subcutaneous route into five Merino lambs about 4 months of age. The remaining supernatant fluid was stored in a refrigerator at 4°C and the same lambs were each inoculated with identical amount of the fluid by the same routes four days later.

The cells from the same flask were washed and resuspended in 10 ml of fresh Eagle's medium to give a concentration of 970 000 cells per millilitre. Five Merino lambs, also about 4 months of age, each received inoculations of 1 ml of the cell suspension by both the intravenous and subcutaneous routes.

The two groups of sheep were placed together in a room with an impervious floor and walls and were kept isolated from other sheep for a period of 1070 days, when surviving animals were slaughtered and necrop-

\*Section of Pathology, Veterinary Research Institute, Onderstepoort. Present address: South African Medical Research Council, Private Bag 380, Pretoria.

\*\*Section of Virology, Veterinary Research Institute, Onderstepoort.



sied. One sheep died during the course of the experiment; it was necropsied.

### RESULTS

The histological examination of the affected lung tissues, part of which was used in the preparation of the tissue cultures, confirmed the macroscopic diagnosis of jaagsiekte.

One of the sheep which had received injections of the supernatant fluid died 252 days after the first inoculation. On necropsy it proved to be negative for jaagsiekte.

The 10 animals in the two groups were of both sexes and were permitted to breed at will; during the course of the experiment eight lambs were born. These were also slaughtered and necropsied at the end of the experiment. On post mortem examination all the sheep with one exception were negative for jaagsiekte. The positive case was an animal in the group which had received inoculations of the cell suspension, and the lesions consisted of a typical jaagsiekte nodule 4×3 cm in size in the right diaphragmatic lobe which extended right through the lung parenchyma from the lateral to the medial side and was surrounded by nine "satellites" varying from 1 to 5 mm in diameter all within a distance of 3 to 4 cm from the larger nodule. The rest of the right lung and the left lung were normal. Histological examination of affected tissue confirmed the diagnosis.

### DISCUSSION

When planning and executing an experiment involving the transmission of jaagsiekte a number of factors must be borne in mind; not only is the incubation period long, but the number of successful transmissions following artificial infection may be low and relatively large numbers of animals must be used before definite conclusions can be drawn. At present, too, there is no known method of ensuring definitely that the experimental sheep used are, in fact, free of the disease before the commencement of the experiment other than ascertaining that the animals originate from a jaagsiekte-free flock. Because of the long incubation period, animals in a transmission experiment should be kept where no other sheep have previously been housed or where

no sheep have been kept for some months at least. In order to prevent them from transmitting this contagious disease to each other before symptoms are manifested, individual sheep in the experiment should be completely separated from each other and from other animals.

In this experiment space was at a premium and therefore all the 10 animals in the two groups had to be housed together; had an animal in the other group also developed the disease, the results would have been inconclusive. The fact that only one animal developed the disease and did not transmit it to any other animal in the experiment despite the close proximity during a period of nearly three years again illustrates the low infectivity of the disease in this country and/or the relatively high resistance of South African Merino sheep.

That this disease is only slowly progressive in some animals was also revealed in this experiment. Tustin<sup>1</sup> has pointed out the possible danger that these sheep with asymptomatic, virtually "static" lesions constitute in transmitting the disease to clean flocks. These slowly developing lesions perhaps may also be related to the high resistance of the infected animal.

Although the number of animals used in this experiment was small and no definite conclusions can be drawn from the results, an indication was obtained that the infective agent of jaagsiekte may indeed be cell-bound; successful transmission only occurred in an animal in the group to which the cells had been administered.

It is impossible at this stage of our knowledge concerning the aetiology of jaagsiekte to determine whether or not the infectious agent multiplied during the 22 days that the tumour cells were grown on tissue culture, although the cells themselves grew well; in fact, the high resistance and/or low infectivity rates referred to above might well have been due to low titres of the infectious agent in the tissue culture inoculum, in other words, only small amounts of the agent might have survived the treatment meted out to it.

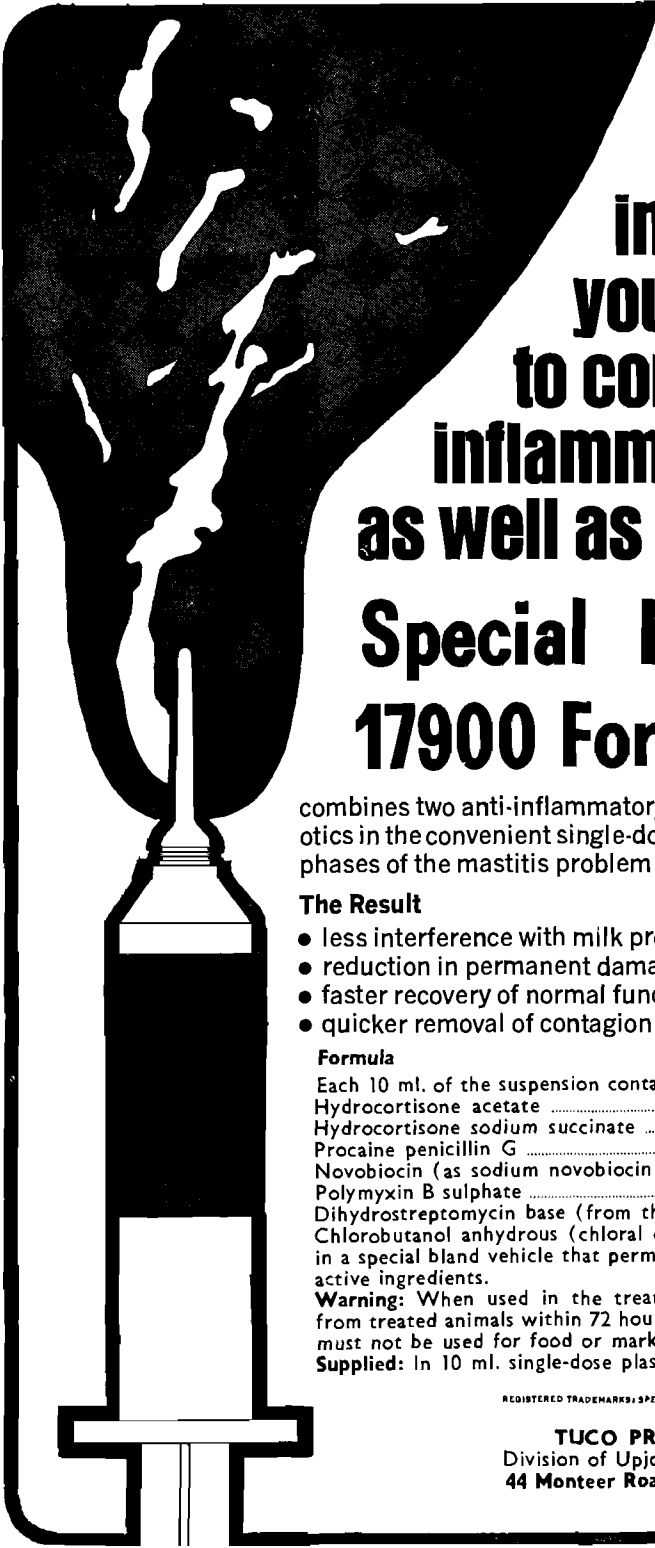
### ACKNOWLEDGEMENTS

The encouragement of Dr. K. E. Weiss and the technical assistance of Mr. W. J. Pienaar are gratefully acknowledged.

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## RESEARCH NOTE

## NAVORSINGSAANTEKENING

# THE PREPARATION OF BROAD SPECTRUM ANTISERA FOR USE IN THE CHARACTERIZATION OF SERUM PROTEINS BY IMMUNODIFFUSION TECHNIQUES

I. S. WARD-COX\*

### SUMMARY

A method is outlined to produce immune sera with wide spectrum precipitating activity against serum proteins by consecutive inoculation of rabbits with globulins and whole serum.

### INTRODUCTION

During the course of analyses of serum proteins of animals by immunodiffusion techniques, it is necessary to use antisera capable of identifying all or most of the antigenic fractions. Such sera should fulfil certain requirements, viz. be of sufficiently high titre for rapid precipitation; be species specific with clear differences when used against closely related species; be capable of full spectrum activity from the  $\alpha$ -albumin through to the  $\gamma$ -globulin region; be of the precipitating type.

The purpose of this investigation was to obtain such antisera. Due regard was paid to the following restrictive conditions:

- (a) The degree of species specificity decreases with the increase in the number of injections.
- (b) The antigenicity of the various fractions of whole serum is apparently governed quantitatively and therefore dominated by albumin.
- (c) Rabbits, being the most suitable laboratory animals for the production of precipitating antibodies, are prone to anaphylaxis when inoculated with large amounts of antigen intravenously.

### METHOD

The method applied in the present study combined the technique of Proom<sup>1</sup> with the

injection of whole serum. Bovine serum served as antigen for the purpose of this article.

Antigen was prepared by diluting 25 ml of normal bovine serum with 80 ml of distilled water and then adding 90 ml of a 10% solution of potassium aluminium sulphate in distilled water. The pH was adjusted to 6.5 by the addition of 5N NaOH, thereby precipitating the globulins. The mixture was centrifuged, the sediment washed twice in 200 ml normal saline containing 1:10 000 merthiolate and the final precipitate made up to 100 ml with the same solution. Rabbits were immunized by injecting 10 ml (equivalent to 2.5 ml serum) of the above antigen subcutaneously at the abdominal wall. At this stage Proom's technique was modified by the inclusion of an intravenous injection of 2.5 ml whole serum after one week. One day prior to this, 1 ml whole serum was introduced subcutaneously so as to desensitize the animal against possible anaphylaxis. Test serum was collected one week later.

For control purposes, separate antisera were prepared respectively against precipitated globulins and whole serum by two injections at weekly intervals of the quantities and by the routes indicated above. Test sera were withdrawn one week after the last injection.

### RESULTS AND DISCUSSION

The antiserum withdrawn one week after the last inoculation with whole serum lacked specificity, apparently due to the fact that more than one injection of the

\*Immunogenetics Unit, Veterinary Research Institute, Onderstepoort.





FIG. 1. Immunoelectrophoretic patterns obtained using an antiserum prepared by inoculating rabbits with whole serum only.



FIG. 2. Immunoelectrophoretic patterns using an antiserum prepared by inoculating with precipitated globulin only.

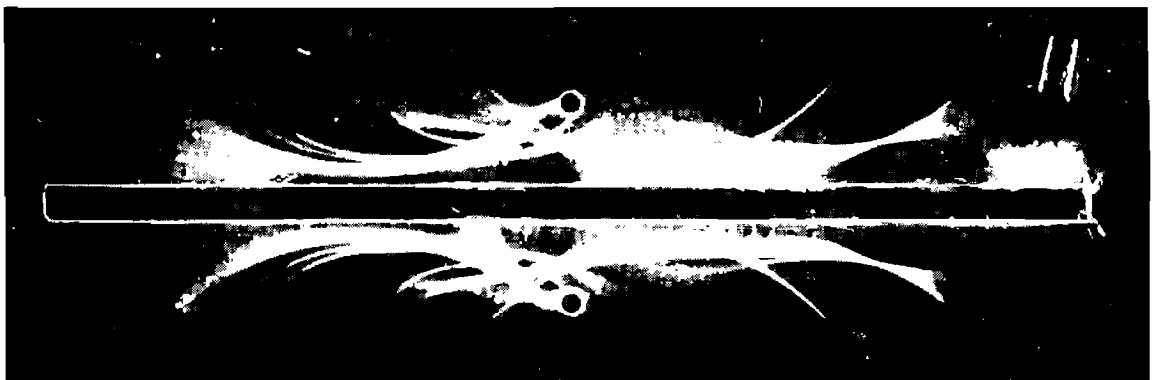


FIG. 3. Immunoelectrophoretic patterns obtained using an antiserum prepared by inoculating rabbits with both whole serum and precipitated globulin.



identical antigen was given. A further phenomenon, illustrated in Fig 1, was the apparent absence of anti- $\beta$ - and anti- $\gamma$ -globulins with a pronounced activity in the albumin and  $\alpha$ -globulin zones.

With Proom's method the absence of anti-albumin and anti- $\alpha$ -globulin activity is striking (Fig. 2). This is to be expected as potassium aluminium sulphate precipitates only the globulins, and more specifically the  $\beta$ - and  $\gamma$ -globulins.

The modified method of Proom used in this study provided sera with a wide spectrum of activity against serum proteins. A typical precipitation pattern is illustrated in Fig. 3. As many as 32 fractions, ranging from  $\rho$ -albumin to  $\gamma$ -globulin have been

identified in bovine serum.

In the past it has been the practice to employ either Proom's technique or whole serum. Either method requires the use of more than one injection of antigen to elicit an appreciable response over a wide range of serum fractions. Using the two antigens together, however, it is only necessary to give a single injection of each with a week's interval between the two, thereby making it the method of choice. Although bovine serum was used in this investigation, this method has been found to be equally effective with sheep, goat, pig and horse serum.

#### ACKNOWLEDGEMENT

I thank Dr. R. D. Bigalke for his helpful criticism of the manuscript.

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## CASE REPORT

## GEVALVERSLAG

### MELANOMA IN THE LARYNX OF A DOG

E. E. McCONNELL\*, J. D. SMIT\*\* AND H. J. VENTER\*\*\*

#### SUMMARY

A case of melanoma occupying the right lateral ventricle of the larynx in a five-year-old Alsatian bitch is reported. It recurred to the extent of causing severe dyspnoea and cyanosis two weeks after the initial excision.

#### CASE HISTORY

A five-year-old Alsatian bitch with a history of progressive dyspnoea, extending over four-and-a-half months and accompanied by loud rasping sounds on both inspiration and expiration, was brought to one of us (R.J.V.) for examination. Two weeks prior to this the animal had been treated for asthma with some favourable results, but signs recurred. Upon examination, very laboured breathing associated with a loud whistling noise was found. The mucous membrane of the oral cavity was severely cyanosed. A tentative diagnosis of either bronchial spasm or a tracheal obstruction was made. Adrenalin administered intravenously gave no apparent relief. A short time later cortisone was given by the same route, again with no effect.

To examine the air passages in detail the dog was given a tranquillizer and 2 ml of a 5% solution of sodium thiopentone. Immediately the anaesthetic took effect, an oesophagoscope was passed into the pharynx. A large elliptical growth (2,5×1,0 cm as measured subsequently) was observed: it grew from the area of the right lateral ventricle and almost filled the laryngeal cavity. The oesophagoscope was then eased

past the tumour into the trachea—a process that could be accomplished only with great difficulty. Once in place, the oesophagoscope now functioned as an endotracheal catheter; the cyanosis immediately diminished, proving that the growth was responsible. The dog was allowed to breathe normally and thus oxygenate itself before the oesophagoscope was drawn back into the pharynx and the examination completed.

Total excision was performed with difficulty. Moderate haemorrhage occurred and was controlled adequately by electrocautery. The tumour was referred to the Section of Pathology of the Veterinary Research Institute, Onderstepoort, for histopathological examination.

#### BIOPSY EXAMINATION

The formalin-fixed biopsy specimen consisted of a single, smooth, greyish, elliptical mass, which, when incised, had a homogeneous, rubber-like texture. The cut surface was yellowish-grey with brownish-black streaks running from the base toward the apex. Microscopic examination revealed a highly vascular tumour composed of bundles of haphazardly arranged spindle-shaped cells, which in some areas appeared orientated around the abundant vascular spaces. The nuclei were uniformly oval and contained finely reticulated chromatin that was slightly hyperchromatic. There were from one to three prominent basophilic nucleoli. The eosinophilic cytoplasm was finely fibrillar and appeared to blend with adjacent cells, making it impossible to distinguish cell outlines. A few cells contained

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\*\*\*155 Oliver Street, Brooklyn Circle, Pretoria.



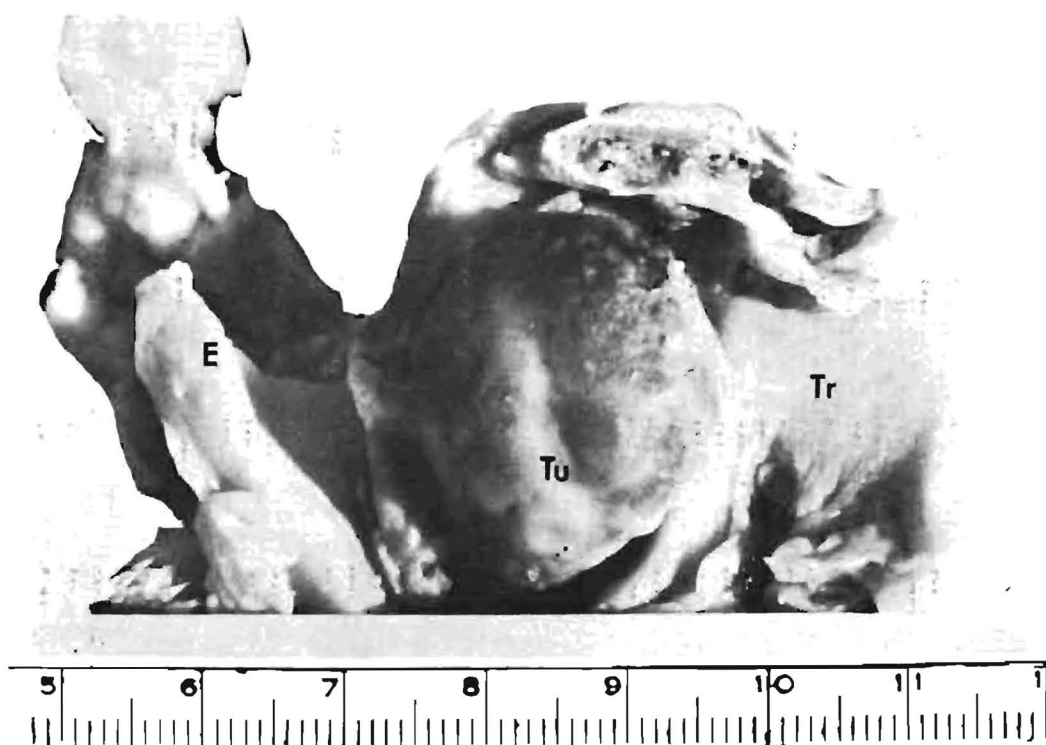


FIG. 1. Melanoma growing out of right lateral ventricle of the larynx. E—epiglottis, Tu—tumour, Tr—trachea.

a fine, granular, yellowish-brown, isotropic pigment, which disappeared with standard bleaching techniques. A few mitotic figures of the normal type were observed scattered randomly throughout the mass. The free surface of the biopsy was composed of flattened tumour cells in various stages of degeneration and necrosis, admixed with extravascular erythrocytes and leukocytes. A diagnosis of melanoma was made.

#### POST-SURGICAL HISTORY

The dog recovered from anaesthesia with no evidence of the dyspnoeic syndrome and was taken home the following day. Unfortunately, within two weeks similar signs had developed and a similar lesion was again observed in the same area. The owners requested euthanasia; the dog was killed by administering an overdose of a barbiturate anaesthetic.

#### POST-MORTEM EXAMINATION

A necropsy was performed immediately after euthanasia. Examination of the larynx revealed a large, pear-shaped polyp attached to the base of the right lateral ventricle. The ventricle was extremely dilated and distorted by the expansive nature of the tumour (Fig. 1) which had forced the vestibular and vocal cords apart; it had completely filled the laryngeal cavity. The mass measured 2.7 cm from the rather narrow base to the apex and 2.0 cm at its widest point. The appearance, colour and texture closely resembled that of the biopsy specimen taken two weeks earlier (Fig. 2). The subjacent cartilage was intact and unaltered by the neoplasm. Microscopic findings on the tumour were markedly similar to those on the biopsy sections; it was considered to be a recurrence of the original melanoma.

A retropharyngeal lymph node found adjacent to the larynx was markedly en-



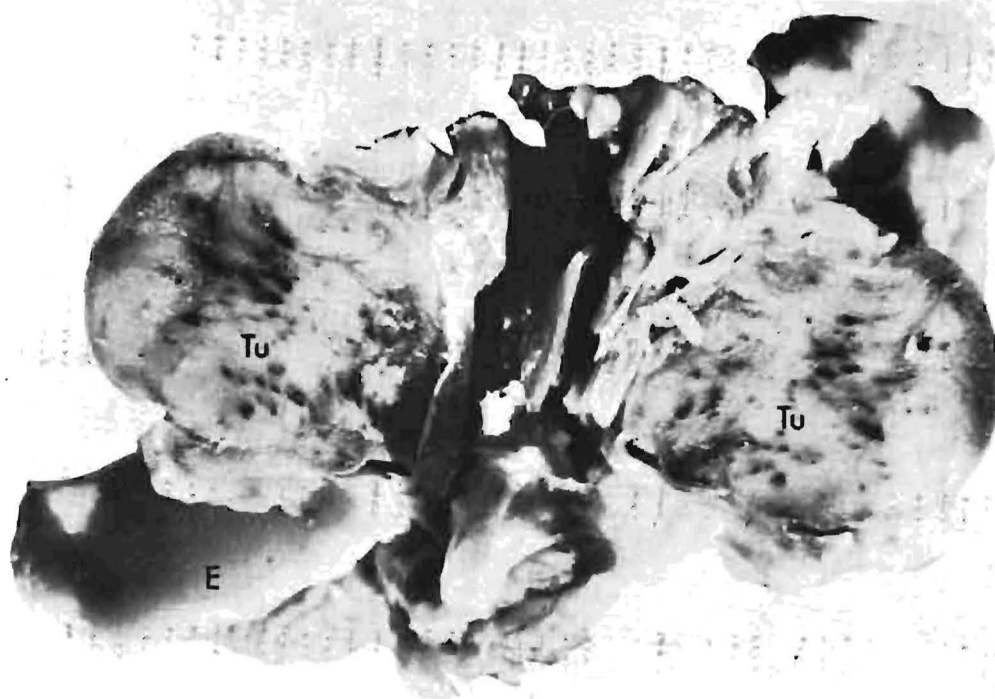


FIG. 2. Cut surface of the tumour showing pigmented streaks. E—epiglottis, Tu—tumour.

larged ( $4,5 \times 1,5 \times 1,0$  cm). Microscopic examination proved this to be a non-neoplastic, inflammatory lesion. The remainder of the post-mortem examination failed to reveal any evidence of metastasis.

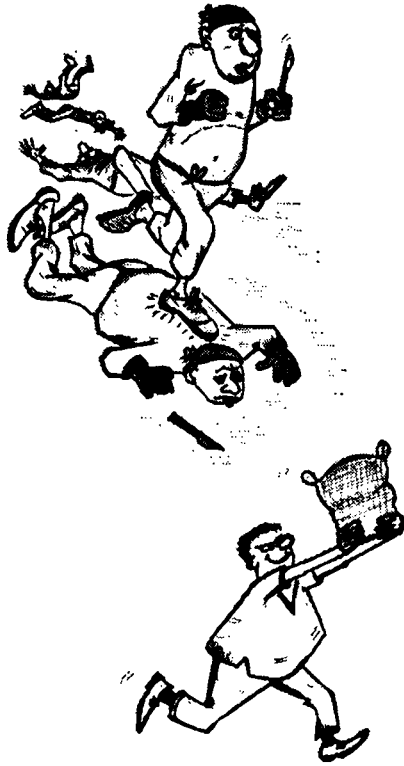
#### DISCUSSION

Tumours of the larynx of animals are uncommon and those found are usually of squamous epithelial origin. Occasional osteomas and chondromas have been observed. Surprisingly, since melanomas are the

most common oral tumour, to our knowledge they have not been described in the larynx. The case reported here presents the common problem of differentiating benign from malignant melanomas. If the tumor had been allowed to progress, it would certainly have caused the death of the dog by asphyxia, despite very little evidence of invasiveness and no metastatic activity. The microscopic indices of malignancy in this case were equivocal, although the minimal production of melanin and moderate numbers of mitotic figures would certainly suggest malignant tendencies.



# CYTOGENETICS - SITOGENETIKA





CASE REPORT

GEVALVERSLAG

PSEUDO-COWPOX (PARAVACCINIA) IN DAIRY COWS

W. H. GIESECKE, A. THEODORIDES AND H. J. ELS\*

Pseudo-cowpox or paravaccinia, also known as milkers' nodules on account of the lesions that may be caused on the hands of milkers of affected cows, occurs commonly in dairy herds. It is mainly significant inasmuch as it lowers milk production, affects udder health, predisposes to mastitis and may infect human beings by contact with the lesions.

In a hand-milked Friesland stud some 48 of the 60 lactating cows suffered from pox-like teat lesions, in various stages of development and involving the base, tip,

external orifice as well as the teat proper. The affection caused some cows to become restless during milking to the extent that



FIG. 1 & 2. Teat lesions due to paravaccinia infection.

some had to be hock-tied, an unusual procedure on this farm.

Lesions occurred on pigmented as well as unpigmented skin, and on a few animals the lesions were acute, consisting of an erythematous eruption or small vesicles. In most instances the lesions were chronic or subacute, circular in shape, 3 to 10 mm in diameter (Fig. 1) and consisted of granulomatous tissue covered with a relatively thick

\*Veterinary Research Institute, P.O. Onderstepoort.





FIG. 3. Paravaccinia virus ( $\times 200\,000$ ).

and rather firmly adherent, slightly elevated, brown scab. As healing progressed centrally, the lesion appeared to extend peripherally, resulting in a ringlike or horseshoe to crescent-shaped mark (Fig. 2). Some teats had circular, compact or extensive lesions covered with a thin yellow, yellowish-brown to grey scab. The process of washing or milking tended to dislodge some scabs and expose the underlying granulomatous tissue.

It is considered significant that none of the calves and heifers, nor the cows which had been dry for more than 2–4 weeks, suffered from any similar lesions. In this instance, no lesions (milkers' nodules) could be found on the hands of the Bantu milkers.

Electronmicroscopy ( $200\,000\times$ ) of scab material stained negatively with 2% phosphotungstic acid revealed paravaccinia virus particles (Fig. 3). The particles are cylindrical with convex ends and measure approximately  $300\text{ nm}\times 150\text{--}180\text{ nm}$ ; the surface is criss-crossed by a regular thread pattern<sup>1</sup>.

#### ACKNOWLEDGEMENTS

Photography of clinical material: A. M. de Bruyn.

#### REFERENCE

1. Lieberman H., Schulze P. & Liebermann H. 1967 *Arch. exp. Vet.-Med.* 21:1419



## CLINICAL NOTES

## KLINIESE AANTEKENINGE

### TREATMENT OF LUXATION OF THE TAROMETATARSAL JOINT OF THE DOG

P. H. LE ROUX\*

Ventral support of the tarsometatarsal joint of the dog is provided by a strong sheet of tissue made up by elements of the medial and lateral collateral ligaments, plantar ligaments and the joint capsule. This support is very rigid; when the joint is subjected to sudden, severe strain this tissue ruptures catastrophically. Such an injury may result when the foot is caught in the top of a gate or fence. Our attempts to reduce and immobilize this luxation by means of a plaster of Paris cast have always resulted in poor recovery. The method described here is very simple and in six cases dealt with in this manner over the past two-and-a-half years it has given perfect results.

Radiographs are taken to ensure that the fifth metatarsal bone is intact and to gauge the diameter of its medullary cavity. The skin is incised over the lateroplatar

surface of the fifth metatarsal bone to expose the bone at approximately one quarter from its proximal end. A 5 mm Steinman pin is used to drill a hole at right angles into the medullary cavity at this point. The metatarsus is aligned with the tarsus and a very sharp Steinman pin of a suitable size is drilled from this pilot hole up the cavity and through the adjacent fourth tarsal bone, while aiming at the medial face of the os calcis. The pin is notched a short distance away from the bone, driven firmly home and snapped off, leaving only a sufficient portion protruding to allow its eventual removal. The wound is closed in the usual way and a padded dressing applied. A plaster cast is applied when the patient is a heavy dog. The pin is removed after six to eight weeks. Healing is firm, with no distortion or excessive scar tissue formation and function is normal after removal of the pin.

### THE USE OF A TEASER TOM TO TERMINATE OESTRUM IN FEMALE CATS

P. H. LE ROUX\*

Female cats on heat provide a problem for breeders of pedigree cats. They are noisy and have to be closely confined to avoid unwanted pregnancy. It is generally considered that coitus will cause ovulation in the female cat in oestrus with rapid cessation of heat phenomena.

An ordinary adult tabby tomcat with a strong physique and placid temperament was prepared as a teaser tom. Under general anaesthesia a bilateral vasectomy was performed under aseptic conditions. Approximately 2 cm of each ductus deferens was re-

moved through two small incisions cranio-ventral to the scrotum. The cut ends were ligated with 2/0 chromic gut without disturbing the bloodvessels. Skin sutures were removed eight days later.

Six weeks after the operation the teaser tom was used for mating and no pregnancy resulted. Since then he had been very much in demand. After coitus the heat cycle reappeared nine weeks later instead of the usual three weeks or less. This is likely to be due to the fact that ovulation and corpus luteum formation had taken place instead of the usual atresia of the follicles.

\*Hermitage Terrace, Richmond, Johannesburg.



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## In Memoriam



WYLE M. J. N. MEESER

Op 30 Januarie 1971 is Meeser Johannes Nicolaas Meeser in die ouderdom van 58 jaar oorlede.

Dit was my voorreg om die oorledene die afgelope sestien jaar te ken, die laaste tien jaar waarvan ek op sy personeel diens gedoen het.

Die oorledene was 'n pligsgetroue amptenaar wat sy werk altyd eerste gestel het. Hy was trots op sy beroep en het altyd gepoog om die aansien daarvan op 'n hoë vlak te handhaaf. In die Afdeling Veeartsenydens was hy bekend vir sy kennis van bek-en-klouseer en veral vir sy waarnemings van die siekte onder wildsbokke. Ook op kulturele gebied het hy 'n leidende rol gespeel. Hy was onder andere lid van die Akademie vir Kuns en Wetenskap en van die plaaslike Skakelkomitee. Verder

het hy ook op vele plaaslike komitees gedien en was hy ouderling van sy kerk.

Die oorledene is op 16 Oktober 1912 te Roodepoort gebore en het sy skoolopleiding te Standerton voltooi, waarna hy na die Universiteit van Pretoria is. Einde 1936 kwalifiseer hy as veearts. Hierna het hy tot die Staatsdiens togetree en was onder andere op Eshowe, Worcester, Upington, Oudtshoorn, Calvinia, Lydenburg en Pietersburg gestasioneer. In 1954 word hy gepromoveer na Senior Staatsveearts en in 1960 na Onderdirekteur, Oos-Transvaal Streek. Op 9 Desember 1939 is hy getroud met Elizabeth Johanna Badenhorst, wat hom drie jaar gelede ontval het. Uit die huwelik is drie kinders gebore. Aan die kinders wil ons graag ons innige meegevoel betuig.

C. C.



## In Memoriam



WYLE P. G. JOUBERT

Die nuus van die skielike heengaan van Dr. P. G. („Smiler”) Joubert op 6 Januarie 1971, enkele dae voor sy 49ste verjaardag, was 'n geweldige skok vir sy baie vriende en kollegas.

Hy is op 8 Januarie 1922 te Smithfield in die O.V.S. gebore. Aan die Universiteit van Pretoria het hy in 1944 die B.V.Sc.-graad verwerf. Daarna was hy as Staats-veearts werksaam te Vryheid, Dundee en Barberton, waartydens hy met onderskeiding aktiewe Ooskuskors, hondsdoelheid, varkpes en bek- en klouseer beheer het.

Sedert 1955 het hy hom op gemengde veeboerdery toegelê in die distrik Dundee. Skaapboerdery was sy eerste liefde en sy ywer is dan ook met veel sukses bekroon. Hy was die stigter van die Dundee/Helpe-mekaar-Wolstudiegroep asook die argitek

van die Dundee-boereunie waar vyf boereverenigings onder sy voorsitterskap saamgesnoer is. Vir sy boeregemeenskap was dit van onskatbare waarde.

Vir georganiseerde landbou het hy onbaatsugtig baie van sy tyd gegee. Hy het reeds geruime tyd in die bestuur van die Natalse Wolkwekersvereniging gedien voordat hy in 1970 as ondervoorsitter verkies is. Hy het ook hierdie liggaam in die Nasionale Wolkwekersvereniging verteenwoordig. Hy was ook raadslid van die Natalse Landbou-unie en lid van die koöperatiewe komitee van hierdie liggaam, waar hy hom besonderlik beywer het vir nadere samewerking tussen die koöperasies van Natal, om daardeur beter dienste aan die boere te verkry. Hy het nooit geskroom om die koöperatiewe gedagte suiwer uit te lewe nie.



Sedert 1965 dien hy in die direksie van die Natalse Landbou-koöperasie waarvan hy sedert 1967 ondervoorsitter was. Ons haal aan uit 'n huldeblyk aan Dr. P. G. Joubert wat in NAUNLU, die amptelike orgaan van die Natalse Landbou-unie, verskyn het: „Natalse boere het 'n onselfsugtige kampvegter verloor. Die koöperatiewe beweging het 'n positiewe leier verloor. Sy vriende het 'n lojale vriend verloor. Sy plek in Natal is leeg.”

As mens was hy 'n leier by uitnemendheid wat oor besondere talente beskik het en nooit geskroom het om 'n positiewe en reguit bydrae deur woord en daad te lewer

waar dit benodig was nie. Sy opinie was dus altyd hoog gerespekteer deur almal. Hy was 'n kranige rugbyspeler en het altyd 'n groot sportgeesdriftige gebly. Met sy heengaan het die veeartsenykundige professie nie slegs 'n waardige lid verloor wat sy plek op vele terreine met groot onderskeiding volgestaan het nie, maar het ons persoonlik 'n ware vriend verloor.

Uit sy huwelik met Fransie Wessels van Dundee is twee seuns en 'n dogter gebore. Aan Fransie en die kinders, Leendert, Frans en Hannatjie, wens ons hiermee ons innige en diepe meegevoel te betuig met hul groot verlies.



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# ACTH CMC\*

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## \* Carboxy Methyl Celulose Karboksimetielsellulose

ACTH / CMC is 'n ACTH wat anders is, anders insoverre CMC 'n helder vloeistof met opaalglans verskaf, wat selfs na berging uiters vloeibaar bly, en vrywel pynloos is. Altyd vir gebruik gereed. **Geen verwarming nodig nie**, selfs op die koudste dag. Dit word maklik deur 'n naald van Nr. 18 of 25 dikte ingespuut.

### BEHANDELING

ACTH bewerkstellig 'n snelle omkeer van simptome en 'n spoedige herstel van die metabolisme wanbalans in ketose van melkkoeie. Weefselontwatering, asidose, verminderde lewerglikogeen, ketonemie, ketonurie, hipoglikemie, verminderde melkafskeiding en verlaagde eetlus kan almal deur ACTH reggestel word.

Dat die werking van ACTH snel geskied word betuig deur die spoedige verhoging van bloedsuikerpeile: betekenisvolle styging geskied binne vier uur na terapie. Binne 24 uur is die bloedglukosewaardes normaal of hoër. Na hierdie aanvanklike styging (afhangende van die hewigheid van die ketose en die dosis toegedien) bly die bloedsuiker op normale peil.

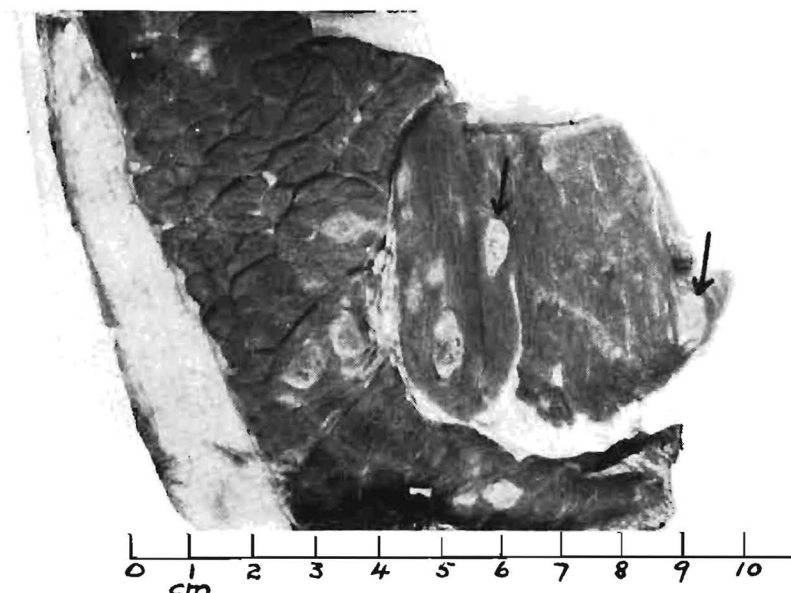
Binne 24-48 uur verbeter die eetlus merkbaar, so ook die algemene voorkoms van die dier. Gewoonlik keer die melkafskeiding binne drie dae na ACTH-terapie na normaal terug.

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#### DEEP INTRAMUSCULAR ONCHOCERCIASIS

Nodules containing *Onchocerca* are quite frequently encountered in the subcutaneous connective tissue and the *M. cutaneus trunci* of slaughtered cattle emanating from bushveld or other subtropical areas. They are characteristically situated on the lower portions of the abdomen and thorax, and are readily recognized on section: the thin filariform worms lie tightly coiled in the connective tissue capsule. Trimming out these superficially situated lesions may mutilate the carcass but leaves it otherwise fit for food.

The figure illustrates a case of bovine onchocerciasis with the nodules situated within skeletal muscle up to a depth of 9,0 cm beneath the surface of the carcass. Because of size, shape and situation they were taken for degenerated *Cysticercus bovis* by a country butcher; a thorough search and extensive excision would be required before an infested carcass could be passed as food.

Degenerative changes precluded specific identification of this *Onchocerca*.

#### DIEP BINNESPIERSE ONKOSERKIASIE

Knoppies met *Onchocerca* word dikwels in die onderhuidse bindweefsel en die *M. cutaneus trunci* van slagbeeste vanuit die bosveld of ander subtropiese gebiede teëgekomp. Hulle is kenmerkend in die laer bors- en buikstreek geleë en is geredelik herkenbaar op deursnee; die dun filaria-vormige wurms lê styf binne die bindweefselkapsel opgerol. Wegsny van hierdie oppervlakkig geleë letsels mag die karkas beskadig, maar laat dit origens geskik as voedsel.

Hier word 'n geval van beesonkoserkiase getoon met knoppies wat in die skeletspier geleë is, tot 'n diepte van 9,0 cm onder die oppervlakte van die karkas. Vanweë hul vorm, grootte en ligging was hulle per abuis deur 'n buitestedelike slagter as gedegeneerde *Cysticercus bovis* aangesien. 'n Deeglike ondersoek en ingrypende wegsnying sou nodig wees voordat so 'n karkas as geskik vir menslike verbruik gekeur sou kon word.

Weens ontardingsveranderings kon geen spesifieke identifikasie van hierdie *Onchocerca* gemaak word nie.

Submitted by: L. W. van den Heever, Dept. Pathology, Fac. Vet. Sci., Univ. Pretoria, Onderstepoort.

Ingestuurd deur: L. W. van den Heever, Dept. Patologie, Fak. Veeartsenykunde, Univ. Pretoria, Onderstepoort.

Photo/Foto: A. M. du Bruyn.