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REDAKSIONEEL

DIE FAKULTEIT VEEARTSENYKUNDE, UNIVERSITEIT PRETORIA, VYFTIG JAAR OUD

Vanjaar, en wel op 28 en 29 September, word die Goue Jubileum gevier van die enigste Veeartsenykundige Fakulteit in Suid-Afrika. Reeds in Maart 1920 gestig, is dit interessant dat die enigste ouer fakulteit met geneeskundige grondslag in hierdie land, 'n skrale twee jaar vroeër sy begin gehad het.

Die relatief-vroeë ontwikkeling van veeartsenykundige onderwys in Suid-Afrika is tot 'n uitnemende mate te danke aan Sir Arnold Theiler. Sy baanbrekende werk nog voor die eeu-wenteling het gelei tot die stigting van die Navorsingsinstituut vir Veeartsenykunde, Onderstepoort, in 1908. Hier is die tradisie van indringende veeartsenykundige navorsing verder uitgebou en dit het die owerheid terdeë bewus gemaak van die besondere belangrikheid van veeartsenykunde. So is 'n ontvanklike gees geskep vir die idee van veeartsenykundige opleiding hier te lande — iets wat weens die unieke aard van die diergeneeskundige probleme in Suid-Afrika essensieël was.

Theiler het 'n belangrike aandeel gehad in sekere vêrsiende besluite in hierdie verband bv. dat die opleidings-organisasie beplan is om akademies in te skakel op universitêre vlak, nl. by die Transvaalse Universiteitskollege van die Universiteit van Suid-Afrika, later die Universiteit van Pretoria. Die Fakulteit Veeartsenykunde was uit die staanspoor 'n volwaardige fakulteit, en Theiler was die eerste dekaan. Hoewel veeartsenykundige opleiding destyds elders oor vier jaar gestrek het, is n vyfjarige kursus onmiddelik hier ingestel.

Om verskeie redes is die Fakulteit by die Navorsings-instituut vir Veeartsenykunde, Onderstepoort, net buite Pretoria, gevestig. Die dosente was dan ook almal navorsers aan hierdie Instituut verbonde. Deur die jare het die Fakulteit 'n besondere reputasie verwerf vir deeglike fundamentele wetenskaplike opleiding, en 'n natuurlike gevolg was dat baie van sy graduandi veel wetenskaplike roem verwerf het.

Aanvanklik het gewoonlik nie meer as tien jong veeartse jaarlikse gegradueer, en vir hulle was daar feitlik net slegs twee rigtings-keuses — navorsing en velddienste in diens van die Staat.

Gedurende die afgelope dertig jaar het die vraag na veeartse in momentum sterk toegeneem en is steeds nuwer en wyer velde betree. Opleidingsfasiliteite moes steeds vergroot word, en in 1955 moes nuwe fakulteitsgeboue 'n paar honderd meters van die Instituut opgerig word om sekere departemente te akkommodeer sodat opname van studente in die tweede jaar na 30 en 1963 na 45, verhoog kon word. Onlangs is besluit dat die land se behoeftes aan veeartse alleen voorsien kan word indien 90 studente per jaar toegelaat word. Sodra die nodige fasiliteite en geboue daargestel is, sal die Fakulteit tot hierdie nuwe fase in sy ontwikkeling oorgaan.

Deur die jare was daar ook 'n geleidelike evolusie met betrekking tot die verbintenis aan die eenkant met die Universitei en aan die anderkant met die Instituut; die verhouding met eersgenoemde het allengs nouer geword. Oorspronklik was die Fakulteit, deur die Navorsingsinstituut vir Veeartsenykunde, administratief staatsverbonde. Al die dosente was deeltyds en akademies verantwoordelik aan die Universiteit Pretoria. Sedert die begin van 1958 het sekere departemente voltyds geword, dosente is op universiteitskaal gesalarieër maar is administratief nog soos voorheen. Tans word gewag op die aanbeveling van 'n kommissie wat ondersoek instel na die wenslikheid dat die Fakulteit geheel onder die Universiteit moet ressorteer. Al word daar ook besluit op gehele akademiese afskeiding van die Instituut, sal die gees van onderlinge kollegealiteit en medewerking, wat tussen die Instituut en die Fakulteit bestaan, geensins versteur word nie, aangesien dit fundeer is op 'n mooi tradisie wat reeds vyftig jaar bestaan.

Die Fakulteit kan met verwagting uitsien na sy tweede vyftig jaar, onderskraag deur die wete dat die professie wat hy dien, in 'n besondere mate diensbaar was aan die wetenskap in die geheel en aan Suidelike Afrika in die besonder. Vir die Veeartsberoep is dit van absolute belang dat veeartsenykundige opleiding steeds van die allerhoogste standaard moet wees, en die S.A.V.M.V. neem hierdie geleentheid te baat om die Fakulteit van harte geluk te wens met die prestasies van die afgelope 50 jaar.

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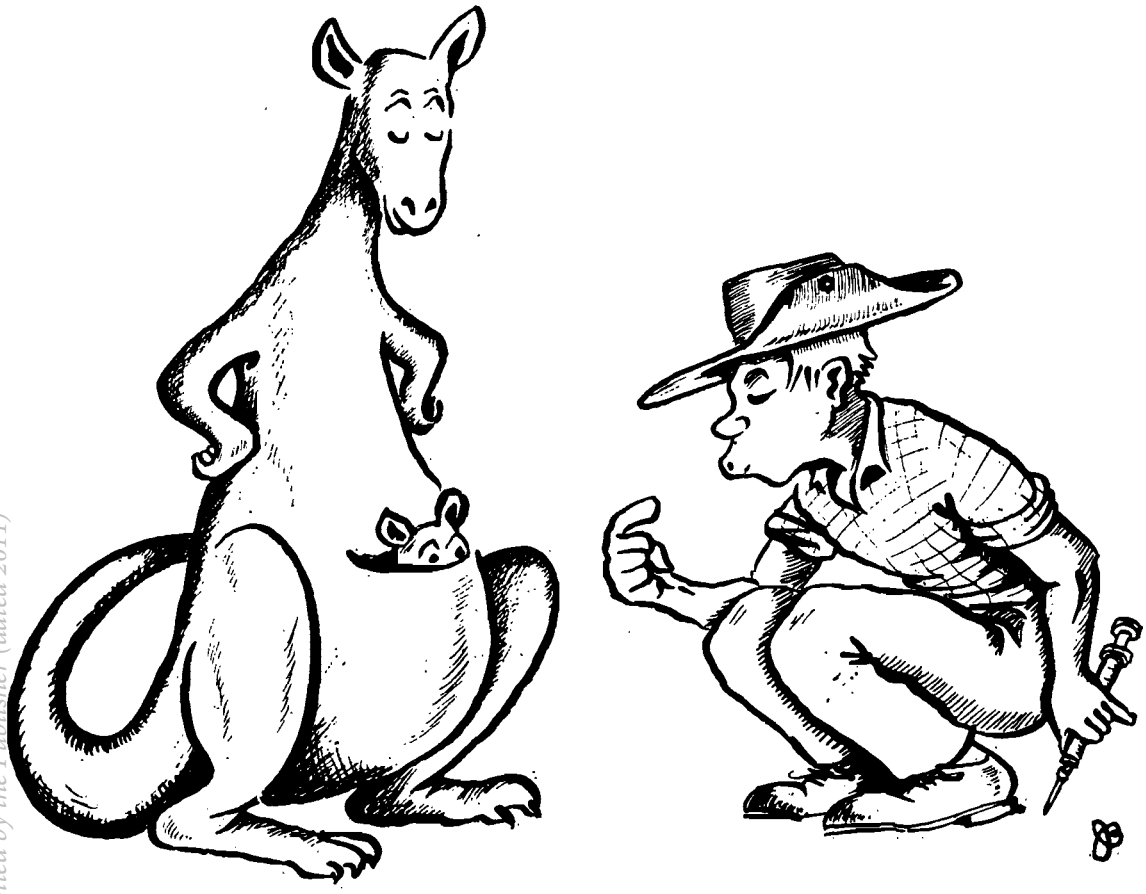
Veterinarians around the World 8



Die Veearts. Wêreldbeeld 8

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EDITORIAL

THE FACULTY OF VETERINARY SCIENCE, UNIVERSITY OF PRETORIA, FIFTY YEARS OLD

This year, on 28 and 29 September, the only veterinary faculty in South Africa will celebrate its Golden Jubilee. Veterinary education in South Africa was initiated in March 1920, only two years after the oldest medical faculty was started.

To a very considerable extent, the relatively early development of veterinary education in South Africa is to the credit of Sir Arnold Theiler. His pioneer work, even before the turn of the century, led to the founding of the Onderstepoort Veterinary Research Institute in 1908. Here the tradition of incisive veterinary research was further augmented, making the powers that be very conscious of the importance of veterinary science. Thus was created an atmosphere receptive to the idea of veterinary education in this country — a matter rendered even more imperative by the unique nature of veterinary problems in South Africa.

Theiler had an important share in the farseeing decisions taken in this connection. Training was planned to form a part of the academic organization at university level — i.e. the Transvaal University College of the University of South Africa, which subsequently became the University of Pretoria. Even though veterinaryy training of the time usually extended over four years elsewhere, a five year course was immediately instituted here.

For various reasons the Faculty of Veterinary Science was located at the Onderstepoort Veterinary Research Institute, on the outskirts of Pretoria. All members of the teaching staff were research workers attached to this Institute. Over the years the Faculty gained a reputation for thorough basic scientific education, and as a natural sequel many of its graduates became famed scientists.

Initially, less than ten veterinarians graduated annually. For many years these young veterinarians had the choice of only two avenues of employment: research or field work in State employ. However, during the past 30 years, the demand and scope for veterinarians has gained in momentum. Training facilities had to be increased from time to time and eventually new faculty buildings had to be constructed a few hundred metres from the Institute to accommodate certain departments so that the numbers of students admitted to the second year could be increased to 30 in 1955 and in 1963 to 45. It has been established recently that the needs of the country can be met only if 90 students are admitted each year. As soon as additional facilities and buildings have been provided, the Faculty will enter this new phase in its development.

Through the years there has been a gradual evolution regarding the relationship with the Institute on the one side and the University on the other, ties with the latter becoming more and more intimate. Originally the Faculty was connected administratively to the State through the Onderstepoort Veterinary Research Institute, all lecturing staff being appointed on a part-time basis and being academically responsible to the University of Pretoria. At the beginning of 1958 certain departments were made full time, and lecturing staff were placed on university scales; administratively there was no change. Now we are awaiting the report of a commission appointed to investigate the desirability of the Faculty becoming solely responsible to the University.

Even should the Faculty secede from the Institute, the spirit of mutual goodwill and co-operation will not be disturbed, founded as it is on a fine tradition of fifty years.

The Faculty can look forward with hopeful anticipation to its second fifty years, secure in the knowledge that the profession which it serves has rendered yeoman service to science at large and to Southern Africa in particular. To the Veterinary profession it remains of the utmost importance that veterinary training be maintained at the highest possible standard. The S.A.V.M.A. congratulates the Faculty on its achievements over the past 50 years.

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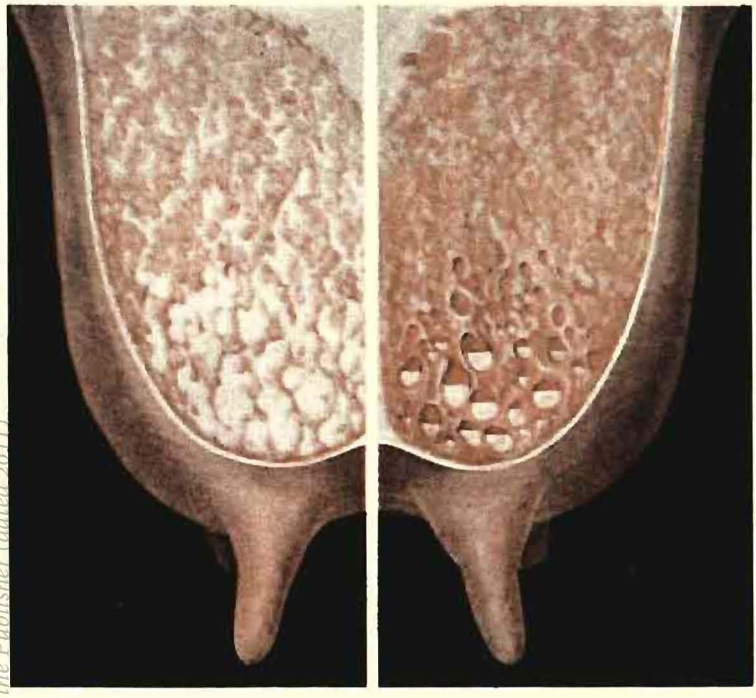
Spring mastitis voor eer u pasiënt nog „Moee" kan sê



Die illustrasie toon aan hoe nuwe Metibiotic-Mastitisskuim, as dit ingespuit 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.

Beproefde formule, verminder weefselbeskadiging. Aktiewe bestanddele sluit in: Twee beproefde antibiotika (penisillien-G-prokaeien 100,000 eenhede, dihidrostreptomisien 300 mg.) en een kortikosteroïde (prednisoonasetaat 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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EDITOR'S NOTE

SYMPOSIUM ON THE PRODUCTION AND USE OF LABORATORY ANIMALS

A symposium on the production and use of laboratory animals was organised jointly by the South African Medical Research Council and the Council for Scientific and Industrial Research and held at Scientia, Pretoria on the 3rd, 4th and 5th of June, 1970.

The papers presented by the various delegates are of great interest, not only to all those engaged in Veterinary and Medical research, but also to general practitioners, teachers and those engaged in other branches of our profession.

We are grateful to the Presidents of the Medical Research Council and the Council for Scientific and Industrial Research, and to the organisers of the symposium, for making available to us a selection of papers for publication in this number. A further selection will be published in the following number.

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Jl S. Afr. vet. med. Ass. 41 (3): 142-146 1970

THE ETHICS OF ANIMAL EXPERIMENTATION*

I. F. H. PURCHASE**

THEN GOD SAID;

"Let us make man in our image and likeness to rule the fish in the sea, the birds of heaven, the cattle, all wild animals on earth, and all reptiles that crawl upon the earth."

New English Bible. Genesis 1:26.

To the fundamentalist ethical considerations are relatively clearly defined; to the ordinary scientist these involve the basic essentials of morality and especially in the field of animal experimentation the scientist is faced with difficult decisions when considering whether his work is morally defensible. Most people will agree that there is nothing ethically wrong in using animals for the betterment of mankind. The reason for this is that man is considered to be at the top of the animal kingdom. His pre-eminent position in the animal world gives him the ability, if not the right, to use other animals for the benefit of all animals, including man.

Those who cannot accept this must consider whether the tremendous strides (which have depended on animal experimentation) made by man in combating disease (polio, plague, tetanus, scarlet fever: to name but a few) should have been prohibited and millions of humans, including probably themselves, allowed to suffer from crippling or mortal disease as a consequence of this attitude. In simple terms, should we be prepared to shorten the lives of laboratory animals in order to have extended the life of Mozart or Einstein?

If we accept that animal experimentation is ethically permissible it does not give us a free hand in doing whatever experiment we like on our laboratory animals. Ethical obligations require us to consider very carefully whether the experiment we propose doing is necessary and particularly whether the pain or discomfort to the animals which is likely to result is reasonable in relation to the type of results we hope to obtain and the benefit which society may gain from the results. In other words, we must be sure that we are not unnecessarily cruel to experimental animals.

Ethics not only requires that we control the type of experiment being executed but demands that management and care of the animals is considered as carefully as is the experiment itself.

The privilege provided by our ability to use animals for experimental purposes carries with it the responsibility of caring for the animals, for when an animal is placed in the artificial environment of the laboratory, it becomes wholely dependent on the animal keeper. The animal keeper then has a definite ethical responsibility to keep the experimental animals under conditions which are comfortable and clean and which are ideally at least as good as those in the natural habitat of the animal. He also has the responsibility to provide living conditions which will provide optimum results from the experiment. Keeping animals for experimentation in suboptimal conditions is therefore unethical and in my opinion can be defined as cruelty.

From the viewpoints described above, it is obvious that ethical considerations are judged on the basis of creulty to animals. It is unethical to be cruel to experimental animals; whether the cruelty concerns experimentation or management is immaterial.

EXPERIMENTAL TECHNIQUES

In considering ethical aspects of experimentation and the possibility of cruelty occurring, it is convenient to examine the various types of experiments which are carried out on animals.

1. Many experiments, particularly those associated with nutrition, breeding, environment and parasitic infestation do not produce discomfort or pain. In many cases the benefit is to the animals themselves and cruelty certainly does not occur.

* Paper presented at the Symposium on the Production and Use of Laboratory Animals.

** Division of Toxicology, National Institute for Nutritional Diseases, S.A. Medical Research Council, Pretoria.

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2. Large numbers of animals are used in "screening" tests, e.g., toxicity testing, diagnosis of infectious diseases or vaccine production. The actual amount of pain and discomfort caused directly by the research worker is very limited. It may involve an oral or parenteral injection or dermal application. The paradoxical aspect of these types of experiments is that discomfort produced by the noxious agent, be it chemical or infectious, may be extreme. However. these experiments are of undoubted benefit to the community and to individual human beings, so that although suffering of the experimental animals undoubtedly occurs, they can be justified. In many experiments the endpoint is death of the experimental animal. While these experiments should be limited to a minimum, the fact that they produce the death of the animal does not mean that they should be abandoned. After all, how many of us do not eat products which can be produced only from animal carcasses?

3. A growing number of experiments are being conducted in the field of psychology where the animals used are nearly always conscious during experimental procedures. In addition, they are often used in experiments when fully conscious after recovery from surgery. Some kind of reward is normally given in such experiments, but often mild punishment is administered. In these cases it is important that the punishment to which the animals are subjected should be kept to a minimum which is compatible with the proper execution of the experiment.

Certain psychological experiments require destruction of the brain. There is no doubt that these can cause considerable discomfort in the form of pain or as a consequence of gross disturbance of locomotion or of producing a constant state of fear. Such experiments, which may give extremely important results, should be performed only after careful consideration of their necessity, and should be of as short duration as possible.

In any case, any experiment using pain as part of the design should be designed with the utmost care so that wastage of animals is avoided. Although inflicting pain on animals may be considered necessary, if the number of animals suffering pain was excessive as a result of poor experimental design, infliction of pain is clearly unethical and would result in cruelty.

4. Physiological experiments and experiments requiring surgery are probably the experiments which are misunderstood by the general public with the greatest ease. Experiments of this type — often referred to as vivisection — provide an extremely emotive subject to those who consider it their task to prevent cruelty to animals. The picture of animals being tortured by white-gowned tormentors with blunt instruments — and all just for the fun of it — haunts the anti-vivisectionist. But here again we have a paradox, because the experimentalist requires good anaesthesia in order to preform his task adequately and suffering or pain is definitely not produced during this type of surgical intervention. At most the animals suffer during recovery from anaesthesia, but again it is in the best interests of the experiment to control pain and discomfort by adequate use of drugs.

In this category fall the experiments on animals by students merely to gain surgical skill. This type of experiment is encouraged in the U.S.A., but is specifically prohibited in Britain. This difference in legislation has led to comments such as the one by Visscher[1] who referred to it as — "... The Cruelty to Animals Act of 1876, which Sir Osler called 'the drastic act which has so hampered medical research in the British Isles'. This characterisation has proved to be tragically correct, especially in surgery." This subject may be debated at length, but my personal feeling is that experiments on animals which are done only to increase surgical skill are not ethical. This comment is particularly true for experiments performed by high school students where such experiments as implantation of brain electrodes, survival surgery and abuse of pregnant animals to produce malformed offspring are commonly executed by students aged 12 to 18 in the U.S.A.[2]

5. Other types of experiments in fields such as endocrinology and pharmacology must be considered in the same light as those described above. The special case in this field concerns experiments which require the infliction of pain for the production of shock. It is possible to quote numerous methods of inducing shock in experimental animals, many of which are certainly unethical by my standards. Making incisions, breaking bones and crushing and burning tissues on animals which are not anaesthetised are certainly unethical. There can be no accept-

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able motivation for experiments of this type and it is doubtful if anyone can assert that the omission of anaesthesia in these experiments is necessary in order to advance medical knowledge.

It can be seen from the foregoing that, by applying the principle that the duration or degree of discomfort to which the animal is exposed must be balanced against the benefits likely to arise from the experiment, we can obtain an acceptable idea of the ethics of inflicting such discomfort. The advances in the evolution of humaneness in animal experiments must largely be due to this type of thinking and to the tendency to judge individual experiments on the basis of experimental animals being "God's creatures" and not just men's tools.

MANAGEMENT

The criterion that the conditions under which experimental animals are kept must be at least as good as those of the animals' natural environment may seem relatively simple. It is complicated by the results of restriction of space in experimental facilities in comparison to natural habitats. The type of "urban" living in experimental animal colonies brings with it diseases seen only in caged animals. Good examples of these are the pneumonias seen in rodents and the enteritis seen in primates, although every species has its own disease. The amount of effort required to eradicate these diseases may be phenomenal as is seen in the S.P.F. techniques and the air-conditioning required in good animal facilities.

A report in 1966 indicated that almost half the animal facilities in the U.S.A. were substandard[3]. The figure in South Africa is, I am sure, much higher. The authorities who finance animal facilities all too often feel that money spent on expensive facilities is wasted. After all, rodent colonies have been kept in unheated, unventilated rooms for years, so why should there be any improvement now? This is borne out by the fact that there is, to my knowledge, only one S.P.F. unit in South Africa at the moment. Fortunately this approach appears to be waning and a more enlightened attitude which considers that animal facilities should be of the same high standard as research workers and results, is replacing it.

Another attitude which parallels the preceding one argues as follows: "I am using 20 of this species per month. When I bring them into the colony half die from disease before I can use them for experiments, so I must order 40 per month." To me this attitude is indefensible and unethical and results in cruelty. To sacrifice the animals by allowing them to die of preventable disease breaches all the codes of ethics and humanity. Not only is there a waste of experimental animals, which in itself is unethical, but the animals are allowed to suffer and the experiments executed on the survivors are bound to be influenced by intercurrent disease. Those who say that nothing can be done about this type of mortality have either not put enough effort into controlling it or are not adequately trained to do so.

Poor management, which allows disease in an animal colony, has another consequence influenced by ethics. Many species suffer from diseases which are transmissible to man. These zoonoses may be fatal to animal handlers and their families as was tragically shown in the outbreak of African Green Monkey disease in Marburg, Germany, recently. It is the ethical responsibility of all those in charge of animal units to design and enforce procedures which will protect their personnel from infection from the animals which they handle. This responsibility overrides any possible cost involved in the protection.

CONTROL OF ANIMAL EXPERIMENTS

There are many ways, both legislative and voluntary, of controlling animal experiments. The variation in control is exemplified by the great variety in the ways that different countries exercise such control. In Britain each experimenter and each institution must be registered before experiments may be performed. In some cases, e.g. the use of relaxants in conscious animals, the experiment itself together with the number of animals must be registered and approved before it is executed. In the U.S.A. control is exercised legally (Public Law 89-544). This Law requires the keeping of records and the setting of standards to govern humane handling, care and treatment and transport of animals by dealers and by research facilities. It is much less restrictive than the British Law and yet it received a tremendous amount of adverse publicity and opposition. Other methods of control in the U.S.A. include the voluntary accreditation of laboratory facilities and the control exercised

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by journals in publishing only research which has been executed according to "Guiding Principles in the Care and Use of Animals" approved by the Council of the American Physiological Society. These state that:—

> "Only animals that are lawfully acquired shall be used in this laboratory, and their retention and use shall be in every case in strict compliance with state and local laws and regulations.
> Animals in the laboratory must receive every consideration for their bodily comfort; they must be kindly treated, properly fed, and their surroundings kept in a sanitary condition.
>
> Appropriate anaesthetics must be used to eliminate sensibility to pain during operative procedures. Where recovery from anaesthesia is necessary during the study, acceptable technic to minimize pain must be followed. Curarizing agents are not anaesthetics. Where the study does not require recovery from anaesthesia, the animals must be killed in a humane manner at the conclusion of the observations.
>
> The postoperative care of animals shall be such as to minimize discomfort and pain, and in any case shall be equivalent to accepted practices in schools of Veterinary Medicine.
>
> When animals are used by students for their education or the advancement of science such work shall be under the direct supervision of an experienced teacher or investigator. The rules for the care of such animals must be the same as for animals used for research."

In South Africa there is at present no law specifically controlling animal experimentation, although one is in preparation.

The imposition of legislation produces a variety of vigorous responses from the public and scientists alike. On the one hand we have the opinion "I know of no reason except prejudice for preferring that an animal rather than a random human being should suffer a given amount of pain." On the other hand, "The lurid stories told by the noe-antivivisectionists about terrible cruelties being inflicted on animals in studying shock, for example, fail to explain that many humans have suffered at least the same degrees of pain and agony, and the same death to which the animals are subsubjected..."[1]. Probably the middle path is the more balanced opinion, "...experiments which cause agony are not justified. Neither are they needed in my opinion. I have never witnessed the extensive burning of unanaesthetized animals or the deliberate production of extreme pain... If experiments of this type must omit anaesthesia in order to advance medical knowledge I am not aware of an acceptable rationalization of them. Whatever knowledge is gained by such experiments is not worth the price"[4].

The legal control of animal experimentation is only as good as its enforcement. In the long run the onus lies on the experimentalist to see that his experiments do not violate ethical standards. Some of the guide lines which might be of interest are described below:—

1. Ethical use of experimental animals includes consideration of their procurement. Animals must be obtained from reputable sources and subjected to the same standards of management and treatment during transport to the laboratory as they receive thereafter. These remarks are aimed mainly at animals which are also domestic pets. It was largely in an effort to control pet stealing that the legislation was introduced in the U.S.A.

2. The experimental worker should be responsible for his experimental animals at all times. It is not necessary to care for them himself; but he should know exactly where they come from and how they have been treated at any point during the time they have been under his care.

3. Animals should always be tamed and familiar with the situation in which the experiment is to be performed. Unless this is done, the animals, by virtue of their wildness and struggling, are bound to suffer discomfort at the very least and at the most they may be injured and may suffer pain or death.

4. The conditions under which the animals are kept must be sufficiently good to prevent experimental work being deleteriously influenced by the health of the animals. In many cases this may require the expert

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advice of a veterinarian or someone with specific experience in this field. Unfortunately, no training course for animal technicians is available in South Africa, and until it is, we shall continue to have trouble in obtaining the correct type of technical assistance.

5. In designing experiments statistical advice is often essential in order to obtain the optimum results from the experiment. In other types of experiments, particularly those in which anaesthesia is used, advice from a qualified person (veterinarian or pharmacologist) may be necessary in order to provide the correct anaesthesia or sedation. This is particularly so for experiments which may be influenced by the anaesthetic and is essential for experiments in which muscle relaxants are to be used. The wish to do experiments does not confer the necessary knowledge of pharmacology and anaesthetics to the experimentalist.

CONCLUSION

The reason for the debate on ethical standards in animal experimentation is that the human being, in common with other animals, does not have the instinct to be kind to his fellow creatures. The only way to develop this humaneness is by education, specifically aimed at providing knowledge of the responsibilities of the scientist and of the optimal conditions under which animals should be kept. Such education is sadly lacking in South Africa and my plea is that the education of people involved in animal experiments should be instituted at two levels. Firstly, we should be in the position to educate animal technicians. Secondly, we should have a specific course for graduates who are using animals for experiments which would educate them in the correct methods of management and anaesthesia. Only when we have adequate education will our ethics be adequate.

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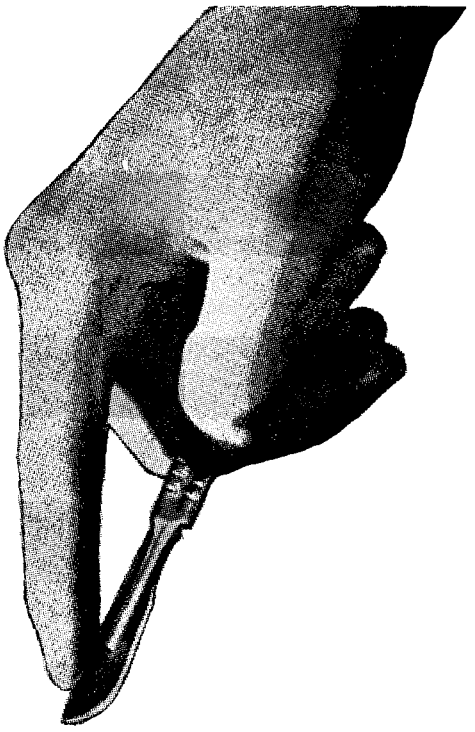

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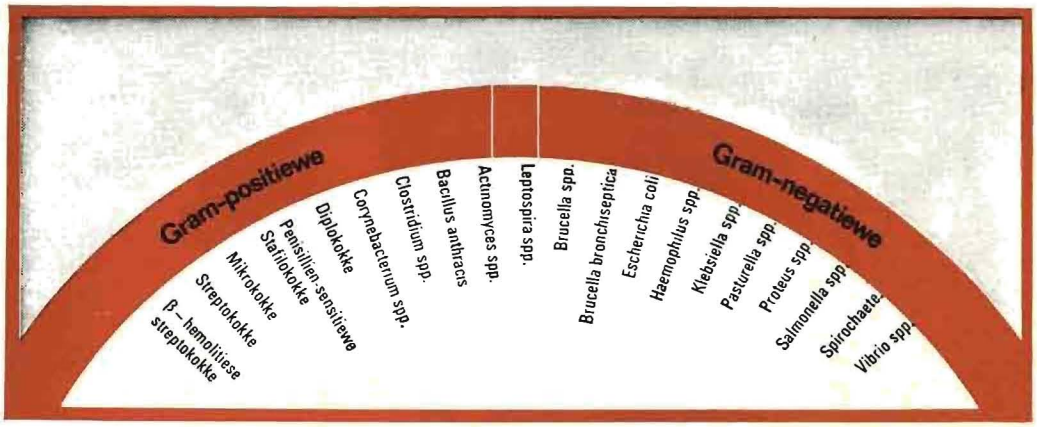

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Jl S. Afr. vet. med. Ass. 41 (3): 149-155 1970

"NORMAL, HARDY ANIMALS"

W. LANE PETTER*

"The value of special strains of animal in short range studies was recognized but the use of such strains in regular toxicity studies was strongly opposed. Strong opinions against the use of pathogen free rats were voiced; these rats have severe liabilities with respect to incidence of spontaneous tumors and lack of resistance to infection. The use of abnormal or specifically diseased animals for safety evalution studies was specifically condemned because of the danger of obscuring and failing to recognize important effects of a drug. Normal, hardy animals were preferred."

This quotation is from the Report[1] of the Commission on Drug Safety in the U.S.A. The Commission certainly knew what it wanted, but the uncompromising expression of its views was disconcerting. What did they mean by the words "normal" and "hardy"? Are these qualities recognized by the animal producer?

The quality of a laboratory animal is governed by the interaction of its genetic structure with its environment. For characteristics of high heritability, such as spontaneous tumor incidences and certain specific susceptibilities to infections or to drugs, the strain is of great importance, while inbred strains will be needed for work entailing homotransplantation of tissues. But in much other work, such as toxicity testing or the screening of new compounds, strain is of much less importance; as the subcommittee said, a normal, hardy animal is wanted, and this will be achieved for the most part by regulation of the environment in which the animal is raised and kept.

THE ENVIRONMENT

The main factors in an environment are physical conditions, care, nutrition and infection. The physical conditions include temperature, humidity, light, noise and the quality of the air to which the animals are exposed. Books and codes of recommendations have been written about the design and construction of animal houses, the conditions that should obtain within them, and they way they ought to be conducted. Many of the recommendations accepted today are valid, but they rest to a large extent on deductive reasoning rather than on observation and experiment, and must thus be continually open to revision. Perhaps some of what follows in this paper will lead to reconsideration of certain aspects of the physical environment, but no detailed discussion of bricks and mortar is appropriate here.

Animal care is a vast subject in its own right. In Great Britain an Animal Technicians Association came into being in 1950, entirely as a result of the efforts of animal technicians: this association has now become the Institute of Animal Technicians, and it holds examinations and grant diplomas at three levels of competence, which are recognized through the country. Other countries are also taking animal care seriously, and offering training and qualifications to those to whose care they entrust their laboratory animals. But for the purpose of this paper it will be enough to state, categorically, that good animal care is indispensable, and without it no other perfections in the animal house will ensure good animals.

NUTRITION

Nutrition can have a profound effect on the quality of the laboratory animal. Since laboratory animals are totally dependent on the food that is provided for them, and have no possibility of fending for themselves, it follows that their diet must be complete and adequate in every respect.

* Paper presented at the Symposium on the Production and Use of Laboratory Animals.
** Consultant: Scientific Advisor to the Huntingdon Research Centre.

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If this is a truism, it still needs stating, because even today signs of dietary deficiency are not rare in laboratory animals colonies.

Most diets are in the form of compound pellets, the formula for which is designed to contain every known essential nutrient for the species, in proper amounts. However, a formula gives only the appearance of standardization, because ingredients can vary according to source, season and conditions of storage, and the keeping qualities of the finished product are also variable. Moreover, even today the last word has not been said about the nutritional needs of any species of laboratory animal, and it is also evident that there are strain differences within the same species. Thus, a strain of rat generally known as CFE appears to have a requirement for vitamin K considerably higher than that of other strains, though it is not known whether this is an inherent characteristic or dependent on other non-genetic factors[2,3].

A recent handbook published under the auspices of the Laboratory Animals Science Association[4] gives a useful review of the nutritional requirements of rats and mice, and further handbooks dealing with other species are on the way from the same source. This handbook does not however, give recommended formulae: this is left to the compounder to work out for himself.

Given a satisfactory formula, the problems of making up a good diet have only begun. The choice of ingredients is wide, and varies in different parts of the world. The compounder has to choose ingredients that are themselves of good and known quality; are readily obtainable at all times; are mutually compatible when mixed; will endow the mixture with good keeping qualities; are palatable; do not contain toxic substances, antibiotics or other things that will adversely affect the experimental animal; will compound well, forming a good pellet; and are, given the other requirements as economical as practicable. Compounding feeds for laboratory animals is rather more exacting than compounding for farm animals. although not quite up to pharmaceutical standards. And when the diet has been formulated and compounded, it has to be tested. Too often a diet has been presented, and even captured a substantial market, that has been tested for a limited time on a relatively small number of animals under conditions of little or no stress. The use of such a diet for longer periods, on animals intensively bred or experimentally stressed, is much more likely to bring to light its deficiencies. A new diet cannot be regarded as adequate unless it can support maximum growth and reproduction over at least two generations without any evidence of deficiency, and unless it is beyond criticism in physical presentation, palatability, storage quality, and in the ability to withstand sterilizing procedures. If after all this it is cheap, either a miracle or an illusion should be suspected[5].

INFECTION

In recent years the greatest attention has been given to the problems of infection in laboratory animals. All laboratory animals are associated with microflora (unless they have been rendered germfree) and the nature of this microflora influences the quality of the animals often profoundly. Germfree animals are by definition "free of all demonstrable micro-organisms and parasites", and to remain so they must be kept in a sterile closed system; in short, in isolators. Germfree animals are one class of gnotobiotic animals, which are defined as those "of which the microfauna and microflora are specifically known in their entirety"[6]. The definition of gnotobiotic animals necessitates that they shall always be kept in isolators; when the isolator breaks down or the animals are removed from it they immediately cease to gnotobiotic, because in any open system they are exposed to and acquire an unknown collection of micro-organisms. It should be emphasized that an open system consists of anything short of an efficient isolator; partial barriers that discourage but do not absolutely prevent the passage of micro-organisms, are thus open systems.

Such barriers may, however, constitute a very useful degree of discouragement, and animals that start by being gnotobiotic and are placed within them may acquire additional microflora at a very slow rate. Sacquet[7] has discussed the concept of pathogen-free animals. He states that "the pathogen-free animal should under no circumstances contain any germ capable of being pathogenic" and he goes on to say that "the differentiation between pathogens and non-

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pathogens has become consequently very difficult indeed". Townsend[8] has reported on the Laboratory Animals Centre's scheme for categorizing laboratory animals according to the presence or absence of a list of micro-organisms that may be regarded as pathogens, as demonstrated by regular sampling and screening. In the absence of clear definitions of pathogens and non-pathogens (which may not be mutually exclusive, for in different circumstances the same organism can be either) it is difficult to attach merit labels on the basis of limited investigations, and indeed the whole concept of microbiological screening is now at issue. What is a sufficient sample? In the case of an infection with high incidence, a small sample is adequate, but if this incidence is, say, 10% in the colony, and the chance of obtaining a positive culture from an infected sample is 20% (for some infections these are reasonable suppositions) the chance of spotting the infection is 1 in 50, and for a negative result the sample size would have to be rather large to carry conviction.

In the bacteriological laboratory there is always a risk of false negative results and, perhaps less frequently, of false positives, and these are to be added to the uncertainties of sample size in microbiological screening. It should be evident, therefore, that an open system, even with a barrier of the kind associated with the term "SPF". is always an unknown quantity. The peripheral barrier, consisting of filtered ventilation, hyperchlorinated water, sterilized food, bedding, cages and other materials, and showers for staff, promises some measure of protection aginst invasion by pathogens, but sooner or later they will penetrate it. When this happens, the pathogens will find a virgin host population, and may run riot, so that a "pathogen-free" colony rapidly becomes converted into a virtual pesthouse.

THE BARRIER CONCEPT

Experience with many such barrier maintained units has shown that eventually a breakdown always occurs, usually after years rather than months. It is likely to occur earlier when the colony is large, the density is high or the precautions against infection are not very strict, but chance or misfortune is a factor in all cases. In fact, what happens in such units is that the inventory of micro-organisms present in the animals tends to grow by the casual acquisition of new types, among which sooner or later will be numbered a frank pathogen. But what of the potential pathogen whose arrival precedes that of the frank pathogen? At what point does the colony cease to be "pathogen-free" or "SPF"? It is impossible to give a sensible answer to this question, because of the ambiguity of the terms being used and the difficulty of exactly defining the state of the colony.

The barrier round such a unit cannot be regarded as analogous to the walls of a germfree isolator, but rather as an obstacle to invading pathogens, whose purpose is to increase considerably the period before breakdown. In this way a colony may be expected to sustain its pristine state of health, what may be called its golden age, for a useful period of time, and it can be eliminated and replaced by a newly founded colony before and not after a breakdown. Anything that can prolong the golden age will contribute to the provision of "normal, hardy animals".

PUTTING THE BARRIER ROUND THE CAGE

We have recently been re-examining the barrier concept in the light of the foregoing considerations. The point of attack of a pathogen is the animal, and it seemed sensible to place some sort of barrier as close to the animal as possible; that is, if possible, round the cage. But to put a solid wall — even a plastic sheet — round each cage would deny or unduly hinder access to the animals, and would from many points of view be unworkable.

For many years the filter cap described by Kraft[9] has been used to provide a barrier round the cage. This is nothing more than a cover or cap of filter material that allows gaseous exchanges to take place, but prevents the passage of solid particles. An efficient filter always offers some obstruction to gaseous exchange, and in practice it is found that under a filter cap there is an appreciable increase in the levels of humidity, carbon dioxide, ammonia and temperature. To manipulate the animals kept under a filter cap necessitates removing the cap, but even if the animals then have no protection from the hazards of exposure to the air of the room, the period of exposure is so short that the risk, according to Kraft, is probably negligible.

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THE FILTER RACK

But the filter cap is somewhat laborious in use, except on a small scale, and is not the whole answer to the problem of putting the barrier round the cage. The development of laminar air flow systems has shown that a curtain of air is a very effective barrier against infection, and it has the advantage that as fast as it is broken it reforms. So we developed a type of cage rack in which each cage was ventilated individually, using air that either came directly from the ventilating system, or was taken from the room, high up where the dust and bacterial load is lowest, and filtered before being delivered to the cage.

The air enters a plenum chamber at the back of the rack, from which it emerges in a horizontal sheet through narrow slits behind the cages and at a level between the top of the cage and the underside of the shelf above. In this way the cages are well and uniformly ventilated, and the animals are not exposed to dust-laden air that has come from other animals in the same room. At the same time there is no hindrance of access to the cages. Experience so far suggests that the golden age of a pathogen-free colony can be substantially prolonged, even though many of the "SPF" routines that are usually considered necessary, such as changing and showering, are abandoned.

The use of a filter rack is practical, since access to the cages is as easy as with a conventional rack. Whenever the animals have to be examined or manipulated, the cage is removed from the rack and either exposed to the hazards of the room, or preferably looked at on a type of laminar flow clean bench. The cost of the rack is little higher than that of many conventional racks. Bearing in mind that the filter rack enables one to dispense with many features of a rigidly barriered building, the extra cost of the racks may well be offset against savings on the building. The clean bench can be a much simplified version of a true laminar flow clean bench, for high efficiency filtration of air does not seem to be necessary.

But perhaps one of the most useful applications of the filter rack is in the experimental laboratory. It has often been pointed out that to breed animals in semi-isolation, so that their state of health is beyond reproach, is a waste of effort if they are later to be transferred to a laboratory or experimental room where the precautions are necessarily less stringent. The filter rack overcome this difficulty because it can provide the protected environment wherever it is used, without the necessity for specially constructed or equipped buildings.

TERMS AND DEFINITIONS

It should be quite clear that terms like "SPF", "pathogen-free", "Caesarian derived" and "minimal disease" cannot have any exact connotation. "When *I* use a word", Humpty Dumpty said in rather a scornful tone, "it means just what I choose it to mean — neither more or less."[10]. And when we use the sort of terms that have today become our jargon, we are being latter-day Humpty-Dumpties — too often without a properly scornful tone. For these imprecise terms represent loose concepts only. While we are still searching for "normal, hardy animals" they may be an ephemeral convenience, in that they indicate a derivation or a system of management that may be expected to have certain general results; but that is all.

In fact, no discontinuity in the spectrum of quality exists between the immediately post-gnotobiotic animals used to found a new colony, and the same colony that has acquired a full burden of infections, pathogenic and non-pathogenic alike. The only definable condition is the gnotobiotic state, which implies isolators and the techniques associated with their operation; and the definition is of course limited by the ability to detect the presence or prove the absence of micro-organisms. The use of gnotobiotic animals requires justification in every case, if only because of their much greater expense, and in any event they can by no means be regarded as "normal". Yet they will always remain as the essential starting point of a new colony under an open system, the inception of such a colony's golden age.

A gnotobiotic rat or mouse costs about 10 times as much to produce as a rat or mouse raised in an open system. The cost of raising such animals in a strictly barriered unit with full shower routine, autoclaving and so on, compared with a conventional unit with no pretensions to special hygenic precautions, differs by a factor of less than 2; indeed, the better food conversion ratios, higher productivity and negligible mortality and morbidity of a colony during its golden age may make the animals coming from it no more expensive than their backyard

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counterparts. It would seem, therefore, a a wise policy to aim to have such animals, but to avoid calling them "SPF"; and they will probably answer as nearly as any to the call for "normal, hardy animals".

NORMALITY

Animals of this kind are normal, in the sense that they are free from lesions due to pathogenic infections, although degenerative and other non-infective lesions may be present. But are they hardy? When exposed to the rigours of the experimental laboratory and the infectious hazards that may abound there, will they go down like skittles to the first assault of a pathogen?

Dubos[11] and others have suggested that an animal with an optimum balance of autochthonous micro-organisms will have a built-in homeostatic resistance to invasion by foreign organisms. Within limits this may certainly be true, but the limits are set by the nature of the autochthonous flora, which itself is influenced by many environmental factors, not least of which is nutrition. If such a flora is to be able to resist attempts to change it, it may be expected that the animals will need to be kept under optimal conditions. But what are optimal conditions? Is there, indeed just one right way, and countless wrong ways, to keep a rat? It would be surprising if this were so, particularly with such an adaptable animal.

Another approach is to consider what diseases or infections are likely to interfere with the experiments for which the animals are to be used, and to take specific precautions against these and only these. For rats on long term studies, respiratory disease is a major disadvantage, and therefore it must be excluded. The presence of Sendai virus in mice, with or without lesions, is an absolute disqualification for work on influenza viruses, but not for most other work.

If this more logical and particular approach to the choice of animal quality is made, the "strong opinions against the use of pathogen-free rats" are silenced.

DEFINING THE ANIMAL

Possibly the main source of confusion has arisen through the attempt to generalize in attributing standards of quality to laboratory animals. To ask for "normal, hardy animals" is almost meaningless since these two adjectives defy definition; but to ask for helminth-free rats is answerable; more particularly to specify demonstrable freedmo from a named pathogen such as *Mycoplasma pulmonis* is quite precise, and begins to approach pharmaceutical standards.

If the user is, therefore, precise in specifying what he needs, the breeder knows what he has to produce. Better still, if the two get together to discuss what is wanted in relation to what can be had and what it will cost, the problem of matching the quality to the need is on the way to being solved, without the extravagence of producing animals more expensive than their use demands, or the frustration of using animals with inconvenient susceptibilities. If in the course of an experiment lesions develop in the treated animals the experimenter wants to be sure that they are at least *prima facie*, the result of the treatment. But if similar lesions appear in the untreated controls, the whole experiment may be vitiated.

The reluctance to use specifically diseased animals, animals that are microbiologically far removed from conventional conditions, or special strains of animals, is understandable, because all such animals must be regarded as atypical; and an atypical animal may be expected to give atypical responses to experimental treatments. But when it comes to "study individual animals in depth", which the Commission on Drug Safety thought of great and growing importance, there can be no escape from seeking exact definitions of the animal in all its parameters. It would seem that "normal, hardy animals" should not be atypical, because they would not then be normal; but the study of individual animals implies that each animal shall be representative, presumably in a demonstrable way, and this can only come about through an artifically manipulated uniformity of the stock from which the animal was taken.

SUPPLY OF ANIMALS

There remains one further problem, namely the supply of animals. It is not enough to produce the right animal for the job; it must be available in the right weight or age range, the right numbers, at the time it is wanted. For many purposes the cost of the animals is perhaps one twentieth of the direct cost of the experiment for which they are to be used; and the cost of the experiment can rise sharply if all the experimental conditions are set up, but the animals

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are not there when they are wanted. The breeder, therefore, must aim to avoid on the one hand keeping the user waiting and on the other producing a surplus which has to be destroyed. To achieve this with perfect nicety is difficult, perhaps one of the most difficult tasks of the breeder.

In Great Britain the total number of laboratory animals used is rising by about 5% per annum, and this is probably a fairly representative figure for many countries. At least 80% of these animals are mice or rats. In view of the increase in the volume of medical, including pharmaceutical, research that is everywhere occurring, this increase is not expected to slacken; rather the reverse. It is perhaps time to ask whether the same results could be obtained by using fewer animals; whether the same number of animals could do more work; or whether other methods could to some extent replace animal experiments. When the pharmacopoeia specifies 10 animals, might not 5 suffice? And if 5, why not 4? Perhaps they would, if the 4 animals conformed to a much tighter specification than the 10.

There are many instances where work, such as the assay of some of the vitamins, that was formerly done on animals is now done on micro-organisms, tissue cultures or in test tubes. How far can this replacement go? There is in England a newly created Fund for the Replacement of Animals in Medical Experiments; and a resolution is before the Commission of Science and Technology of the Council of Europe which makes the same suggestion: namely, that laboratory animals are becoming obsolete.

Few if any scientists whose work compels them to use animals would agree with this suggestion, and not many would subscribe to the implication behind the suggestion that animal experiments are, of their very nature, undesirable and therefore should be abolished. There are certain propositions that need to be stated from time to time, and they are as follows:—

1. Animal experiments have provided valuable information in the past, do so today and will continue to do so as far as can be seen in the future. There is therefore a positive obligation to use animals so long as this holds true[12].

2. Most animal experiments are associated with nothing more than trivial discomfort or transient slight pain for the animal, of a degree less severe than is considered acceptable in farm husbandry. There is thus no fundamental objection to using animals for experiment.

3. The small number of experiments that are seriously distressing to the animal, and which therefore raise deep moral issues, are or should be controlled by legislation (as in Great Britain), by explicit ethical codes (as in U.S.A.), by the consensus of scientific opinion (almost everywhere), or by the conscience of the scientist (who is no worse a fellow than the layman).

Inhumanity of any kind is not compatible with a truly civilized society, however sophisticated. The experimental biologist has come in for more than his fair share of vituperation in this connection, but his case is so strong that he should not shrink from defending himself. At the same time, if his work is in any respect open to criticism on the score of inhumanity, he is the best person, and is usually also the first, to put it right.

CONCLUSION

When a group of eminent scientists asks for "normal, hardy animals", their request must be taken very seriously, even though the demand is ill-defined and apparently based on questionable assumptions. In 1964 this demand appeared almost incomprehensible, but the last six years have seen great progress in understanding the meaning behind these misleadingly simple words.

Those whose main preoccupation is with the laboratory animal, as a subject of study in its own right, have been equally guilty of paralogism, through inventing and using terms like "specific pathogen-free". They — I should say we, for I am one of them — have drawn false analogies from gnotobiotic methodology; we have imperfectly appreciated the limitations of microbiological techniques; we have had too facile an approach to nutrition; and we have almost totally ignored the study of population dynamics, especially in relation to the spread of infection (which has been regarded solely as a matter of hygiene) and the effect of the social milieu of the animals on behaviour and responses to experimental procedures.

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Today we are beginning to put our house in order, but it is only a beginning. The next 20 years will see a transformation in our attitudes and our animal laboratories that will be even more profound than that which has occurred since 1950.

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BOOK REVIEW

SYMPOSIUM RADIOAKTIVITÄT UND STRAHLENBIOLOGIE UND IHRER BEDEUTUNG FÜR DIE VETERINÄRMEDIZIN

Hannover 19-21 June, 1968. pp 292, figs 171, tables 59. Price: DM 54, Verlag Paul Parey, Berlin.

This symposium consists of 43 scientific papers ranging over a very wide field. The papers are arranged under four groups:

Group 1, The action of ionizing radiation of the animal.

Group 2. Radio-activity in the animal, in animal byproducts and in feeds of animal origin.

Group 3. The preservation of food of animal origin by irradiation.

Group 4. Application of radionuclides and ionizing radiation in veterinary medicine.

It is patently impossible to review individual articles or the groups in detail. Suffice it to say that each article is supported by an impressive array of quoted literature. A great deal of information is presented of, inter alia, the research worker, the public health and food hygiene specialist, and the clinician with strong bearings towards radiology and radiotherapy.

The contributors hail from the atomic energy agencies, veterinary research institutes and other research centres for a large number of countries in Europe (East and West) and Scandinavia.

C. F. B. H.

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Jl S. Afr. vet. med. Ass. 41 (3): 157-164 1970

THE MANAGEMENT AND USE OF LABORATORY PRIMATES FOR MEDICAL RESEARCH*

C. R. Coid**

It is well-known that during the last fifteen years there has been a large demand for simians in many countries for biomedical research. During the year 1968 a total of 8642 primates of the Family Cercopithecidae were imported into the United Kingdom and these represented the majority of primates used for scientific research, including the production and testing of vaccines. (Summary of Statistics 1968).

This present communication deals mainly with the management and husbandry of this family of primates in general and in particular *Macaca irus (M. fascicularis)* and *Macaca mulatta.* In addition a brief outline is given of some of the work for which these species have been used in Britain in recent years. It is not, of course, possible to cover all aspects, but a few selected topics of current interest are discussed.

MANAGEMENT

The basic principles of animal husbandry applicable to all species of laboratory animals for medical research also apply to the management of simians. There are, however, additional factors which must be considered when organizing facilities for using the various species. The most important fact is that many simians used in laboratories today, may carry and transmit infectious agents, sometimes fatal, not only to their own kind but also to man. It is apparent therefore, that systems of management employed to run a primate unit must take into consideration the nature of the human and animal pathogens likely to be involved, and their modes of transmission.

Principal Pathogenic Micro-organisms of Simians

The principal bacterial infections of non-human primates which present a hazard to man are those caused by *Mycobacterium tuberculosis* Shigella and Salmonella.

Tuberculosis is a relatively common disease in *M. mulatta.* A survey of the literature tabulated by Ruch[24] shows that the disease in monkeys is caused mainly by the bovine and human type of organisms, both of which cause infections in man. Pulmonary lesions in monkeys frequently cavitate and bacteria may be expelled by coughing. Such animals must, of course, be considered a serious hazard to personnel and other monkeys in contact.

The spread of Shigella infection to a human from simian species has been documented by Carpenter and Sandiford[3]. The incident occurred when a child was watching monkeys, in a "pets' corner" of a large store, and at the same time eating an ice-cream. One of the animals put out its paw to grab the cornet, in so doing, touched the ice-cream. Before the mother could throw the cornet away, the child had licked the ice-cream. Two days later the child appeared off colour and, after four days the faeces contained blood and mucus. *Sh. flexneri* 103Z was isolated from the child and also from the faeces on the floor of the monkey cage. Other cases of Shigella infections associated with monkeys have also been documented[11, 22, 24].

Transmission of Salmonella species to handlers of non-human primates must also be considered a possibility, but evidence that this has definitely occurred is lacking[23].

Certain intestinal protozoa and nematodes may also be infectious to man. Three organisms considered by Neal[19] to be handling hazard are *Entamoeba histolytica, Giardia intestinalis* and *Balantidium coli.*

The most serious microbial hazards to personnel are those created by the viruses. The principal agents known to cause disease,

* Paper presented at the Symposium on the Production and Use of Laboratory Animals.

** Medical Research Council Clinical Research Centre, Harrow, Middlesex, England.

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frequently fatal in man, are Rabies, *Herpes virus simiae*[25] and the "Marburg" agent of vervet monkeys[26].

Of 15 workers who died from *Herpes virus simiae* infection 7 had been scratched or bitten by monkeys[21] and in the case of the "Marburg" agent of vervet monkeys it seems possible that infection could have occurred through the unbroken skin[26]. It is also important to note that *Herpes virus simiae,* which appears to be of low pathogenicity for monkeys belongs to the same group as *Herpes virus hominis* which, when latent, may be activated by various stimuli[1].

During experiments in which monkeys were given immuno-suppressive treatment with antilymphocyte serum[28], the workers suggested that viral infections in the animals might have been due to *Herpes virus simiae* and after injection of antileucocytic serum (Coid and Burgos, 1967, unpublished observation) observed a herpes-like vesicle on the lip of a treated animal of the species *Macca irus.* It is important, therefore, that even after the quarantine period, when under experiment, risks should not be taken when handling monkeys or their tissues.

There are many other viral agents to be found in simians, but from the point of view of practical management it is probable that the precautionary measures adopted to reduce the risk of agents previously mentioned should be adequate to control other organisms.

Quarantine

Monkeys for experimental use are still principally obtained by capture from the wild. The crowded conditions experienced by these animals between capture and arrival at the laboratory are such that infectious diseases can easily become established and spread within a group. It is essential, therefore, to isolate new intakes from existing conditioned animals in a separate section of the building for a suitable length of time[10]. The quarantine period must be sufficient for recently-infected animals to develop signs of disease or become immune to inapparent infections. Previously it has been suggested that this should be not less than 6 weeks where not more than 2 animals are kept in a cage[9]. However, Boulger[2] has demonstrated rabies in a recently-imported rhesus monkey and in view of this it would be desirable, since the incubation period for this disease, generally speaking, is 2-8 weeks and sometimes longer[32] to extend the time of quarantine to not less than 12 weeks when animals are kept in pairs, and not less than 8 weeks where they are held in single cages. It is highly desirable to keep different species in separate rooms.

It may be of interest to know that the Ministry of Agriculture does not now permit the importation of "exotic" animals into the United Kingdom and this includes non-human primates, except to approved establishments. Such establishments include research institutes which must have their premises approved before permission is granted.

Procedures during Quarantine

Following the importation of Macaque monkeys the greatest incidence of morbidity usually occurs during the first three months. After arrival it is essential that the animals are rested and little should be done to them other than providing food, water and medication if necessary, during the first 48 hours. When treatment of animals with antibiotics is not incompatible with subsequent experimental work, prophylactic doses of a tetracycline drug may be helpful in controlling enteric infections. Some of these drugs may be given in the drinking water[6].

After a reasonable rest period the animals should be examined for evidence of *Herpes virus simiae.* This examination should be made on all species of simian. Any animal showing evidence of herpes lesions manifested by ulceration in and around the oral cavity, and sometimes conjunctivitis, should be destroyed and the carcase incinerated. Such examinations should be made after they are effectively sedated with, for example, phencyclidine hydrochloride.

During the quarantine period it is desirable to carry out a tuberculin test and recommended procedures for this examination have been published[13]. This recommends the use of an intradermal test, although there is doubt about the value of this test; and a chest X-ray for each animal.

Building Design

Buildings for monkeys should be designed, or adapted, in such a way that the risk of transmitting infections to their own species or to the staff (or, indeed, the risk

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of the staff infecting the monkeys) is reduced to a minimum. This can be achieved to a large extent by providing facilities for the staff to change into clothing which is worn only within the animal unit, and also for them to wash and shower before leaving the building. These changing rooms should be designed in such a way that, in relation to the building as a whole, staff can pass through to the animal quarters without having to retrace their steps. Close to this area it is very useful to have a small self-contained laundry room to deal with staff clothing.

Quarantine Areas and Experimental Rooms

The rooms for quarantine of new arrivals must be well separated from those housing the conditioned healthy animals. This also applies to rooms holding animals experimentally infected with organisms which are pathogenic for healthy monkeys. Each of these areas should, preferably, be self-contained with its own ancillary accommodation. They should also have subsidiary changing facilities: these need not be elaborate but should provide adequate room for changing shoes, gowns and other accessories. The entrance to the main animal holding rooms should also have a subsidiary changing area where outer garments worn in the animal rooms may be kept.

All animal rooms should be well ventilated and have 12 to 15 air changes per hour, with an allowance of approximately 1.274 m air space for each animal. The method of heating rooms is not critical, but in the event of a breakdown of the heat source the thermal lag assocciated with underfloor heating provides a useful safeguard against a sudden drop in temperature.

The structure and strength required of animal room walls depend very largely on whether racking is free-standing or fixed to support cages on a cantilever system. The latter method requires strong walls since they have to bear not only the weight of the cages and racking but also the increased stress due to the frequent vigorous shaking activities of monkeys confined in cages.

If economy of floor space is important, the height of the rooms should be sufficient to allow for two tiers of cages. Experience shows that for Macaques a height of 2.7 m allows for this arrangement with cages not more than 0.9 m high.

The space between opposing rows of cages is an important factor in the layout of a room and should be sufficiently wide to allow for comfortable working, particularly where large cages have to be handled. Too narrow a space, for example, could preclude the use of a fork-lift truck for moving and lifting cages. Although it is not possible to give exact figures desirable for all conditions, a distance of 2.1 m between opposing cages is satisfactory and preferably this should not be less than 1.8 m. It is also worth remembering, when considering this dimension, that staff should be able to walk between cages without the fear of being molested by the grasping hands that dart with amazing rapidity out of the cages.

The doors of monkey rooms should not be less than 1.3 m wide and corridors should certainly not be less than 1.5 m wide to allow for easy movement of traffic. Across the full width of each animal room door it is desirable to incorporate in the floor a shallow disinfectant trough for footwear and trolley wheels.

It is well to remember that monkeys can sometimes escape from cages, and windows should be protected by wire mesh or be strong enough to withstand the full force of an escaped animal propelling itself at considerable speed against this possible escape route.

ANCILLARY AREAS

In addition to the areas already mentioned, ancillary areas may be considered as usually consisting of rooms for cage washing, food preparation, storage, surgical procedures, autopsy, service laboratories and offices. These need not differ significantly from equivalent areas in other animal units, but must be large enough to accommodate bulky equipment such as cage washing machines.

Less frequently, research laboratories are incorporated within the ancillary area of a primate unit. This can be a satisfactory arrangement and is certainly an advantage when the work involves frequent examination of the animals. Furthermore it enables the scientific worker to have ready access to his experimental animals, and, at the same time, creates additional interest for the staff responsible for the care of the animals.

STAFF

Because of the potential microbiological hazards to animal staff and laboratory

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workers when dealing with monkeys or tissues of simian origin it is essential to ensure that they are adequately trained, and understand the procedures necessary for handling potentially infected material. Personnel should have suitable protective clothing, and changing facilities. It is desirable that the staff in a primate unit should have a complete change of clothing daily. Suitable outer protective clothing consists of a gown, disposable mask, goggles or vizor, rubber boots and rubber or plastic gloves. If disposable gloves are not available each pair should be washed in a suitable disinfectant immediately after use.

Since laboratory primates may be infected with *Mycobacterium tuberculosis* medical advice about immunization of personnel against this agent is necessary.

If a person is scratched or bitten medical attention should be obtained[13], and it is recommended that the wound be scrubbed clean immediately with copious supplies of soap and water and made to bleed. It is also recommended that all personnel coming into contact with simians should carry a card to inform the doctor that the individual works with these species.

EQUIPMENT

Cage design and handling of simians

In designing cages for simians it is important, apart from experimental requirements, to consider the welfare of the animals. In this respect the provision of cages as large as possible is desirable. Unfortunately these dimensions must be decided largely on a subjective assessment. However, a cage found to be suitable for a pair of adult *M. irus*[9] measures 77.5 cm x 76.2 cm x 91.4 cm.

Frequently boredom is observed in captive primates, and where possible animals should be caged in groups, even if only in pairs. In most institutes it is usually possible to do this. In the wild, most of a primate's working time is spent in search of food. Napier[18] suggests that food-getting activities should be devised to alleviate boredom. He also suggests that it should be possible to mix food pellets or natural food objects into the deep gravel or sandy floor surface of the cage in order that the animals can spend many hours of the day hunting for food.

To avoid injuries to the operator arising from bites, cages for simians should, ideally, have a movable partition so that the animals may be restrained at the front, thus allowing sedatives to be administered by injection. This detail of cage design is probably the most important factor related to the safe handling of primates and the arrangement used in conjunction with a parenterally administered sedative, for example, phencyclidine hydrochloride, eliminates the risk of bites.

The use of catching boxes, nets, and manual restraint has also been described[7, 9, 21], but such procedures, and other methods of catching monkeys, should only be used when it is not possible to sedate them in the first instance. It may, of course, be necessary to restrain monkeys manually, and without sedation as described[9] when escapes occur. One of the occasions when such escapes are likely to happen is at the time of transfer from one cage to another; this applies particularly when monkeys first arrive at the laboratory in their travelling crates and are being introduced to their permanent quarters. If at this time animals succeed in escaping they should, preferably, be guided into a baited cage, and manual restraint used only as a last resort.

Problems of capture will arise when an animal escapes outside the building. In such circumstances it may be desirable, and safest for all concerned to have it shot by an expert marksman. This procedure though drastic, may be the most successful method of avoiding accidents and the undesirable publicity which can occur following the escape of an experimental animal of this kind.

Disposal of Waste

Where simians are maintained in cages with droppings trays containing absorbent material, the waste and excrement is best disposed of by incineration. When cages are suspended over traps flushed periodically into a communal sewage system it is obviously desirable to discuss the implications with the appropriate authorities. If, of course, the sewage system is unable to deal with potentially infected material or excreta and other effluent likely to be infected with pathogens, it is necessary to sterilize the waste by heat or chemicals.

Handlig and Washing Cages

Apart from the possibility of infection arising by direct contact with monkeys or

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their tissues, injuries may occur from contaminated equipment. For example, scratches can occur from cages which are handled manually for cleaning and washing. To overcome this hazard, such equipment may be handled mechanically by a fork-lift truck[9]. In addition, monkey cages often weigh 40 kg and sometimes more, and easy handling of these heavy items makes it possible to clean them more frequently than might otherwise be possible, thus contributing to the overall cleanliness of the establishment. The danger of infection arising from scratches may be reduced further by the employment of a cage-washing machine. A number of such machines are available, and one found to be very satisfactory for cleaning cages has been described previously[9].

Other aspects of management which should not be overlooked are aesthetic considerations. It is recognized, as mentioned previously, that staff should be provided with the best possible working environment and facilities for maintaining a high standard of personal hygiene. People work better under clean and pleasant conditions and, furthermore, elimination of much of the unpleasant drudgery associated with cage washing means an improvement of the efficiency and speed of working all round, thus releasing staff to carry out more productive and probably more pleasant duties.

Anaesthesia

Macaques do not demand any special considerations as far as choice of anaesthetics are concerned. Inhalation anaesthesia is normally well tolerated and a very satisfactory method using halothane administered with an endotracheal tube has been described[4].

FEEDING

Compounded diets similar to those available commercially for other species of laboratory animals, are perfectly satisfactory for primates. These usually are supplemented daily with fruit or vegetables. It is also possible to feed simians with foodstuffs normally purchased for human consumption[5].

CAPTIVE BRED SIMIANS

In recent years the demand by research workers for pregnant and new-born simians has shown an increase and initially attempts were made to satisfy this demand by the importation of pregnant animals. In certain instances this may be satisfactory, but experience shows that there is a very high foetal wastage due to abortion and stillbirths. Furthermore, where "timed" pregnancies are required this method of supply is not acceptable and there is, of course, an increased risk of disease by the purchase of pregnant females from the wild, as there is with other categories of monkeys.

Fortunately the breeding of monkeys in a laboratory on a large scale is now a well-established procedure[30]. It is possible to obtain infant monkeys by caesarean derivation thus obtaining a "pathogen-free" animal comparable with those of other species. The hand-rearing of new-born baby *M. irus* is relatively easy compared to the difficutly of hand-rearing, say baby mice, and for this work it is desirable to employ women who have had children of their own and employ techniques similar to those used for rearing human babies. The value of obtaining captive-bred baby monkeys is already appreciated. For example, Valerio *et al.*[30] describe the use of captive-bred baby simians under rigidly controlled conditions for long-term studies on leukaemia, while van Bekkum *et al.*[29] state that problems arising from infections after irradiation can be avoided by the use of laboratory-raised monkeys. These latter workers are also of the opinion that the large-scale production of pathogen-free monkeys for certain specific studies should be attempted, and they consider that this approach seems realistic both from the financial and technical point of view.

To present a list of the type of experiments for which simians are currently being used in medical research would be of little value since there are many publications readily available which illustrate the wide field and variety of investigations being covered. However, in recent years there have been some investigations in the United Kingdom which are of interest to me and, at the same time serve to illustrate the type of investigations which benefit from having captive breeding colonies available.

Reproductive Studies

The usefulness of rhesus monkeys for studying reproductive problems related to the use of an intra-uterine device is shown by the investigations made by workers at the University of Birmingham. For example

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Kelly and Marston[15] demonstrated in rhesus monkeys the contraceptive effect of an intrauterine device. In their investigation fifteen of 24 "control" animals became pregnant following 68 compatible matings, three animals conceiving twice, and none of 21 animals fitted surgically with a small plastic IUD conceived after 77 compatible matings.

Infant Behaviour

For ethical reasons it is frequently not possible to carry out investigations in human subjects. This applies in certain instances to the application of some experimental techniques in behavioural studies[16]. To overcome this kind of difficulty Hinde[14] describes the use of rhesus monkeys in experiments on mother-infant interaction and the consequences of maternal deprivation. The investigations were based on studies of small captive groups, each consisting of a male, three of four females and their young. The results of these experiments carried out at the University of Cambridge, have precise parallels in human behaviour. In particular, the responses of infant monkeys to a period of separation from their mothers closely resemble the responses of human children under similar circumstances.

Nutritional Investigations

Many different species have been used for comparative nutritional studies. However, when an animal species has to be selected as a model for growth experiments which for one reason or another, cannot be carried out in man, the use of simians should be considered. Firstly the growth curve of the rhesus monkey and chimpanzee approximates more closely that of man than the curve for non-primates, and secondly the slow growth of the foetuses of all primates suggests that a long maturation period may be a common characteristic of the whole primate order[20].

Dental Research

In recent years *M. irus* monkeys have been used extensively by workers in the Department of Dental Science at the Royal College of Surgeons of England for investigation into dental caries. This disease in *M. irus* resembles caries in man clinically, radiographically and microscopically[5].

Although much useful work has been done with young monkeys caught in the wild, captive bred animals of this species for dental research have the advantage that from birth they may be weaned and maintained on a strictly controlled diet — cariogenic or otherwise. Furthermore, as with other species of laboratory animal, it is possible, if time-mated animals are available, to obtain healthy young in the "germ free" state by caesarean operation. Such animals are useful in microbiological studies of the oral cavity.

FUTURE SUPPLIES

For economic reasons it seems unlikely that all the primate animals required in the immediate future for medical research purposes, including the testing of vaccines and drugs, could be provided by breeding in a controlled environment. However, where finance is made available a high proportion of animals of good quality could be produced by this method. One of the major difficulties besetting the production of primates in laboratory colonies is the relatively long time that captive animals take to reach breeding age. Female macaques are not likely to be sexually mature before three years of age, and male animals are likely to take longer. To overcome such difficulties it may be necessary to accept a compromise and import animals born in the wild, on the point of maturity, and after suitable medical treatment and conditioning, use these as breeding stock.

For projects requiring a large number of primates it may be worthwhile considering the use of marmosets *(Callithrix)*. The advantage of this species is that they are small with a head and body length range in the male of 173 - 220 *mm*[17] and the adult male animal weighs only 175 - 360g. Twins are born in 90 per cent of births and cage-bred animals are sexually mature at 15 months of age. As a research animal the marmoset has already been used in many fields, including reproductive physiology, virology and immunology[12].

Conservation of Wild Primates

Apart from the provision of better animals for medical research the captive breeding and propagation of primates is important, because of the need for conservation of wild life. Already, the golden tamarin *Leontideus rosalia* is said to be near extinction in Brazil, and in Sierra Leone it has been estimated that for every young chim-

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panzee exported for research or for zoos between four and six mothers have to be killed[18].

In addition, Napier[18] also points out that "Primates are rare animals, and it should be a matter for profound thought before the decision is made to remove an animal from the wild. Medically useful primates must be bred in captivity in sufficient numbers so that the cause of scientific conservation is not imperilled by the extravagant demands of a sister science". Clearly is it important that all biological scientists, particularly those of us using primates for medical research, should be concerned about conservation of the various species.

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BOOK REVIEW

HANDBOOK OF VETERINARY PROCEDURES AND EMERGENCY TREATMENT

R. W. KIRK AND S. I. BISTNER

W.B. Saunders Company, Philadelphia, London, Toronto. 1969. Pp. xxi & 474, 76 tables and 53 figures. Price R7.95.

This very useful pocket-sized handbook is designed, as stated by the authors, to be within easy reach of the busy small animal clinician, be he student or experienced practitioner.

The text is divided into six sections. The first section on emergency care covers the majority of the facets which relate to any emergency with a large part devoted to the treatment of specific clinical conditions and poisonings. In the second section on the interpretation of the signs of disease, the authors review a number of clinical conditions with the associated diagnostic considerations. Section III deals with the special examination of each organic system, while the fourth section presents a series of diagnostic and therapeutic procedures. The penultimate section covers the interpretation of many laboratory tests and the final section is composed of a very interesting series of charts and tables which serve as a source of a great number of useful facts. Examples of the latter include rosters of normal values, canine and feline growth curves, nutrient requirements of dogs, immunization procedures, poisonous substances in household items, common drug doses etc.

The information is presented in outline form with many cross references and is not intended to be complete and detailed discussion although the reader is referred to standard bibliography where pertinent. The telegraphic style has in fact permitted the inclusion of an immense amount of material which could be made use of daily by a small animal practitioner.

Amongst the general contents of this handbook there are some particularly interesting subsections, namely, radiation therapy, physiotherapy, nebulization therapy, electrocardiography, measurement of central venous pressure, simplified fluid therapy, growth curves for fifteen dog breeds, the import-export requirements for pets in many foreign countries, and a list of potentially dangerous ornamental plants.

There are, as one would expect, a few subsections which are not applicable to South Africa, e.g., the list of poison control centres in the United States and the interstate shipping regulations for dogs and cats. However, these represent only a very small part of the text and in no way lessen its overall usefulness.

The book is well printed although some of the sketches illustrating the placement of the unipolar chest leads for electrocardiography in the dog are not too distinct.

"Handbook of Veterinary Procedures and Emergency Treatment" would indeed be a beneficial addition to the library of a veterinarian engaged in small animal practice.

W. L. J.

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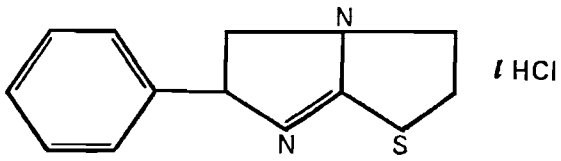

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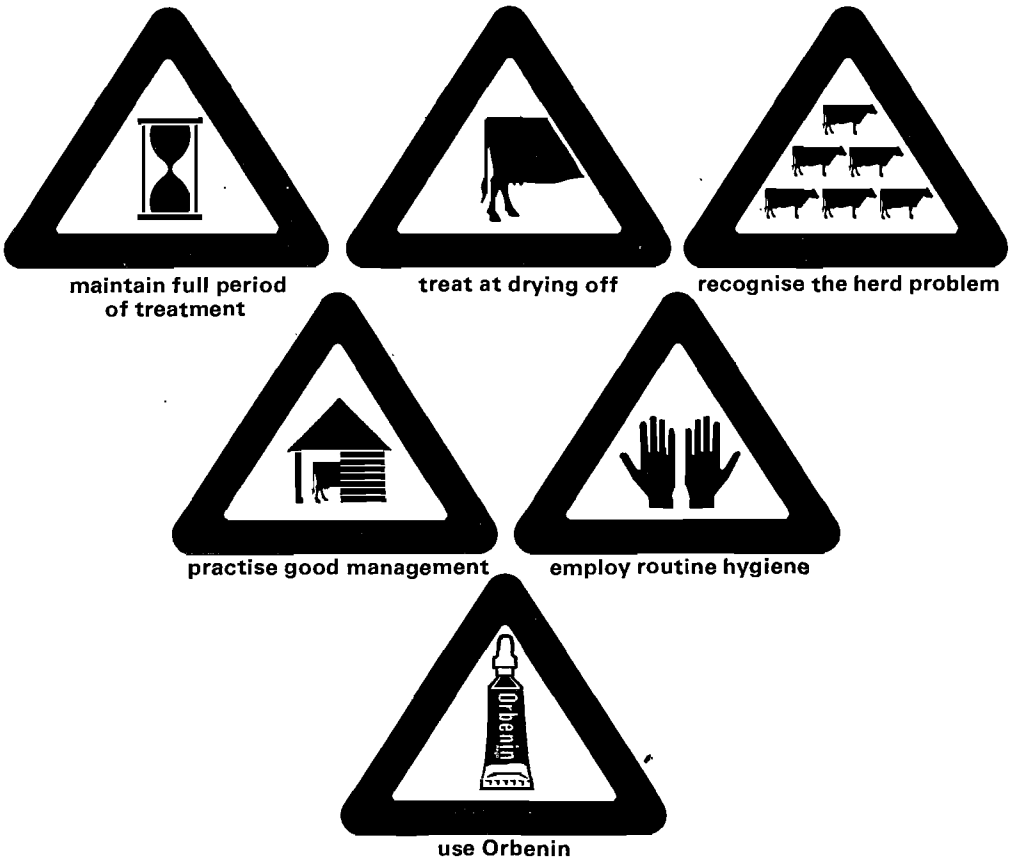

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A REVIEW OF THE EXPERIMENTAL PROJECTS SUPPORTED BY THE UNIVERSITY OF STELLENBOSCH PRIMATE COLONY*

J. H. GROENEWALD**

The University of Stellenbosch Primate Colony is possibly unique in the world in as far as supplying large primates to a variety of acute and relatively acute surgical experimental projects. The purpose of this paper is to present a short review of the various surgical projects supplied and uses to which a large number of baboons handled by this colony, were put.

From July 1967 to December 1969 over 3,099 baboons were handled by the colony. These consisted of Cape Chacma baboons *(Papio ursinus)* of both sexes and all age groups. The animals were procured mostly from the Western Cape region but some came from the Eastern Cape from distances up to 500 miles away.

The mainstay of the colony has been to supply baboons for experimental projects related primarily to kidney transplantation but also to many other forms of organ transplantation. In the kidney transplantation project alone 1182 animals were used in studies directed towards finding newer and better immunosuppressive agents. Amongst the more important findings that emerged from these studies was that kidney graft survival can be prolonged and rejection postponed by the administration of, and especially with pre-transplantation treatment of, subcellular kidney cell fractions[1] (Table 1). As indicated in Table 1, survival times for animals treated with these fractions could be prolonged to a mean survival of 20.0 days, as compared to 9.6 days for untreated controls. By mechanically converting the electric charge of these sub-cellular kidney cell fractions (SKCF) we created what we termed "contra-antigens"[2]. These so-called contra-antigens, (Table II) proved to be even better than SKCF in prolonging renal allograft survival, and furthermore, gave good histological evidence of suppression of the rejection phenomenon[3]. As indicated in Table II survival in these groups treated with contra-antigens, as in the SKCF treatment groups was dose related. The maximum mean survival time of 16.5 days, achieved with contra-antigens was further increased to 26.5 days, with pretreatment using similar dosages (Table II).

Amongst the other interesting immunosuppressive agents evaluated was Chloroquine which gave histological and biochemical evidence of immunosuppression but did not prolong allograft survival beyond a mean time of 11.8 days. Thalidomide, on the other hand, prolonged allograft survival on a dose related basis up to a mean of 21.2 days (Table III) but showed no histological evidence of immunosuppression[4].

During an extensive series of experiments directed towards finding the most successful means of organ preservation, close to 200 organs were preserved by various means for periods up to 24 hours. A further number of over 200 kidneys were subjected to isolated perfusion from 1 to 3 hours[5]. These studies confirmed that even under hyperbaric (3ATA) and hypothermic (4°C) conditions oxygen is still utilized in maintaining metabolism even when employing helium as an environment gas[6]; further more that helium might not be an inert gas and can be used for successful organ preservation under hyperbaric conditions when organs are preserved in physiological solutions[7]. These studies also confirmed the toxicity of hyperbaric oxygen[8]. It was also shown that up to 90% of kidneys could be successfully preserved for 24 hours in freeze-thawed defribriginated serum under hyperbaric (3ATA) hypothermic (4°C) conditions without any perfusion[9]. Indeed that perfu-

* Paper presented at the Symposium on the Production and Use of Laboratory Animals.

** Department of Surgery, Faculty of Medicine, University of Stellenbosch and Karl Bremer Hospital, Bellville, S.A.

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sion under these circumstances is probably harmful and that what little oxygen is needed to maintain metabolism under these conditions will probably diffuse into the preserved organ through an intact vasculature[10]. The importance of maintaining the microcirculation during the period of ischemia that the organ removed for transplantation is subjected to prior to re-establishment of its blood supply, was also emphasised[11]. Results achieved with transplantation will almost certainly be improved if many of these conditions are met during the vital ischemic interval.

Extensive studies were also performed in evaluating anti-lymphocyte serum (ALS) and anti-lymphocyte globulin (ALG) destined for human use in a baboon model prior to human application[12]. Because of the lack of a suitable in vitro test for the evaulation of such ALS/ALG preparations prior to clinical application, the baboon model of evaluating the effects of these agents on first and second set skin grafts survival has proved to be invaluable[13]. Not only can the ability of these agents to prolong first and second set skin graft survival be measured and then serve as a means of predicting the potential clinical value of such preparations, but their in vivo effect on thrombocytes and other possible toxic manifestations[14] can also be sought and batches possessing such possible toxic effects be eliminated from human application.

Baboons from the colony have also been supplied for various protocols in other types of organ transplantation. These include studies on cardiac and lung transplantation performed to evaluate amongst other parameters the serological patterns and processes of rejection in these types of transplantation[15]. Corneal transplants were performed not only to study them as a means of homotransplantation, but xenografts, transplanting baboon corneas to man, were also done[16]. Liver transplantation is not only an important issue on its own, but because of the immunological favouritism that the liver probably enjoys the whole immunological pattern associated with liver transplantation is at present being extensively investigated. Pancreas transplantation is also under investigation.

Whereas these transplantation projects, of which the kidney project has been by far the largest, have formed a major part of the research for which the University of Stellenbosch Primate Colony has supplied animals, other equally important surgical research projects have also been supplied. Only a few of these will be mentioned and some shortly discussed.

As part of the development of the University of Stellenbosch mitral valve, extensive evaluation of this valve was performed by mitral valve replacement in baboons[17]. Because baboons tolerate cardiopulmonary bypass well, and even lengthy perfusions of up to three hours using high flow rates of 100 to 125 ml per min per kg body-weight are followed by prompt and complete recovery, the baboon is the ideal experimental animal for this type of research. Of importance here too as well as in the kidney and other transplantation projects, is the fact that baboons can be typed with human ABO serum and therefore, transplantation between compatible animals at this level, as well as blood transfusion is achievable in such major procedures[18].

Another major project at present receiving heavy support is *ex vivo* extra-

Table I: SURVIVAL TIMES OF UNTREATED CONTROLS AND BIOLOGICALLY TREATED ALLOGRAFTED BABOONS*

Treatment	Dosage Schedule	Survival in days
Untreated controls		9.7± 5.6
SKCF (6)	0.02 ml single dose im	12.0± 4.0
SKCF (13)	2.0 ml im 7 days; 2 ml in every second day	20.0±21.0**
SKCF (5)	20.0 ml im at operation and seventh postoperative day	7.0± 4.0
SKCF (6)	0.2 ml at operation and every second day im	16.0± 9.0**
SKCF (6)	0.02 ml pre- and postoperatively every second day	7.0± 5.8
SKCF (6)	0.02 ml pre- and postoperatively every second day im	9.0± 6.3
SKCF (4)	2.0 ml pre- and postoperatively every second day im	12.6± 5.8

* All values are mean SD.

** Statistically significant $P<0.05$.

( ) Number of animals in each experimental subgroup.

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corporeal liver perfusion of baboons with experimentally created hepato-cellular damage. Although this study is still in its infancy and hampered by such problems as the experimental creation of hepatocellular damage in the experimental model, more than pleasing results have already been achieved so far. These will be reported later this year.

Other studies that have previously been reported and will only be mentioned here include studies on the anatomy and physiology of the baboon prostate[19], and studies on the renal and systemic effects of experimentally created aortic coarctation[20].

Table II: SURVIVAL TIMES OF UNTREATED CONTROLS AND BIOLOGICALLY TREATED ALLOGRAFTED BABOONS*

Treatment	Dosage Schedule	Survival in Days
Untreated controls		9.6± 5.6
Contra-Antigens	(6) 0.2 ml im every second day	11.8± 6.9
Contra-Antigens	(6) 5.0 ml im every second day	16.5± 7.1**
Contra-Antigens	(6) 20.0 ml im every second day	12.4± 7.3
Contra-Antigens	(6) 2.0 ml. im pre- and post-operatively every second day	26.5±19.5**

* All values are mean SD.

** Statistically significant $P<0.05$.

( ) Number of animals in each experimental subgroup.

In having only shortly discussed the major projects of surgical importance, this report has not attempted to allude to massive studies in serology, immunology, bacteriology, chemistry, pharmacology, neurology, radioisotopes, and other fields where baboons were supplied for by the colony both at the Karl Bremer and Groote Schuur Hospitals.

The valuable asset of having the Cape Chacma baboon available in large numbers for these projects is obvious. Because of its similarity in many aspects, both anatomically and physiologically to man, and because of its availability, this makes the baboon, we think, the ideal experimental animal for our purposes.

Table III: SURVIVAL RATES OF BABOON ALLOTRANSPLANTS*

Treatment	Dosage Schedule	Mean Survival Rate	Total dose
Thalidomide Saline Suspension	10 mg/kg at operation	21.2**± 10.7	4
Thalidomide Saline Suspension	10 mg/kg postoperatively	11.1 ± 2.7	6
Thalidomide Saline Suspension	100 mg/kg at operation	7.7 ± 2.2	4
Thalidomide Oil Suspension	10 mg/kg at operation	6.1 ± 1.5	7
Thalidomide Oil Suspension	10 mg/kg postoperatively	4.7 ± 1.2	3

* Values mean ±1 SD.

** Statistically significant $P<0.01$.

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BOOK REVIEW

CATALOGUE OF EIMERIIDEA (PROTOZOA, SPOROZOA), SUPPLEMENTUM I

L. P. Pellerdy

Akadémiai Kiadó, Budapest 1969 pp 80, Price $2.40.

This book is the first supplement to ap-ır following the original publication of ıtalogue of Eimeriidea" in 1963. Together y give the most complete and up to date available of the Coccidia thus making m indispensable works of reference to the tozoologist working on coccidiosis. The plement is of the same format as the ginal work, very well produced and easy ıse.

Index I lists, in alphabetical order, the v species described in the different genera between 1963 and 1968, as well as some omitted from the original catalogue and others on which new or revised data have become available. It also gives the sizes of the oocysts of the various species and the names of the hosts.

Index II contains an important list of synonyms and incorrect names of organisms and Index III gives the names of the hosts.

A. de V.

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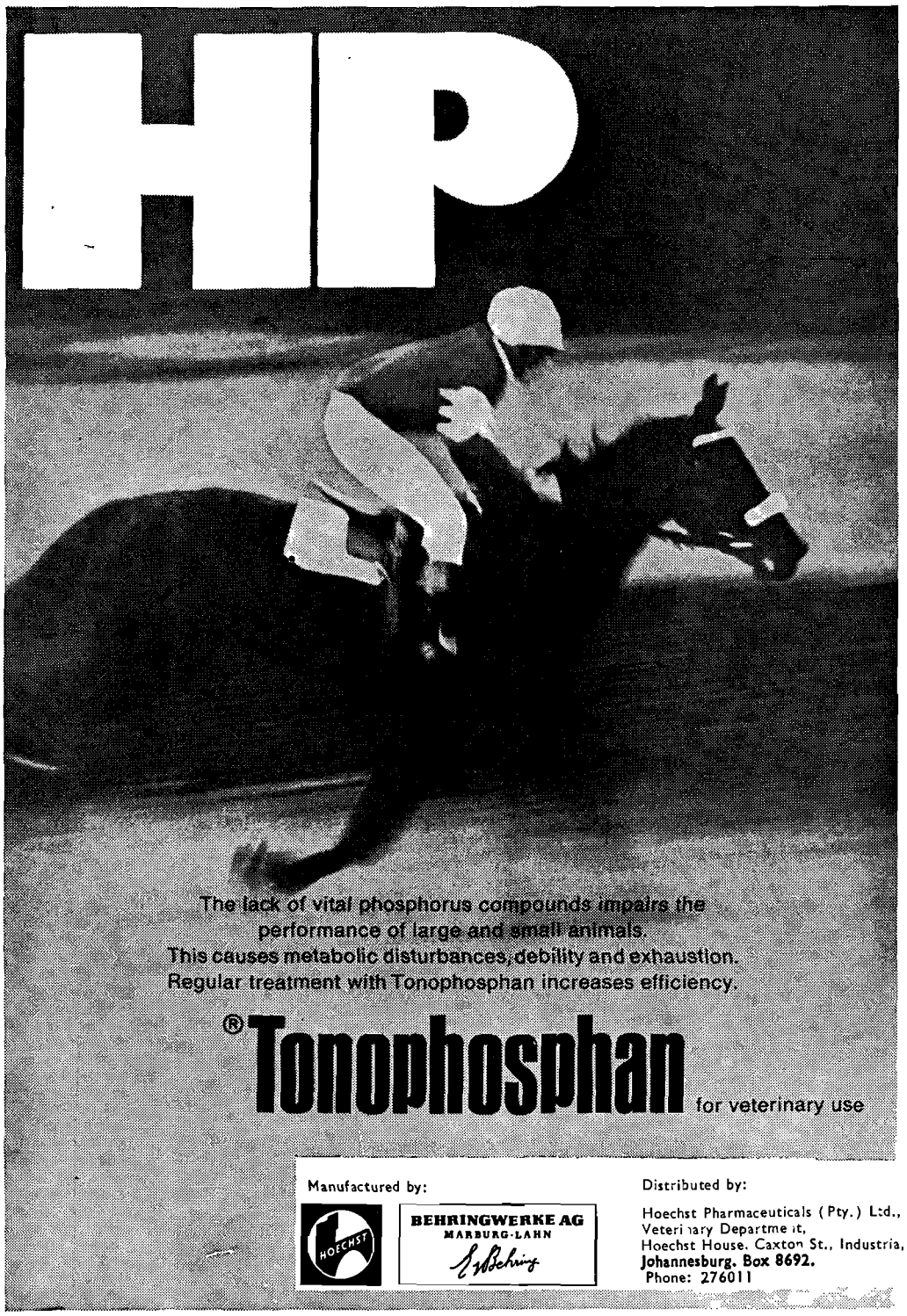

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SACCOSTOMUS CAMPESTRIS PETERS, 1946 AS A LABORATORY ANIMAL*

R. J. PITCHFORD** AND P. S. VISSER**

SUMMARY

Of thirteen rodent species tested *Saccostomys campestris* was found to be the most suitable for the routine maintenance of *Schistosoma haematobium* in the laboratory. The only disadvantages are the aggressive nature of the female towards the male during the breeding season and the confined breeding season. They are easy to handle, not expensive, require no special diet, long lived and are highly susceptible to *S. haematobium* without being killed by heavy worm loads. They do not show signs of overcoming *S. haematobium* infection for long periods.

Easy routine maintenance of bilharzia parasites in the laboratory depends to a great extent on the suitability of the definitive mammalian host. In an attempt to find a suitable definitive host for maintaining *Schistosoma haematobium* nine indigenous South African rodent species which included *Aethomys chrysophilus, Lemniscomys griselda, Mystromys albicaudatus, Otomys tugelensis, Praomys (Mastomys) natalensis, Rhabdomys pumilio, Saccostomus campestris, Steatomys pratensis* and *Tatera brantsi* were used in addition to *Arvicanthus niloticus, Rattus rattus,* hamsters and white mice.

The criteria for a suitable animal for this purpose were that it should be sufficiently susceptible, should not be killed by heavy loads of the parasite, should be easy to breed in a confined space, easy to handle and the established parasite infection should not show evidence of being overcome by the host.

Of the above rodents *Lemniscomys griselda, Mystromys albicaudatus, Rhabdomys pumilio, Tatera brantsi* and *Rattus rattus* were discarded as not being sufficiently susceptible. White mice were discarded because of a high mortality with heavy infections and *Praomys (Mastomys) natalensis* was discarded because it showed evidence of overcoming the infection within a comparatively short time. Of the remaining six species, four, *Aethomys chrysophilus, Otomys tugelensis, Steatomys pratensis* and hamsters were discarded in spite of being sufficiently susceptible, because they would not breed, or bred with great difficulty in the laboratory. *Arvicanthus niloticus* was discarded because of its size (up to 180 g), extreme viciousness, and, not being an indigenous rodent, because of the trouble it might cause if it escaped[1]. Of the animals tested therefore *Saccostomus campestris* was the most suitable for routine maintenance of *Schistosoma haematobium.*

Saccostomus campestris, the pouched mouse, is a small rodent weighing up to 90 grams, occurring "from the Cape to north of the Equator in East Africa. ... It is not a common species but is widely distributed, inhabiting savannah bush country. It is nocturnal" and lives in underground burrows often in association with gerbils and tends to avoid water. It requires no special diet[2].

Saccostomus has been bred in the London Zoo; and the first breeding stock in South Africa originated from Zululand in 1955. This colony died out through fighting[1].

These rodents are docile and extremely easy to handle even when first brought in from the field. The female however tends to become vicious during the breeding season and may bite. The breeding season is well defined from September to March during

* Paper presented at the Symposium on the Production and Use of Laboratory Animals.

** Bilharzia Field Unit, South African Medical Research Council, Nelspruit.

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which period up to four litters are born varying in size from 3 - 10 but usually from 5 - 7. Females up to two years old, are reasonably good mothers though occasionally the first litter in September/October is eaten. Unfortunately the pregnant female attacks and will kill the male unless they are separated. She may also attack him at other times and consequently the sexes must be kept apart during the non-breeding season. The correct timing of mating is uncertain and at the moment is still largely a matter of trial and error. There appeared to be no difference in results when the male was put into the female's cage or she into his. Occasionally the male is not attacked and the pair can be left together during and after pregnancy. Good mothers produce an average of 23 offspring per season.

The breeding cages are metal from which most light is excluded and the breeding pair is left undisturbed as far as possible. The floor of the cage is covered with wood shavings and sawdust which is changed about once a month or less frequently. They are clean in their habits and appear to defaecate and urinate in one section of the cage away from their nest of wood shavings. Only one pair is placed in a cage. The male is removed when the female is pregnant.

The length of the gestation period is three weeks. The young are left with the mother for at least four weeks after which they are removed and housed in open mesh wire cages resting on wood shavings until two months old. They can then be paired. *Saccostomus campestris* lives for 3 - 4 years in the laboratory and the most active breeding occurs during the first two seasons. After two seasons the litters are smaller and less frequent. They appear to be less tolerant to extremes of temperature than for example *Praomys (Mastomys) natalensis.*

The Nelspruit strain as been maintained without field replacements, since 1958 and has met all the requirements needed for maintaining *S. haematobium;* it has however not been sufficiently prolific to use for field work where several thousands are needed per annum. Previous to 1969 numerous partial albinos were born; the first total albinos were born during 1969 but it is too early to draw any comparison between them and the normal animal.

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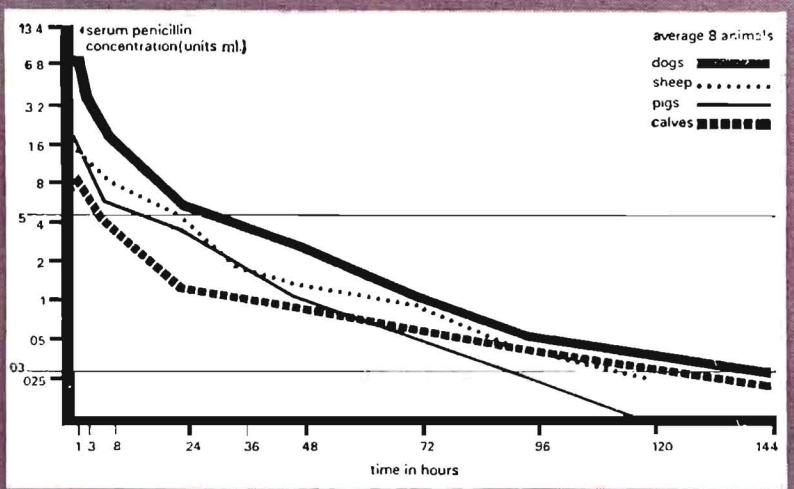

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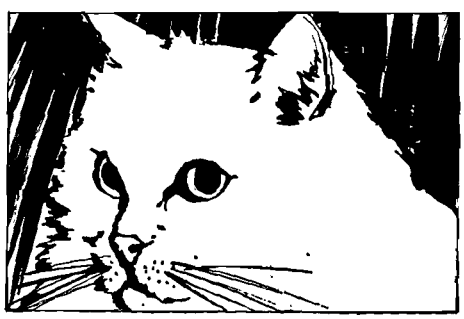

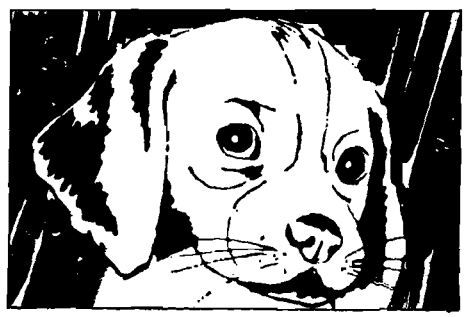

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THE VIRUSES OF VERVET MONKEYS AND BABOONS IN SOUTH AFRICA*

H. MALHERBE** AND MARGARET STRICKLAND-CHOMLEY**

A knowledge of the viruses of the vervet monkey has been essential in the production and safety testing of poliovirus vaccine in South Africa. Viruses have been isolated from the throat, rectum, kidney, salivary glands and spinal cord of vervet monkeys. The isolates include some new viruses, as well as a herpesvirus (SA 8) related to the dangerous B-virus of macaque monkeys, and an adenovirus which is highly oncogenic for hamsters.

The viruses of baboons are now being investigated to assess the hazard to personnel working with these animals. Viruses already identified include adenovirus, reovirus, enterovirus, cytomegalovirus, myxovirus and two herpesviruses of which one is the SA 8 virus.

The vervet monkeys of South Africa comprise two species: the common vervet or "blou-aap" *(Cercopithecus aethiops pygerythrus)* found in savannah and forest-fringe areas, and the samango monkey *(Cercopithecus mitis)* which lives in forests. When a vaccine against poliomyelitis became possible through cultivation of poliovirus in tissue cultures by Enders, Weller and Robbins in 1949[1], the more numerous *Cercopithecus aethiops* monkeys were selected for production and testing of the vaccine to be made in this country. Ability to recognize the viruses of this animal became imperative; and since 1954 our laboratory has made a special study of the subject.

The common baboon of South Africa is the chacma *(Papio ursinus)*. In recent years this animal was being increasingly used by a number of research centres, while virtually no attention was given to the viruses infecting this primate. We therefore included baboon viruses in our research programme, and some interesting findings have been made.

It is difficult to assess whether a virus recovered from a monkey or baboon is essentially a simian virus, or whether it was acquired from another species in nature or from human handlers subsequent to capture. When viruses of special interest have been found, we have sent them to the Simian Virus Reference Center at the Southwest Foundation in San Antonio, Texas, for comparison with other simian and human viruses; and several South African viruses have been accepted as prototype strains.

In the United States, simian viruses were, for a number of years, sent to Dr. Robert Hull, of Eli Lilly and Company, who classified them in an SV series[2]. For the sake of convenience in our laboratory we grouped our isolates in a series designated SA (for Simian Agent), and the viruses we had recognised up to 1963 were described in two papers[3,4]. Comparison of the SV and SA series is still proceeding, but for practical reasons the SA designations of our isolates tend to be retained. Our grouping was based mainly on distinctive changes produced by the viruses in vervet kidney tissue cultures, and it is clear that different serotypes can be found within a number of the groups. A useful review of simian viruses classified in categories generally accepted for human viruses was published by Hull in 1968[5]; but the situation is still fluid, the data incomplete, and the classification of some unusual viruses not yet possible.

1. SOUTH AFRICAN VERVET MONKEY VIRUSES

Since 1954 we have studied tissue cultures prepared from the kidneys of more than 3000 monkeys, and we have become reasonably familiar with the viruses harboured in renal cells. Numerous blood samples have been checked for viraemia, usually with negative results. A limited

* Paper presented at the Symposium on the Production and Use of Laboratory Animals.
** Poliomyelitis Research Foundation, Johannesburg.

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number of other tissues have been examined for viruses; but the main source of our isolates have been throat and rectal swabs. We will not consider here evidence of arbovirus infections in monkeys, but will confine ourselves to those viruses to which monkey handlers may be exposed.

A. VIRUSES FROM THE KIDNEY

Evidence is lacking as to whether these were latent within renal cells or were also being excreted in the urine. It may, however, be reasonably assumed that a virus recovered from kidney cells can at some stage be found in the urine.

(1) The most commonly encountered virus, SA 1, produces syncytia without inclusions. It is related to the "foamy viruses" often found in monkey kidney cultures elsewhere in the world, and belongs to the myxovirus, or influenza-virus-like, group[5].

(2) Reovirus (SA 3) is an occasional contaminant of kidney cultures. It can be recognized by its distinctive cytoplasmic inclusions, and the report from our laboratory[3] comprised the first description of reovirus inclusions[6]. The SA 3 prototype strain is related to but not identical with Type 1 human reovirus and two SV reovirus strains isolated in the United States.

(3) SA 4 virus has been found only in uninoculated kidney cultures. It has the characteristics of a picornavirus and is slightly related to the SV 4 picornavirus which has commonly contaminated kidney cultures in the United States.

(4) The cytomegalovirus SA 6 reveals itself in kidney cultures only after prolonged cultivation, and its presence can be recognized by the characteristic nuclear inclusions.

(5) A unique virus, SA 12, has been found only once, in a kidney culture. It produces basophilic nuclear changes resembling those induced by the SV 40 virus of macaque monkeys which does not occur in Africa. Unlike SV 40, however, SA 12 does not produce tumours in hamsters.

(6) SA 15 virus belongs to the herpes group and was found on one occasion only in a kidney culture held for a prolonged period. It produces syncytia with pleomorphic nuclear inclusions.

(7) Although measles-related viruses have been found relatively frequently in kidney cultures in the United States, we have encountered only one virus producing similar changes. The relationship of this agent, provisionally called VK 625, to measles virus has not yet been investigated.

B. VIRUSES FROM SALIVARY GLANDS

(1) Typical SA 6 cytomegalovirus inclusions were seen in histological sections of the parotid glands of 26 out of 40 monkeys examined in one series in our laboratory. The virus was recovered in tissue cultures from each of three pools of parotid tissue taken form a total of 15 of these animals.

(2) We have recently isolated a reovirus from the submandibular salivary glands of a vervet monkey.

C. VIRUS FROM SPINAL CORD

The herpesvirus SA 8 was recovered on two occasions, separated by a year, from the cords of two monkeys inoculated intraspinally with formalin-inactivated poliovirus vaccine. It is assumed that the virus was latent in the cord and was activated by the trauma of the intraspinal injection, since virus could not be recovered from the bloods of the monkeys or from the vaccine inocula. The monkeys became paralysed and showed severe lesions of both grey and white matter in the cord.

SA 8 virus was then found to be related to the B-virus first described in 1934 by Sabin and Wright[7] which occurs in macaque monkeys and is highly pathogenic and fatal for man. B-virus has not been found in African monkeys, but the presence of a related virus which produces in rabbits and monkeys lesions identical to those of B-virus serves as a warning to those who handle South African simians.

D. VIRUSES FROM THE THROAT

(1) SA 1 foamy virus can frequently be isolated from throat swabs. It does not appear to be associated with overt illness in the monkey.

(2) SA 3 reovirus has been recovered from throat swabs on a number of occasions.

(3) Adenovirus isolates have frequently been made from monkey throats.

(4) A virus, SA 10, was obtained from the throat of a samango monkey. It was found by Harwin[8] and by Heberling[9] to be closely related to Type 3 human parainfluenza virus.

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(5) From the throat of a *Cercopithecus* monkey another virus, SA 13, also related to the human Type 3 para-influenza virus, was isolated; but it differed from SA 10 virus in its haemagglutinating properties[8].

E. VIRUSES FROM THE RECTUM

(1) SA 5 is an enterovirus recovered from a rectal swab. It is serologically distinct from the other picorna-virus SA 4 which was isolated from kidney cells.

(2) Adenoviruses are often obtained from rectal swabs. The vervet prototype virus SA 7 strain C8 was studied by Hull and his associates[10], producing tumours within 40 days in all suckling hamsters inoculated by them with the virus. This adenovirus has proved to be one of the finest models for the study of viral oncogenesis, and tissue culture in vitro methods for the detection of hamster cells transformed into tumour cells within a week after inoculation of the cultures with SA 7 virus have been developed by several groups[11, 12, 13].

The well-established fact that oncogenic viruses can produce tumours in species other than the natural hosts is of direct relevance to all who handle animals.

Two other adenovirus serotypes have thus far been distinguished among the numerous isolates stored by us, but neither of these is oncogenic for hamsters.

(3) SA 11 virus, recovered from a rectal swab, produces distinctive loculated cytoplasmic inclusions. It is virtually identical to a virus isolated by us on several occasions from abattoir efflent in Johannesburg, which we called the O Agent[14]. These two viruses comprise a new group of RNA enteric viruses[15].

We thus have vervet monkey viruses which can be classified with other animal or human viruses; but certain of the monkey viruses isolated in our laboratory appear to have no counterparts elsewhere.

2. SOUTH AFRICAN BABOON VIRUSES

Only limited investigations have been carried out by us since 1968, on animals from two catching sites and three research centres. Our findings thus far comprise:

(1) From the throat: SA 1-like foamy virus; adenoviruses; and an SA 15-like herpesvirus.

(2) From the rectum: adenoviruses; a reovirus; an enterovirus; and the SA 8 herpesvirus.

(3) In the salivary gland: histological evidence of cytomegalovirus infection, but virus has not yet been recovered.

For specimens collected from baboons in the Kruger National Park we are indebted to Major E. E. McConnell, of the U.S. Armed Forces Institute of Pathology, who is at present temporarily seconded to the Veterinary Research Institute at Onderstepoort. Through the courtesy of Professor H. D. Brede of Stellenbosch University we have obtained specimens from baboons in the Cape. Professor W.B. Becker, of the Virus Research Unit at the University of Cape Town Medical School, has also been investigating the viruses of baboons; his isolates including adenoviruses and an SA 8-like herpesvirus.

Thus within a short period of time there has been recovered from the chacma baboon an assortment of viruses including the B-virus-related herpesvirus SA 8. The use of baboons should therefore be undertaken only with a clear understanding of the potential hazards, particularly in transplantation studies when immunosuppressive drugs may enhance infections.

3. MARBURG VIRUS

We have carried out extensive investigations[16, 17, 18, 19] to ascertain whether the Marburg virus, which caused the death of eight laboratory workers in Germany and Yugoslavia in 1967 following contact with vervet monkeys from Uganda, was active in South Africa. We have not found evidence of this virus in wild simians here; but the fact that serious infection with a previously unknown virus can occur suddenly should serve as a warning to us that constant vigilance is necessary.

In South Africa we do not have a laboratory devoted exclusively to the infections of sub-human primates, nor do we have adequate facilities for the handling of dangerous viruses; and failure to provide these may have serious consequences. For the present we must rely on improving methods for the handling of monkeys and baboons, while the importation of primates from other countries should be subject to rigid control. Our simians have been spared

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infection with B-virus, haemorrhagic fever virus, and poxviruses, all of which have caused much trouble elsewhere; and it would be a major tragedy if these were to be conveyed to our indigenous monkeys and baboons.

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HELMINTH PARASITES OF SMALL LABORATORY ANIMALS AT THE VETERINARY RESEARCH INSTITUTE, ONDERSTEPOORT*

ANNA VERSTER** AND DEIDRE BROOKER**

INTRODUCTION

Laboratory records of helminths in the small animal colonies of the Institute consisted of isolated reports of *Cysticercus fasciolaris*, the larval stage of *Taenia taeniaeformia* (Batsch, 1786), in white rats; *Hymenolepis nana* (von Siebold, 1852) in white mice and golden hamsters, and an oxyurid, *Passalurus ambiguus* (Rudolphi, 1819) in rabbits. A systematic survey was therefore conducted to determine the incidence of helminths in the various colonies.

MATERIALS AND METHODS

Material was obtained from three sources from September to December, 1969:

a. Unplanned and incidental deaths of mice and rabbits.
b. Golden hamsters and ferrets autopsied after discharge from other experiments.
c. Redundant white rats, adult males, from the breeding stock.

With the exception of the ferrets, 25 or more animals of each species were examined. There was no attempt to select at random for either age or sex.

Bloodsmears were examined for microfilaria. The alimentary canal was removed and opened in saline and finally the thoracic and the remaining abdominal organs were examined. *C. fasciolaris* was fixed in 70% alcohol at room temperature; the other helminths were fixed in hot (70°C) 70% alcohol with 5% glycerine.

RECOVERIES

These are listed in Table 1.

Table 1: HELMINTH PARASITES RECOVERED FROM SMALL LABORATORY ANIMALS AT ONDERSTEPOORT

SPECIES	No. examined	No. positive	CESTODES			NEMATODES		
			I. madagascariensis	*H. nana*	*C. fasciolaris*	*P. ambiguus*	*A. tetraptera*	*P. muris*
Rabbit	25	8				8		
Guinea pig	29	0						
White rat	25	20	5		19			
White mouse	25	11		10			1	1
Golden hamster	39	1		1				
Ferret	10	0						

* Paper presented at the Symposium on the Production and Use of Laboratory Animals.

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

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DISCUSSION

Oxyurids

In rabbits *Passalurus ambiguus* was recovered in 32% of the animals slaughtered. This high incidence is to be expected as this and other oxyurids have a direct life cycle. *Aspiculurus tetraptera* (Nitzsch, 1821), however, was recovered from one mouse only, while *Syphacia obvelata* (Rudolphi, 1802) was entirely absent. This differs from the observations of Sasa, Tanaka, Fukui & Takata[1] who found *S. obvelata* common in rat and mouse colonies in Japan.

Spirurids

Protospirura muris (Gmelin, 1790), uses various insects (e.g. cockroaches) as intermediate host. The recovery of this helminth from a single mouse indicates that cockroaches, etc., have access to the mouse cages. In this instance the insect may have become infested from faeces from a feral or wild rodent.

Cysticercosis

Cook[2], Sasa *et al.*[1] and Tuffery & Innes[3] record *C. fasciolaris* from both rats and mice. Although this parasite is common in the white rats (76%), it has not been found in white mice at this Institute.

Infested cats void the eggs of *T. taeniaeformis* in their faeces. When the eggs are ingested by rodents they develop into a strobilocerci in the liver.

Their presence in this colony must be due to contamination of their rations by cat faeces, prior to or after preparation of the rodent food. In the preparation of feed cubes the heat used is inadequate to destroy *Taenia* spp. eggs. Moreover cats are used to control wild rodents in the yard and feed storeroom at the Institute, constituting a further source of infestation.

TAENIASIS

Hymenolepis nana was recovered from ten out of 25 white mice and from one of 39 golden hamsters examined. The cysticercoid may develop in an insect intermedfate host or auto-infestation takes place by one of two methods. Rodents may swallow eggs which develop to cysticercoids in the gut wall, or eggs laid by adults in the gut enter the mucosa of the villi to develop into cysticercoids. Subsequently the cysticercoids re-enter the gut to develop to the adult stage.

Inermicapsifer madagascariensis (Davaine, 1870) occurred in 20% of the rats examined. This is a common parasite of various species of rodents and has been reported in children[4]. Collins[5] has recovered it from feral *Rattus rattus* Linnaeus, 1758 trapped in the grounds of the Institute. The life-cycle of *Inermicapsifer* spp. are unknown, but an invertebrate probably acts as an intermediate host. If this is the case, housing white rats out of doors probably facilitates the completion of the life-cycle.

None of the 29 guinea pigs nor any of the ten ferrets examined were infested. Natural infestations in these hosts have been reported by Neveu-Lamaire[6].

ACKNOWLEDGEMENTS

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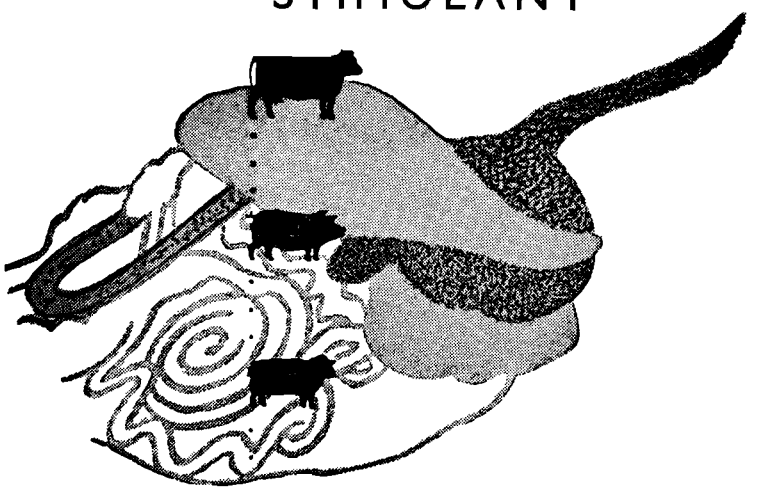

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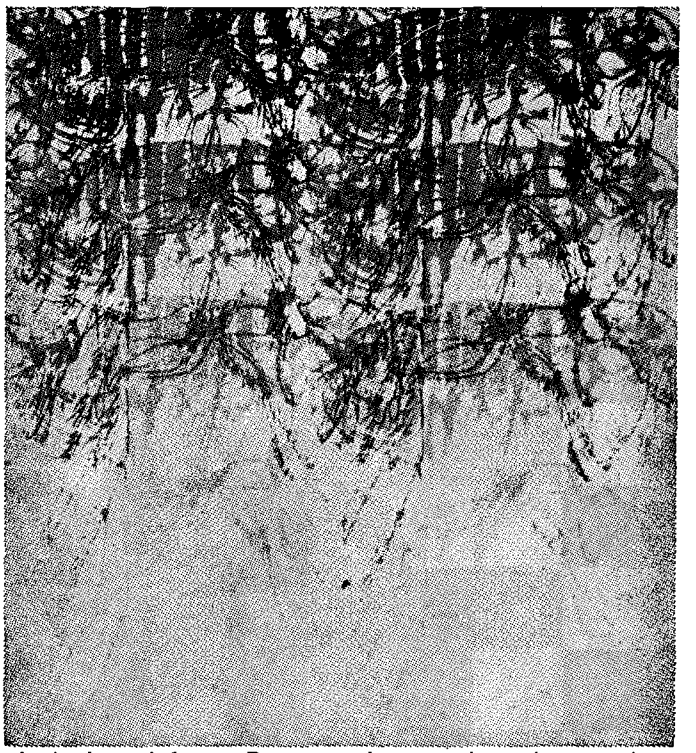

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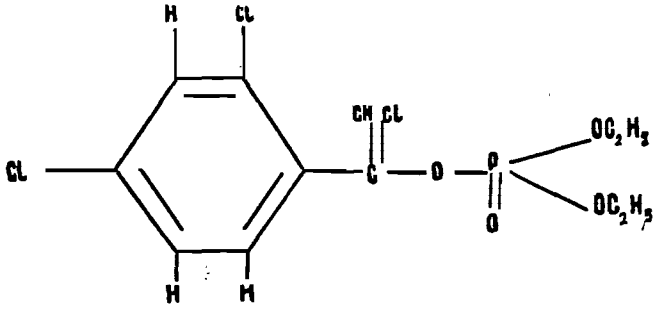

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COCCIDIOSIS OF RABBITS AT ONDERSTEPOORT*

A. J. De Vos**

DESCRIPTION OF OUTBREAK

Rabbits are bred for research purposes at the Veterinary Research Institute, Onderstepoort. From 1500 to 2000 young are born annually. The females are housed individually in hutches from 4 weeks prior to parturition until 8 weeks thereafter at which stage the litters are weaned. The hutches consist of an enclosed nesting compartment with a concrete floor and a small exercise run with a wire mesh floor. Bedding is provided. The hutches are not cleaned during the first 3 weeks after parturition, but once or twice weekly thereafter.

Severe outbreaks of enteritis, which caused heavy losses mainly among 6 to 8 week old rabbits, have periodically occurred in this colony. The condition was more prevalent during summer, but was not seen consistently every year. One particularly severe outbreak, with a mortality rate of more than 80 per cent in some litters, occurred during the first four months of 1969. In most cases the disease had a very acute course and many animals died overnight although they appeared to be perfectly healthy the previous afternoon. Symptoms, although seldom observed, included severe listlessness, diarrhoea, polydipsia, anorexia and grinding of the teeth. In a few instances the disease had a more prolonged course, and the animals were in an emaciated condition when they eventually died. Only rarely did any of the affected animals recover.

The macroscopic lesions seen at autopsy were limited to the digestive tract but varied somewhat in appearance. In many cases the wall of the small intestine was hyperaemic and somewhat thickened and in some instances the caecal wall was severely congested with multiple haemorrhages visible through the serosal surface. The contents of the large intestine were usually softer than normal, but a few cases were seen where it was either of normal or increased consistency. The intestinal contents frequently contained small amounts of blood and in some cases large volumes of gelatinous mucus practically filled the large intestine. A few cases of tympany were also seen. In addition, small foci of hepatic coccidiosis were seen in a few animals.

Coccidial oocysts were present in the intestinal contents of all the affected animals. Counts ranged from 10,000 to 3,000,000,000 cysts per g of faeces with an average of approximately 700,000 per g. A number of different *Eimeria* spp. were identified but *Eimeria magna,* considered to be one of the most pathogenic *Eimeria* spp. of rabbits[14, 21, 25, 27, 34], was by far the most prevalent. The endogenous stages of these organisms were plentiful in the epithelial cells of the intestinal mucosa of most of the animals and were frequently situated proximally to the nucleus.

The counts recorded in the affected animals were on the average considerably higher than those in unaffected animals and the symptoms and lesions resembled those described for coccidiosis by Chapman[6] to a very large extent. Intestinal coccidiosis was therefore incriminated as the probable cause of death. However, the very acute course and high mortality rate are rather unusual for this disease of rabbits. In most reports a more prolonged course with a less fatal termination[3, 14, 25, 27, 31, 36], resembling typical coccidiosis in many other animals, is described.

THERAPEUTIC STUDIES

In an attempt to find an effective chemotherapeutic agent the following experiment was conducted. All available litters between the ages of 6 and 8 weeks, amongst which

* Paper presented at the Symposium on the Production and Use of Laboratory Animals.
** Veterinary Research Institute, P.O. Onderstepoort.

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mortalities were being reported, were divided into three groups of about 40 each. The first group received sulphadimidine† in the drinking water for 14 days at a final concentration of 0.2 per cent. The second group received amprolium‡ in the drinking water at a level of 0.025 per cent for 5 days and thereafter at 0.0125 per cent for a further 14 days. The third group acted as untreated controls. The mortality rate in the different groups and oocyst counts of the various litters were used as criteria of the effectiveness of the drugs.

Sulphadimidine caused a considerable reduction in the death rate but could not eliminate it entirely. The oocyst counts also dropped appreciably and remained at a low level from the eighth day after the start of therapy until the third day after withdrawal of the drug (Fig. 1). Thereafter the counts again rose to a moderate level. Amprolium at the higher concentration had no effect on the mortality rate, but caused a reduction in the numbers of oocysts discharged (Fig. 2). The lower concentration, however, had no effect on either.

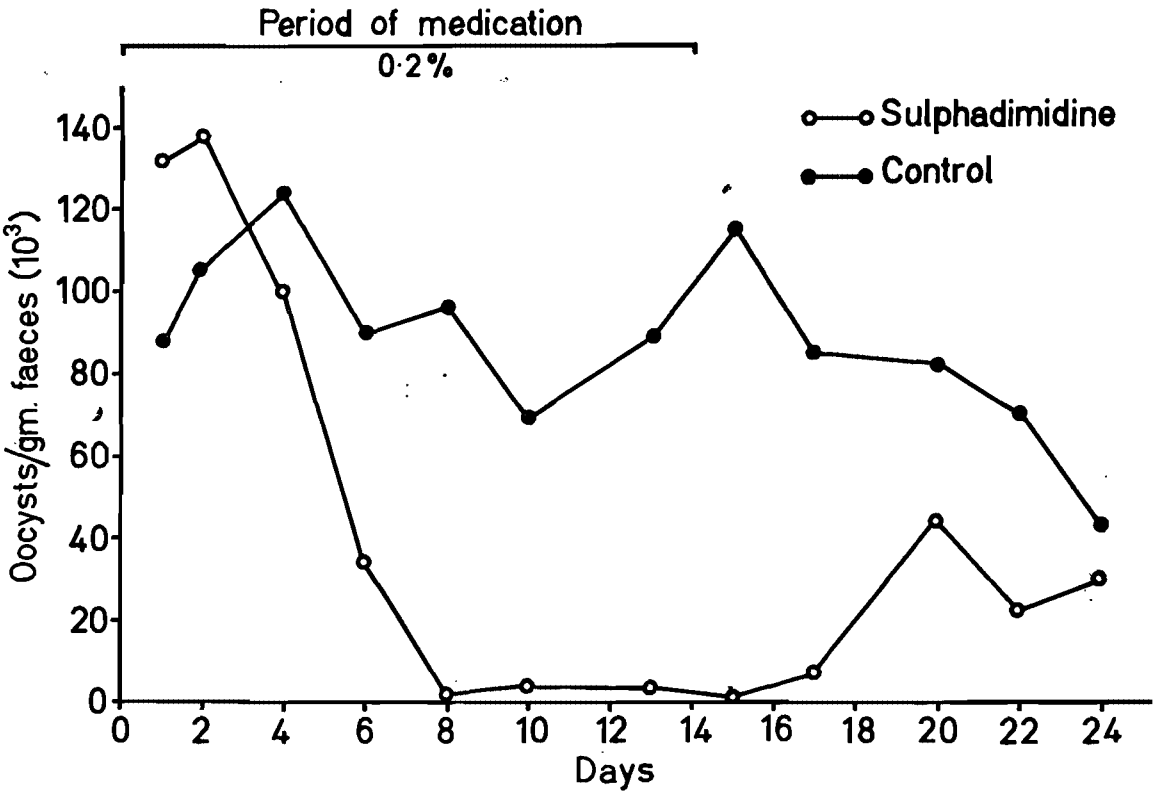

FIG. 1. The effect of 0.2 per cent sulphadimidine in the drinking water on the numbers of cocysts discharged by young rabbits.

† Sulfa-Dim Sol. 33⅓%, MHS.
‡ Amprol 20%, MSD.

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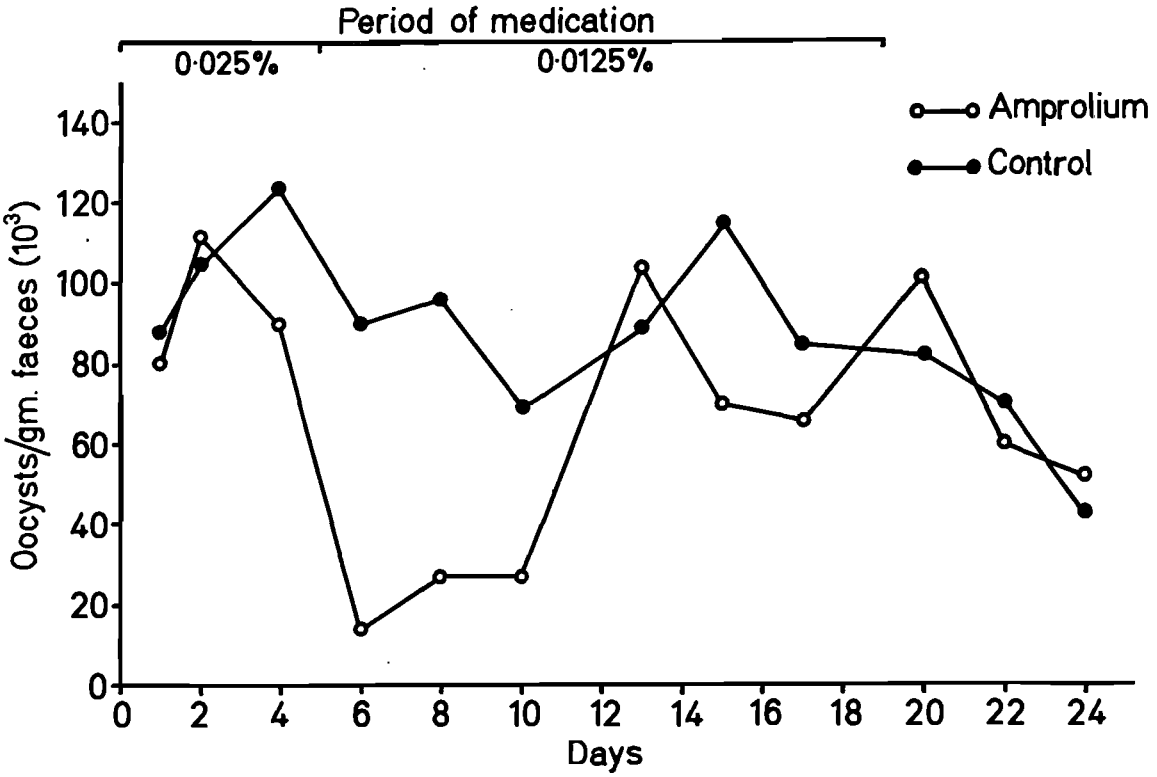

FIG. 2. The effect of 0.025 and 0.0125 per cent amprolium in the drinking water on the numbers of oocysts discharged by young rabits.

In uncontrolled experiments sulphachloropyrazine† and sulphaquinoxaline‡, both at a level of 0.03 per cent in the drinking water and given for 7 and 14 days respectively, were found to have approximately the same effect as sulphadimidine.

The results obtained with the sulphonamides are in accordance with observation by other workers[8]. Amongst others, sulphadimidine[7, 14, 29], sulphachloropyrazine[18], sulphaquinoxaline[14, 31, 36], sulphadimethoxine[37], sulphamonomethoxine[37], sulphamerazine[14], sulphamethylthiazole[29], formosulphathiazole[16, 18] and phthalylsulphathiazole[20] have been shown to be effective against intestinal coccidiosis of rabbits. No reference relating to the effectivity of amprolium against intestinal coccidiosis of rabbits could be found, but Soulsby[35] reported that it had no effect on hepatic coccidiosis.

Nitrofurazone plus furazolidone† was also used at one stage at Onderstepoort, but the results were disappointing despite the fact that nitrofurazone has been reported to be effective against intestinal coccidiosis of rabbits[4].

Based on these results all litters were given either sulphadimidine or sulphachloropyrazine in the drinking water for 2 weeks on reaching the age of 6 weeks. The mortality rate was considerably reduced in this way, but a small number of young animals still died after showing symptoms similar to those mentioned above. Essentially the same picture was again seen at autopsy but, in addition, small circumscribed haemorrhages were noticed in some cases under the serosal surface of the stomach. Only small numbers of oocysts were found in the faeces of these animals and very few of the endogenous

† Esb 3, Ciba.
‡ Embazin, M & B.
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stages were seen in histological sections. Coccidiosis could therefore not be definitely incriminated as the cause of death and it seemed as if some additional agent was involved.

Acute enteritis of rabbits, commonly known as "mucoid enteritis", has been reported by various authors[1, 3, 9, 10, 11, 19, 22, 24, 26, 31, 32, 33, 36, 38, 39] and is generally considered to be considered to be an important disease of rabbits[10, 22, 26, 33, 36]. The symptoms and lesions, as reported in the literature, however, vary considerably. In general it seems as if the disease mainly effects young rabbits from 5 to 10 weeks of age and has a very acute course and high mortality rate. The most prominent symptom is a diarrhoea and the faeces frequently contain blood or a large amount of gelatinous mucus. Additional symptoms such as anorexia, dullness, polydipsia, grinding of the teeth, tympany and a subnormal temperature have also been reported. At autopsy the lesions vary from a catarrhal or mucoid to a haemorrhagic enteritis and haemorrhages are frequently present in the gut wall. The entire intestinal tract may be affected but the most prominent lesions are usually seen in the caecum. Haemorrhagic ulcerations in the fundic mucosa of the stomach are also common. The intestinal contents vary from scanty liquid material, often mixed with blood, to large amounts of gelatinous mucus, fluid or gas.

Despite the fact that mucoid enteritis has been known for many years its aetiology still remains obscure. A number of possible causes have, however, been suggested, amongst others, nutrition. Ostler[26] reported cases where the disease occurred shortly after a change in the food. Kurilov[1], on the other hand, considers early weaning an important cause of the disease while McCuistion [23] regards the main cause to be an amilase deficiency of young rabbits due to agalactia of the doe. Arrington & Wallace[2] could, however, not confirm the latter observation.

Some workers are of the opinion that the aetiology might be of a contagious nature[3, 32], but attempts to transmit the disease generally gave inconclusive results[22]. Greenham[11] found *Escherichia coli* to be much more numerous in affected animals than in unaffected ones, but he was uncertain whether or not the organism was a cause or an effect of the disease. Similar observations were made by Löliger, Matthes, Schubert & Heckmann[17], Yuill & Hanson[39] and, to a certain extent, Ostler[26]. Pridham[30] found *Vibrio* spp. to be very common in cases of mucoid enteritis, while Morcos[24] reported a *Clostridium* sp. from cases of "infectious tympanitis" and Van der Schaaf, Van Dorsen, Donker-Voet, Frik & Van Maanen[38] *Clostridium welchii* from cases of acute gastroenteritis. Attempts to isolate a virus have so far been unsuccessful[22] and reports on a possible fungal cause are variable[22].

Various attempts have been made to treat this disease and favourable results were reported with the use of nitrofurazone[31], chloramphenicol[1, 13], chlortetracycline[5, 15, 36], oxytetracycline[5], formosulphathiazole[18], sulphachloropyrazine[18] and phthalysulphathiazole plus streptomycin[9]. Greenham[11], however, found oxytetracycline, chlortetracycline and nitrofurazone plus furazolidone to have little effect on the mortalities. Similarly Hagen[12] stated that sulphaquinoxaline did not influence the incidence of enteritis as a whole although it reduced the incidence of haemorrhagic enteritis. Alteration of the diet[11] and improved hyygiene [36] apparently had little effect on the mortalities.

Based on this information it was assumed that mucoid enteritis was the cause of those mortalities seen at Onderstepoort which could not be ascribed to coccidiosis. In view of the fact that these cases occurred despite sulphonamide therapy, treatment was attempted with oxytetracycline†. It proved to be highly effective when given in the mash for 7 days at a concentration of 0.01 per cent. All deaths due to enteritis stopped and did not recommence after withdrawal of the drug. Attempts to eliminate the losses by thorough daily cleaning of the hutches and disinfection proved worthless in the absence of antibiotics. Deaths ceased to occur during the autumn of 1969 and have not recurred since. No attempts were made to isolate bacteria from affected animals, but the favourable results obtained with the antibiotic seem to indicate that bacteria were involved.

† TM 10 (Terramycin), Pfizer.

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DISCUSSION

The resemblance between mucoid enteritis and coccidiosis as seen at Onderstepoort is striking, especially as far as the symptomatology, age susceptibility and macroscopic lesions are concerned. It would appear that the two diseases occurred concomitantly in the original outbreak. Coccidiosis, however, seems to have been the main cause, since elimination thereof caused a considerable reduction in the mortality rate. To what extent mucoid enteritis complicated the clinical picture is unknown, but the disease was more acute and severe than coccidiosis as it usually manifests itself in rabbits.

Numerous publications exist which deal with either coccidiosis or acute enteritis, but in only a few the possibility of a combination of the two is considered. Löliger *et al.*[17] were able to produce acute deaths in young rabbits with a combined infection of *E. coli* and *Eimeria* spp. whereas separate infections caused less severe reactions. These authors conclude that uncomplicated coccidiosis is less important than a mixed infection with *E. coli*. They consider the *E. coli* infection to be of primary significance with coccidiosis playing a lesser role. Similarly Gordon[10] suggests that coccidiosis may have been an associated factor in cases he observed and was not the primary cause of death. According to the Annual Report of the Los Angeles Livestock Dept., 1943[19] enteritis was seen either alone or complicated with coccidiosis or pneumonia. Pellérdy[28], however, mentions the possibility of secondary bacterial infections in cases of rabbit coccidiosis, but apparently considers the latter more important.

The observations made at Onderstepoort seem to support the contention that coccidiosis complicated by another condition is more important than the uncomplicated disease. The exact interrelationship between the two in the causation of disease, however, remains obscure and requires further investigation.

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A COMPARISON OF CERTAIN RAT STRAINS WITH RESPECT TO SUSCEPTIBILITY TONEPHROCALCINOSIS*

D. B. Du Bruyn**

INTRODUCTION

The rat is frequently used as a model for testing the safety of foods, not only in respect of recognized toxic ingredients but also with regard to the balance of nutrients in the relevant foodstuffs or diets.

The literature contains an abundance of data on the nutritional factors, such as mineral and vitamin excesses and deficiencies, which can cause nephrocalcinosis in rats. Although most of the fundamental work has been done with rats of various strains, very little attention has thus far been paid to the question whether or not there is a strain difference in the response to consumption of a nephrocalcinogenic diet.

Cousins and Geary[1] have reported from the University of Otago Dental School, New Zealand, that the females of Wistar-derived albinos, of pure lines of white and black rats, and of a pure-line black-and-white Wistar-derived strain were found to be highly susceptible to nephrocalcinosis, whilst the males of these breeds appeared non-susceptible. Goulding and Malthus[2] confirmed the very high susceptibilities to calcium phosphate calcinosis of the females of the above strains, and also studied a Spraque Dawley strain from the United States. However, the latter workers found that also the males of all the above strains were susceptible, although incidences and severities were more variable in males than in females. In concordance with the latter observations we found in our preliminary investigations[3] of the relationships between dietary calcium, phosphorus and magnesium content and the nephrocalcinogenicity of the diet, that in Wistar-derived random bred males developing nephrocalcinosis, there were marked differences in severity between individuals within a particular group. Using a strain derived from Charles River CD rats, Gershoff[4], working in the Department of Nutrition, Harvard School of Public Health, however, supplied evidence indicating that the deposition of oxalates in rat kidneys in pyridoxine deficiency occurs more readily in males than in females.

From the investigations cited above it can be seen that both sexes from all of the strains used were to some degree susceptible to nutrional nephrocalcinosis. It is, however, not possible to establish conclusively on the basis of the available information whether or not there were differences in the suceptibilities of the different strains.

The present report has a bearing on results obtained in a preliminary investigation of the relative susceptibilities of certain rat breeds that were available to me at the time of the study.

MATERIALS AND METHODS

Both sexes of the following rats were examined: Wistar-derived random-bred albino rats; two inbred strains BD V and BD IX; and the F_1 generation of a cross between the two inbred strains. These rats were fully described in the paper presented by Dreyer[5]. The animals were bred at the NNRI and raised on a stock diet which did not produce nephrocalcinosis in any of the above rats when fed for a period of at least 40 days after weaning.

Experimental groups of 3 males and 3 females were fed an otherwise well-balanced semi-synthetic diet containing magnesium at either a 150 or a 50 mg % level for 40 days, the Ca : P ratio of the diets at both magnesium levels being as recommended by NRC[6], *viz.* 1.2:1. Full details on the composition of both diets (Diets I and II) are shown in Table 1.

* Paper presented at the Symposium on the Production and Use of Laboratory Animals.
** National Food Research Institute, C.S.I.R., Pretoria.

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The rats were housed in screen-bottomed cages (3 rats per cage) in an air-conditioned room at 28±2°C and 50±5% relative humidity.

At the end of the 40-day feeding period the animals were anaesthetized with ether for removal of kidneys. The kidneys were fixed in buffered formalin (pH=7.2) and sections of 10-micron thickness were stained with alizarin for the identification of calcium structures.

Table 1: CONSTITUENTS OF EXPERIMENTAL DIETS

Constituent	Weight in g/100 g of Air-dry Diet	
	Diet I	Diet II
Casein	15.0	15.0
Sunflower seed oil	10.0	10.0
Agar	2.0	2.0
Sugar	10.0	10.0
Vitamin mixture[a]	2.0	2.0
Trace element mixture[b]	2.6	2.6
Salt mixture[c]	5.7	5.7
Magnesium oxide	0.13	0.40
Dextrin	52.6	52.3

(a) Standard synthetic vitamin mixture supplying vitamins as suggested by Cuthbertson[7] plus vitamin A and E as recommended by NRC[6].

(b) Supplying Zn, Fe, Mn, Cu and I salts in quantities recommended by NRC[6]. Citric acid was included in mixture (0.8%) to prevent rancidity[8] in diet. K_2SO_4 was added to raise SO_4 content to level suggested by Bernhart and Tomarelli[8].

(c) Supplying Ca and P in amounts recommended by NRC[6]. Special allowance was made for the phosphorus present in the casein used. K and Na supplied by this mixture were 3X and 8X, respectively, the amounts recommended by NRC[6].

RESULTS AND DISCUSSION

The results of the incidence of nephrocalcinosis in the 4 breeds of rats are shown in Table 2.

Table 2: INCIDENCE OF NEPHROCALCINOSIS IN THE DIFFERENT RAT BREEDS

Rat breed	Sex	Diet I (Mg=50 mg%)	(Diet II) (Mg=150 mg%)
Wistar-derived	Male	+	−
Wistar-derived	Female	+	−
BD V	Male	+	−
BD V	Female	+	+
BD IX	Male	+	−
BD IX	Female	+	+
F_1 generation	Male	−	−
F_1 generation	Female	+	+

Note: (+) = Nephrocalcinosis observed in at least one member of group of 3 rats.
(−) = Normal.

It will be seen that kidney calcification at the 50 mg% dietary magnesium level (Diet I) occurred in all groups, except in the males of the F_1 generation of the cross between BD V and BD IX rats. At the higher magnesium level (Diet II) all female groups except the Wistar derived albinos were susceptible, whilst all males remained free from signs of nephrocalcinosis. It therefore appears that apart from the dietary factor (inadequate intake of magnesium) genetic factors as well as the sex of the animal are involved in the calcification precess, since rats which are genetically equal but of different sex as well as rats of the same sex differing in genetic make-up frequently differed in their response to a given experimental diet. The results obtained with rats in a comparison of the relative nephrocalcinogenicities of diets will therefore depend on the rat strain in the relevant trial.

Another important implication of the data shown in Table 2 is that rats of different genetic make-up appear to differ in respect of the amount of magnesium required for full protection against calcification of the tissues.

NRC[6] has recommended a diet containing 40 mg % magnesium and calcium and phosphorus at respectively 600 and 500 mg % levels. This recommendation was based solely on the growth performance of rats. Diet I contained magnesium at a level slightly in excess of the NRC recommendation and calcium and phosphorus exactly as recommended. Diet I afforded protection against calcification only in the case of the F_1 generation males, its magnesium content being inadequate for all the other breeds studied. It is also noteworthy that Diet II, which contained magnesium at 3.75 times the level recommended by NRC, was not quite capable of affording full protection to all rats from the various breeds, the kidneys of the females from the two inbred strains and of the F_1 generation showing signs of calcification. One would therefore be inclined to suggest that the magnesium level of diets containing calcium and phosphorus at the levels recommended by NRC should be even higher than 150 mg % to protect all rats of the breeds used in the study against kidney calcification.

Goulding and Malthus[9] have succeeded in preventing nephrocalcinosis in all the rat strains investigated by them by raising the

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dietary magnesium level to 450 mg%. Magnesium chloride as well as magnesium carbonate proved successful for prevention of calcification, but magnesium carbonate supplementation furnishing a dietary magnesium level of the above magnitude resulted in death because of magnesium phosphate deposition in the urinary tract. It therefore appears that the vehicle of the magnesium ion should be selected with caution since certain anions can have undesirable side-effects.

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BOOK REVIEW

AN INTRODUCTION TO VETERINARY PHARMACOLOGY

F. ALEXANDER

Second Editon. E. & S. Livingstone Ltd., Edinburgh and London. 1969. Pp. x & 331, 78 figures, 6 tables. Price 58s net.

This book has been written by Prof. Alexander to provide veterinary undergraduates with a concise account of the pharmacological properties of the principal drugs used in veterinary therapeutics. The twenty-five chapters cover most of the material which one would expect in a general textbook of veterinary pharmacology.

A commendable aspect of the book is that a large number of the seldom used and obsolete remedies have been ignored, while the author has concentrated on providing information about the drugs in current use and on explaining the principles of drug action where these have been established.

A new feature in the second edition is the appendage at the end of each chapter of a short list of selected references to more detailed accounts of the particular subject matter of the chapter.

Unfortunately, however, the overall presentation is not particularly good and there are sections which could be considerably improved, e.g., the biotransformation of drugs, the muscle relaxants and the analgesics. Furthermore, the text suffers serious drawbacks because of a number of equivocal statements, spelling errors, carelessly presented structural formulae and poorly reproduced illustrations. Exampes of these criticisms may be found on page 2, where it is not clear that it is the concentration of the nonionized moiety which is primarily responsible for the rate of drug passage across a biological membrane; on pages 30 and 31 (fursemide) and page 268 (arsanilic acid) where there are spelling errors; on page 142 (betamethasone), page 199 (oestradiol and testosterone), page 231 (sulphadimidine) and page 245 (chloramphenicol), where the structural formulae are not well drawn; finally on page 182 where Fig. 14.3 is very indistinct.

Veterinary undergraduates could perhaps make use of this book provided that they were guided in their studies, but it would be of dubious use to veterinary practitioners as a reference manual.

W. L. J.

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Jl S. Afr. vet. med. Ass. 41 (3): 201-204 1970

EFFECTS OF HIGH LEVEL COPPER SUPPLEMENTATION ON GROWING-FINISHING PIGS

P. A. Boyazoglu* and E. L. Barrett*

SUMMARY

Copper additions of 150 parts per million to the diet improved carcase quality and reduced feed requirements on higher protein levels (16%) when portion of the protein consisted of fish meal. Low protein of 13% and the replacement of fish meal by sunflower oil cake meal at both protein levels, despite vitamin B_{12} supplementation, gave lower performance figures and resulted in the accumulation of copper in the liver. These high copper concentrations probably contributed to the lowering of performance even at the 16% dietary protein level in the absence of fish meal.

At raised copper concentrations of 150 and 300 ppm added to the diet the Cu content of liver markedly increased, iron was reduced (300ppm Cu), zinc was increased and manganese and cobalt were unaffected.

It is apparent that the ration formulation particularly with regard to the type of protein used, has a major role in determining whether feeding levels of copper will have a beneficial or detrimental effect.

INTRODUCTION

The minimum copper requirement of pigs is in the range of 5 - 10 mg/kg feed[1] depending on age. This concentration is met by most standard concentrate formulations without copper supplementation.

Additions of copper to pig rations of 250 parts per million (ppm) and even higher in the form of copper sulphate improved growth rate and feed conversion[2,3] to a degree similar to the growth promoting effects of several antibiotics as used in growing-finishing rations. The beneficial effects of the addition of 250 ppm copper were studied by Braude[4] and a summary of results of 83 lots involving 1215 pigs gives a reflection of anticipated responses. These pigs averaged improvements of +8.1% (−12% to +25.2%) in daily weight gain and +5.4% (−5.2% to 12.6%) in improved efficiency of feed utilization over nonsupplemented controls[4]. Concurrently there have been observations that 250 ppm copper intake precipitated chronic toxicity and poor performance, and reliable reports consider this recommended level as toxic[1].

This distinct discrepancy could be the result of basic differences in feed formulations. One such difference is the protein source which may be predominantly of oil-cake meal origin or of fish meal origin. Fish meal has a higher biological value and the presence of considerable quantities of the sulphur containing amino-acids could result in a protective effect in the livers of pigs performing well on high copper feeding.

A further consideration related to high copper feeding is that when a nutrient is fed in excess of the actual requirements it is likely to affect related compounds as in the case of trace elements.

The experiment reported here was planned to evaluate the relationships between graded copper levels and proteins in the ration of bacon weight pigs by measuring the effects on chemical and physical changes.

MATERIALS AND METHODS

A factorial design 3 x 2 x 2 was planned with four replications per treatment as in the following table.

Large White (Yorkshire) pigs bred at the Institute were weaned simultaneously, two replications at a time, at 6 weeks of age. Each pig was individually fed starting with creep ration containing 18% protein for a pre-experimental period of two weeks. Exceptionally good or mediocre feeders were eliminated. The remaining pigs were divided into groups as indicated in Table 1, each

* Nutrition Section, Veterinary Research Institute, P.O. Onderstepoort.

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Table 1: EXPERIMENTAL DESIGN

Copper added	Sunflower oil cake meal			Fish meal		
	0 ppm	150 ppm	300 ppm	0 ppm	150 ppm	300 ppm
Protein 13%	4*	4	4	4	4	4
Protein 16%	4	4	4	4	4	4

group containing the same number of barrows and gilts in equal numbers for all groups.

Pigs were fed once a day with amounts commencing at 1 kg/pig/day and gradually increasing to 3 kg/day as the pigs approached bacon weight (85 kg).

The diets fed are shown in the following table 2.

The pigs were slaughtered when they had reached or just exceeded a weight of 85 kg. The liver and kidney weights were taken at slaughter and samples of the liver were preserved in 10% formalin. Evaluations were made 24 hours after slaughter on the refrigerated side.

Mineral analyses were done on the formalinized liver using a Beckman 979 atomic absorbtion spectrophotometer. The liver specimen was dried on blotting paper, a 1 gm sample of parenchyme taken and wet ashed in a mixture of sulphuric and perchloric acids with the addition of nitric acid. Lanthanum oxide was used to avoid interference of related minerals. The sample was processed with the appropriate mineral lamp in position to supply the reading for each particular element.

RESULTS

The grand mean values of the entire group of animals in the experiment are given in Table 3.

Table 3: GRAND MEAN VALUES

Physical Characteristics

Carcase weight per cent of live weight	77.4%
Fresh liver weight	1.37 kg
Fresh kidney weight	254.4 g
Eye muscle area	27.2 sq cm
Fat over eye muscle	1.9 cm
Feed conversion	3.5

Mineral content of livers, ppm on wet basis

Copper	151.5
Manganese	6.24
Zinc	188.8
Cobalt	24.6
Iron	158.7
Magnesium	176.9

The effect of elevating the protein level from 13% to 16% irrespective of protein type resulted in increases in a) liver weights from 1.299 kg to 1.431 kg,** b) kidney weights from 238 gm to 270.8 g** and c) eye muscle area from 25.17 sq cm to 29.27 sq cm.** At the same time there was an improvement of the feed conversion rate which was lowered from 3.73 to 3.29.** The change in protein level also resulted in a reduction of the liver iron content from 182.1 ppm to 135.3 ppm.

When fish meal was replaced by sunflower oil cake meal there was a reduction of a) eye muscle area from 28.79 sq cm to 25.65 sq cm** and b) the carcase percentage from 78.67 to 76.10**. Increases were recorded in (i) poorer feed conversion from 3.22

Table 2: EXPERIMENTAL RATIONS

	13% Protein						16% Protein					
Yellow mealie meal	65	65	65	65	65	65	65	65	65	65	65	65
Wheat bran	16	16	16	21.5	21.5	21.5	3	3	3	16	16	16
Lucerne meal	10	10	10	10	10	10	10	10	10	10	10	10
Sunflower oil cake meal	6	6	6				19.5	19.5	19.5			
Fish meal				2	2	2				8	8	8
Limestone powder	1	1	1	1	1	1	0.75	0.75	0.75	0.5	0.5	0.5
Dicalcium phosphate	1	1	1				1.25	1.25	1.25			
Salt	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
$CuSO_4.5H_2$ g*		58.6	117.2		58.6	117.2		58.6	117.2		58.6	117.2

*Per 100 kg feed as tabulated.
All twelve formulations had the following additions per 100 kg feed: B_{12} 1,100 mg; Vitamin A 1,320,000 iu, Vitamin D 200,000 iu.

** ($P < .01$)

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Table 4: SUMMARY OF TREATMENT EFFECTS

		Liver g	Kidney g	Fat over eye muscle cm	Eye muscle area sq cm	Feed Conversion	Carcase %	Liver: Mineral content in parts per million on wet basis Cu	Fe	Mn	Co	Zn	Mg
Protein Level	16%	1.457	271	1.8	29.27	3.29	77.0	138.3	135.3	6.4	25.0	188.2	173.2
	13%	1.299	238	2.2	25.17	3.56	77.7	164.3	182.1	6.1	24.2	188.5	174.1
Protein Source	O.C.M.	1.335	247	1.83	25.65	3.80	76.1	205.2	160.1	6.3	24.6	189.7	181.6
	F.M.	1.395	262	2.0	28.79	3.22	78.7	97.4	157.3	6.2	24.6	187.5	165.7
Copper Level	0	1.329	252	2.0	25.36	3.55	77.2	23.3	172.4	6.5	25.1	183.1	171.1
	150	1.387	251	1.85	28.54	3.40	77.2	122.8	178.6	5.8	24.85	185.1	172.5
	300	1.380	261	1.8	27.77	3.58	77.7	307.7	125.1	6.4	23.8	197.8	177.7

to 3.80**, (ii) liver concentration of magnesium from 165.7 to 181.6** and (iii) (doubling of) liver concentrations of copper from 97.38 to 205.15 ppm.

The addition of 150 ppm copper as copper sulphate to the ration, compared to unsupplemented formations, resulted in a significant increase in eye muscle area from 25.36 sq. sm. to 28.54 sq. cm.* However 300 ppm did not give a significant change. At the 150 ppm copper addition interaction with 16% protein level the 3.31 feed conversion was significantly lower than the 4.02 of the 300 ppm copper-addition interaction with 13% protein concentration.

Manganese concentrations in the liver were 6.6 ppm which were significantly higher at the 13% protein interaction with 300 ppm copper as compared to the 5.14 ppm of the 13% protein interaction with 150 ppm copper.

Zinc concentrations were also significantly increased to 218.7 ppm* at the sunflower oil cake meal interaction with 300 ppm copper as compared with the grand mean level of 188.8 ppm.

Iron concentrations were markedly influenced with reductions taking place from 172.3 (ppm copper) and 178.6 ppm (150 ppm copper) to 125.1 ppm (300 ppm copper). The sunflower oil cake meal interaction with no copper addition resulted in 210 ppm iron in the liver which progressively declined to 156.3 ppm and 113.6 ppm with the additions of 150 or 300 ppm copper to the sunflower oil cake meal rations. This suggests an inverse relationship between the iron and copper concentrations in the liver which, however, was not evident when fish meal was present in the ration.

The liver cobalt levels appeared to be unaffected by treatment effects as applied in these experiments.

The additions to the basic rations of 150 or 300 ppm copper resulted in highly significant changes in liver concentrations which are given in the following table.

Table 5: EFFECT OF COPPER ADDITION ON LIVER COPPER CONCENTRATION

	Copper addition to feed, ppm		
	0	**150**	300
Liver copper in ppm on wet basis	23.3	122.8	308.3

DISCUSSION

The carcase changes brought about by raising the protein level from 13% to 16% were beneficial and produced more lean weight. The lowering of feed conversion was also an indication of a more efficient feed utilization. Associated with these changes was a lowering of liver iron content which was indicative of increased blood formation.

The replacement of sunflower oil cake meal by fish meal gave further benefit but also reduced the liver copper content level most dramatically from 205.15 ppm to 97.38 ppm. This reduction of accumulated copper by the feeding of fish meal is considered to be the reason why toxicity is not found with high copper feeding when fish meal

*($P < .05$) **($P < .01$)

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containing rations are fed. The advantageous effect of copper feeding at high levels is thought to centre in the digestive tract and not in the body's metabolic pathways which it tends to overload.

The high biological value of protein eliminates such overloading within the body. From these results it appears that supplementation with 150 ppm improved carcase characteristics substantially but at 300 ppm only marginal benefit was noted which indicated detrimental effects taking place especially at the 13% protein level.

The copper concentrations in the liver rose rapidly with increased intake thus confirming that the concentration in the liver is a good criterion of evaluating feed copper intake. The high copper intake however had an inverse relationship with iron concentration which is possibly caused by an increased rate of haematopoeisis draining the iron stores.

The changes noted in magnesium, zinc and manganese as quoted earlier can at this stage not be interpreted efficiently.

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Edited by
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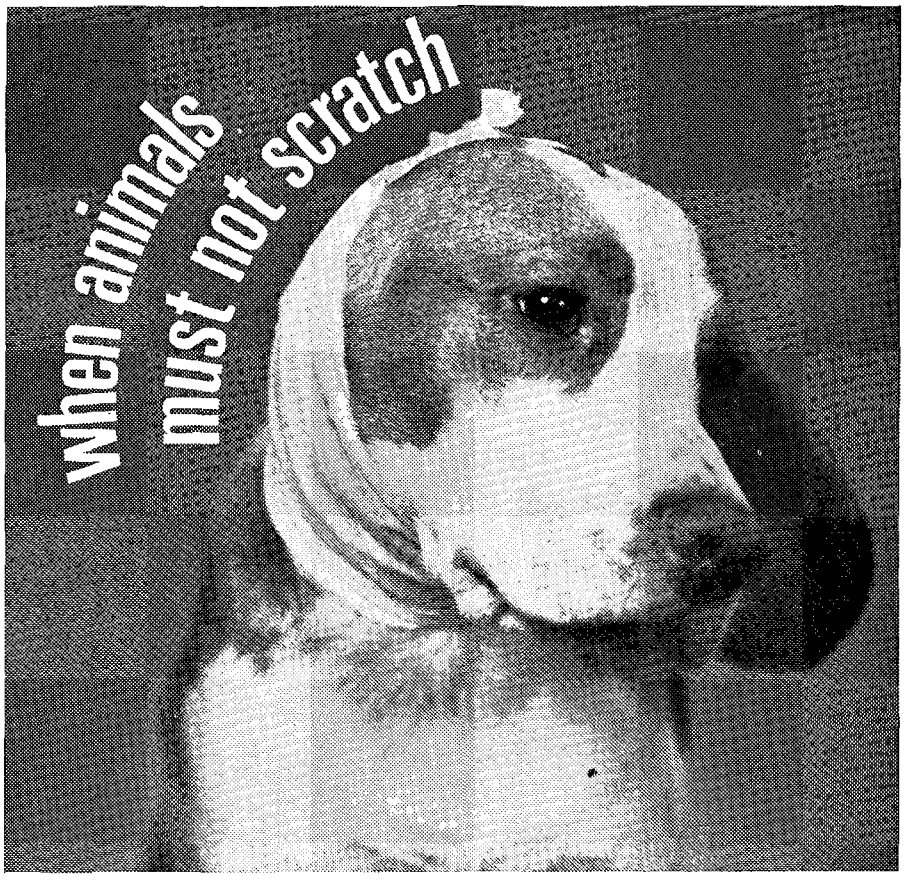

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COPPER METABOLISM IN THE MERINO SHEEP IN SOUTH AFRICA

Normal levels of liver Copper in Sheep from the Cape Midlands

J. A. ERASMUS

SUMMARY

By means of a cumulative relative frequency curve the normal range for liver copper of sheep which were born and bred on a known enzootic icterus farm was found to be 20 - 1400 p.p.m. on a dry matter basis. When compared with the normal range obtained for liver copper in healthy Transvaal sheep, 60% of these data were found to fall out of this range.

Significantly higher liver copper values were obtained from clinically healthy sheep born and bred on known "enzootic icterus farms" in the Cape Midlands than from sheep born and bred on farms on which both enzootic icterus and geeldikkop are unknown in the same area. Both mean values were found to be higher than the mean value for liver copper in Transvaal sheep.

INTRODUCTION

The acute form of enzootic icterus is characterized by an abnormal copper metabolism and an acute haemolytic syndrome. A range of 500 - 1528 ppm (wet matter basis) of liver copper was found in sheep which succumbed to the acute form of the disease in comparison to a suggested normal range of 72 - 372 ppm (on a wet basis) for clinically healthy sheep from the Transvaal. Red cell copper values as low as 11 mcg% have been noted in sheep in the acute stage of the disease.

Assuming the red cell volume in the healthy sheep with a blood-volume of 2,000 ml to be 30%[2, 3, 4, 5] and the red cell copper to be 60 mcg%[6] it could be expected that in a haemolytic syndrome of this nature 228 mcg of copper would be liberated if about 64% of the erythrocytes were to haemolyse. If this liberated copper was then taken up by the liver only, the increase of copper in a liver of 400 gm would be in the order of 0.57 mcg per gram wet material.

From this calculation it would appear that the marked increase in liver copper in the animal which succumbed to the acute form of enzootic icterus is caused by factors other than this final haemolytic crisis. An investigation into the normal range for liver copper in clinically healthy sheep in the Cape Midlands was thus deemed necessary.

MATERIALS AND METHODS

Formalinized liver samples from 96 clinically healthy aged Merino wethers and ewes from a known enzootic icterus farm in the Noupoort district were collected during a period in which no cases of the disease occurred. One gram was accurately weighed, dried and then pulverized. The samples were then wet ashed[6] and made up to 100 ml with distilled water. The copper content of each sample was determined directly by means of a Techtron model AA_4 atomic absorption spectrophotometer using compressed air and acetylene as burner gas.

In a second survey 162 formalinized liver samples from aged, clinically healthy Merino wethers were analyzed for copper by the formation of a coloured complex with biscyclohexanone oxalyldihydrazone (Cuprizone)[7] after wet ashing of the dried material. All readings were made on an EEL model A photo-electric colorimeter using an appropriate Ilford light filter.

The actual values for liver copper were read off directly from previously constructed calibration curves.

Fifty of these 162 samples were collected from two farms on which both enzootic

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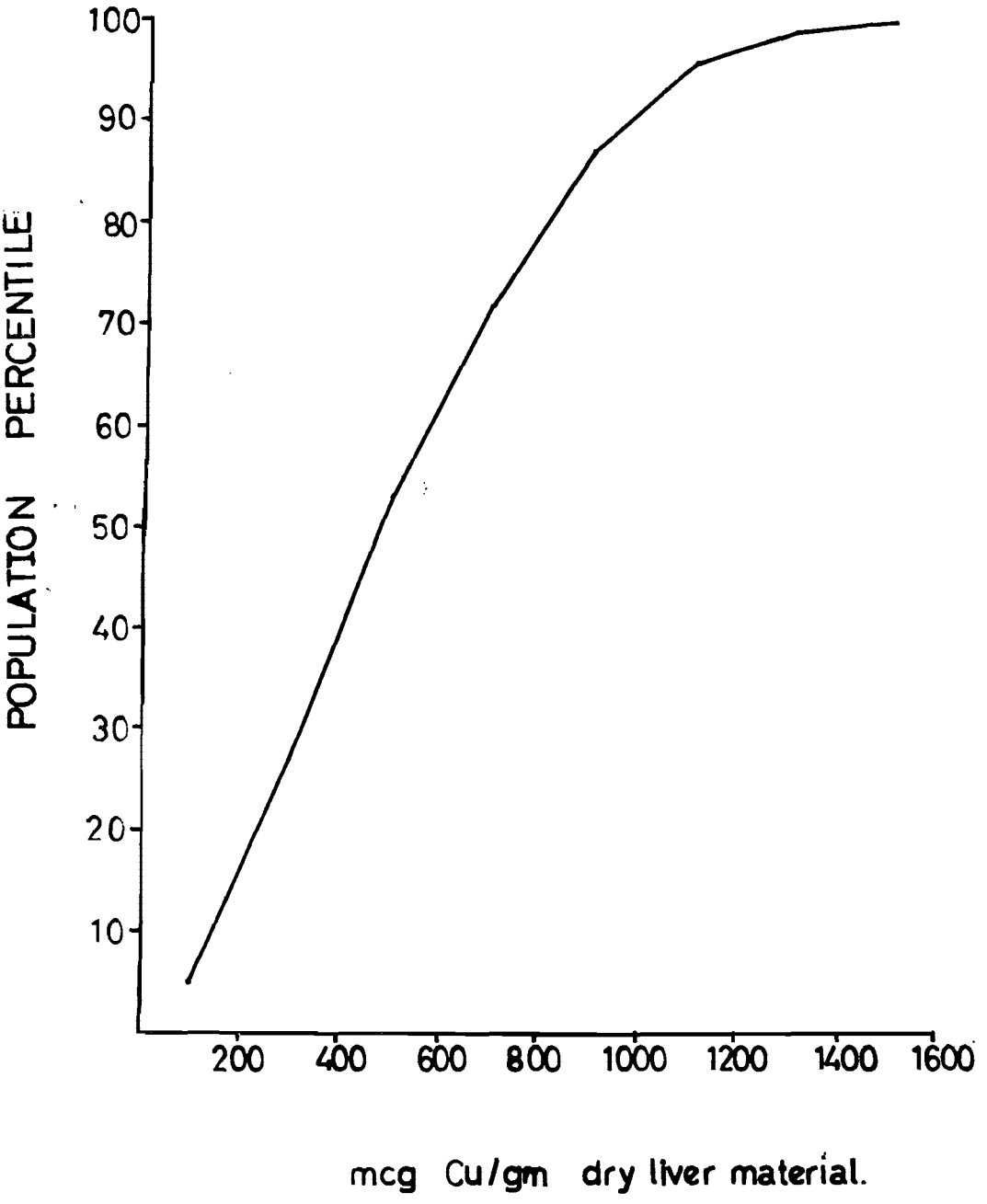

FIG. I Cumulative relative frequency curve for liver Copper (ppm dry matter)

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icterus and geeldikkop were unknown and 112 samples were collected from four farms with a known history of enzootic icterus. All sheep were dependent on natural Karoo vegetation as their only source of nourishment.

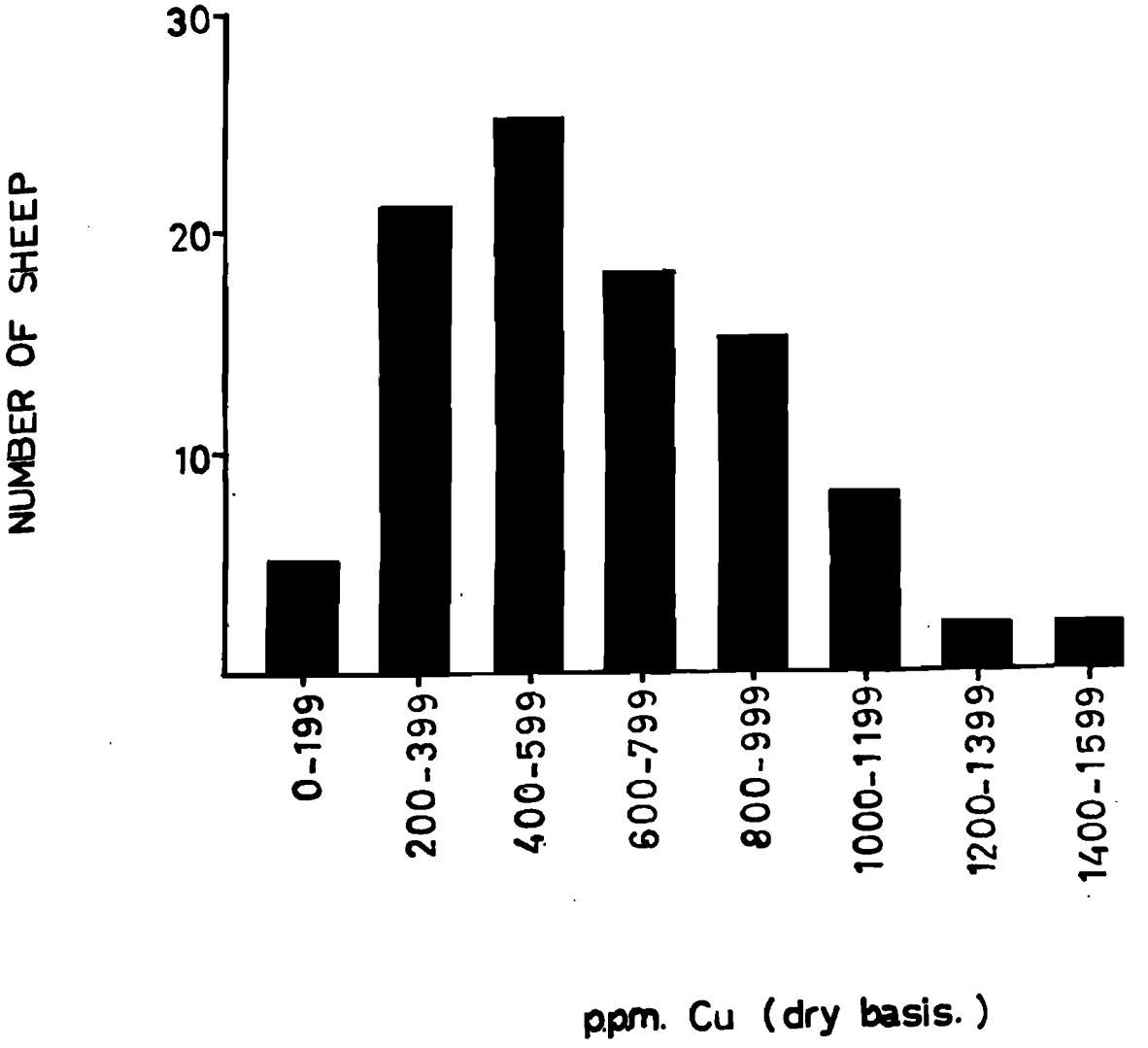
The normal range for liver copper was calculated from the data obtained from the 96 animals in the first survey using a cumulative relative frequency curve[8,9].

RESULTS AND DISCUSSION

The distribution of data and the cumulative relative frequency curve from which the normal range was deduced are shown in Fig. 1 and 2. The normal range is given in Table 1.

Table 1: RANGE FOR LIVER COPPER (P.P.M. ON A DRY MATTER BASIS)

Mean as deduced from median	80% Range	98% Range
475 n = 96	120—970	20—1400



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According to the literature sources available it is apparent that (table 2) marked variations in the normal range for liver copper may occur. The upper range of 1400 ppm found in this work, agrees however well with figures found by Cunningham[12] and cited by Spector[13]. It is also evident that values found for 60% of cases in the first survey were far beyond the copper range for clinically healthy Transvaal sheep.

Table 2: NORMAL RANGE FOR LIVER COPPER IN SHEEP (P.P.M. ON A DRY MATTER BASIS) AS TAKEN FROM THE LITERATURE

Normal range	Scource
123—584	Beck[10], 1956
37—380	Beck[11], 1963
186—1374	Cunningham[12], 1946
100—1350	Spector[13], 1956
200—400	Adelaar[14]

The mean values and standard deviation of the means for both surveys are given in table 3. The mean value for liver copper in sheep from the known enzootic icterus farms in the second survey was significantly higher than those from control farms in the same area. Due to a significant difference in the homogeneity of variance, data from the first survey could not be compared statistically with those of the second.

Table 3: MEAN VALUES AND STANDARD DEVIATION OF THE MEANS ON DATA SUBJECTED TO A SQUARE ROOT TRANSFORMATION

History	Method	Number of cases	Mean	S
Enzootic icterus	Atomic absorption	96	24.4	5.6
Enzootic icterus	"Cuprizone"	112	28.4*	10.7
Control	"Cuprizone"	50	22.7*	9.6

*$P < 0.001$

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THE ANTHELMINTIC EFFICACY OF RESORANTEL

J. G. GAENSSLER* AND R. K. REINECKE**

SUMMARY

Resorantel was highly effective when dosed at 65 mg/Kg *per os* to sheep and cattle infested either with *Moniezia expansa* or *Paramphistomum microbothrium.*

At this dosage rate it cleared 13 of 14 sheep and five calves infested with *M. expansa.* It exceeded 99% efficacy in cattle infested either with immature or adult *P. microbothrium.* In sheep it was more than 80% effective in more than 80% of the treated flock against immature and adult *P. microbothrium.*

It however, had no anthelmintic efficacy at a dose rate of 65 mg nor at 130 mg/k against *Stilesia hepatica, Fasciola hepatica* and *Fasciola gigantica.*

INTRODUCTION

Resorantel*** is the generic name for 2, 6 dihydroxybenzoic acid — 4' — bromanalide. The pure substance has the following properties: It is a colourless powder, stable at room temperature for at least 2 years; turns pink if heated at 100°C; highly sensitive to iron compounds which stain it deep red or violet; resistant to acids and alkalies when heated to 100°C; insoluble in water, hydrocarbons or vegetable oils; slightly soluble in lower alcohols at room temperature and highly soluble in dimethyl foramide.

Resorantel has been shown to be highly effective against cestodes[1,2] in sheep and *Paramphistomum microbothrium* in goats[3]. Moreover, in laboratory and field trials with over 2,000 lambs it was well tolerated[4].

This paper describes critical and controlled anthelmintic tests in sheep and cattle with natural infestations of *Moniezia expansa, Stilesia hepatica, Fasciola hepatica, Fasciola gigantica* and *Paramphistomum microbothrium.* A controlled anthelmintic test in an experimental infestation of sheep with *P. microbothrium,* is also reported.

MATERIALS AND METHODS

Anthelmintic:

Resorantel 75% w/w wettable powder was added to water to make a final concentration of 130 mg/ml.

Experimental animals:

On the day of dosing sheep were weighed and calf weights estimated with the aid of a weighband.

Dosage rate:

With the exception of two groups in trials on *S. hepatica* and *Fasciola* spp. mentioned below, all animals were dosed orally at the rate of 65 mg/Kg.

Experiment 1: *M. expansa in sheep and cattle*

Fourteen cross-bred lambs and five Jersey calves naturally infested with *M. expansa,* were used. Faecal collecting bags were attached to the lambs during the day, but not at night whilst the lambs were suckling. This was done prior to treatment for 2 days and after treatment until slaughter 3 - 6 days later. The tapeworm segments collected from the faecal bags were placed in water in measuring cylinders and the volume of water displaced was noted. In calves the animals were individually penned and the presence of segments in their faeces noted every day, until slaughter 5 days after treatment.

At autopsy, the mesenteric fat was stripped, the entire intestinal tract from pylorus to anus was opened and the ingesta washed through a 100 mesh to the linear inch sieve (Endecott). The mucosa of the entire intestine and the ingesta trapped on the sieve's surface was diligently examined macroscopically for the presence of scoleces and tapeworm segments.

Resorantel removed all the *M. expansa* in 13 of 14 treated sheep (Table 1). All segments were expelled within 24 hours after treating the calves. At autopsy neither sco-

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*** Terenol — Farbwerke Hoechst A.G.

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Table 1: EXPERIMENT 1: CRITICAL ANTHELMINTIC TESTS IN SHEEP INFESTED WITH **M. EXPANSA** AND DOSED WITH RESORANTEL AT 65 mg/Kg

Sheep No.	Weight kg	Dose ml	Volume of segments passed		Scoleces recovered at autopsy
			Prior to dosing ml	After dosing ml	
1	11	5.5	9	80	0
2	6	3.0	0	15	0
3	12	6.0	0	75	1
4	11	5.5	6	70	0
5	11	5.5	6	75	0
6	5	2.5	3	*10	0
7	7	3.5	8	* 0	0
8	10	5.0	3	125	0
9	9	4.5	6	12	0
10	15	7.5	8	* 0	0
11	15	7.5	25	85	0
12	11	5.5	20	20	0
13	5	2.5	0	30	0
14	12	6.0	12	120	0

*Faecal collecting bags lost on the day after dosing i.e. Day + 1.

leces nor segments were recovered from the intestinal tract of any of the calves.

Experiment 2: *S. hepatica* in sheep

Fifteen sheep were selected at random from a flock of Blackhead Persian X Van Rooy sheep known to be infested with *S. hepatica*. Two were slaughtered and the presence of *S. hepatica* confirmed. Six of the sheep were treated with resorantel at 65 mg/Kg and the remaining seven at 130 mg/Kg. Seven to 21 days later all thirteen sheep were slaughtered and their livers examined for *S. hepatica*.

None of the six sheep treated at 65 mg and only 2 out of 7 sheep treated at 130 mg/Kg were free of *S. hepatica*.

Experiment 3: *P. microbothrium in sheep and cattle*

(a) *Immature worms in sheep*:

Twenty paramphistome-free German Merino sheep were housed under worm-free conditions. Each of them was dosed orally with three doses of 500 metacercariae of *P. microbothrium* on the 14th, 12th and 10th days respectively, before treatment. All the sheep were weighed and 11 of them treated. Two untreated controls were killed on the day of treatment. The other 7 controls and the 11 treated sheep were killed 4-6 days later.

At autopsy double ligatures were tied at the pylorus, 9m from the pylorus and at the ileo-caecal valve. The methods of worm recovery described by Horak[5,6] were used with one modification. Instead of 300 mesh, 400 mesh to the linear inch

Table 2: EXPERIMENT 3: CONTROLLED ANTHELMINTIC TESTS IN SHEEP INFESTED WITH **P. MICROBOTHRIUM**

Sheep No.	IMMATURES: experimental infestations		Small intestine: worms recovered post-mortem	Sheep No.	ADULTS: natural infestations		Rumen: worms recovered post-mortem
	Weight kg	Dose ml			Weight kg	Dose ml	
		Undosed controls			Undosed controls		
15	20	—	101	35	30	—	3,721
16	18	—	321	36	27	—	435
17	16	—	103	37	30	—	2,965
18	20	—	97	38	35	—	2,760
19	24	—	278	39	35	—	3,771
20	24	—	306	40	36	—	1,923
21	20	—	90	41	29	—	3,872
22	18	—	83	42	30	—	1,555
23	20	—	269	43	30	—	2,081
	Dosed with resorantel at 65 mg/kg per os				Dosed with resorantel at 65 mg/kg per os		
24	20	10.0	0	44	30	15.0	0
25	18	9.0	0	45	30	15.0	0
26	18	9.0	0	46	34	17.0	1
27	16	8.0	0	47	34	17.0	0
28	24	12.0	0	48	34	17.0	175
29	24	12.0	129	49	34	17.0	0
30	18	9.0	4	50	34	17.0	0
31	16	8.0	0	51	30	15.0	0
32	18	9.0	0	52	34	17.0	107
33	18	9.0	0	53	28	14.0	0
34	18	9.0	0	54	29	14.5	53

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sieves (Endecott) were used to wash the ingesta. All the worms recovered were counted.

The controls had worm burdens ranging from 83 to 321 with a median count of 103 (Table 2). With the exception of Sheep 29 with 129 and Sheep 30 with 4 worms respectively, all the treated sheep were negative. Thus 103 (0.25) = 26 which constitute more than 75% reduction on the control median in 10 out of 11 Sheep that were treated.

(b) *Adult worms in sheep:*

On the basis of faecal worm egg counts on 5 g. of faeces, 20 naturally infested sheep were selected. Nine acted as undosed controls, while 11 were treated.

At autopsy 3 and 9 days after treatment, the rumen was removed, the ingesta was washed through a 100 mesh to the linear inch sieve (Endecott) and the worms counted.

The results are summarized in Table 2. In the controls worm burdens ranged from 435 to 3,872 with a median count of 2,760. With the exception of four sheep with worm counts ranging from 1 - 75, seven of the treated sheep were negative. Since 2,760 (0.25) = 690 all the treated sheep showed more than 75% reduction in worm burdens on the control median.

(c) *Immature worms in cattle:*

A natural outbreak of paramphistomiasis occurred in the Nigel district with clinical signs of severe emaciation, projectile diarrhoea, anorexia and mortalities in six calves. Two undosed controls were killed on the day of treatment and another three, six days later. The weights of the calves were estimated with a weighband and five of them were dosed. Five days later they were killed.

Procedures at autopsy[5,6] and worm counts[7] were similar to those described for immature worms in sheep but a 200 mesh to the linear inch sieve (Endecott) was used instead of a 400 mesh sieve to wash the ingesta, and three, one-thirtieth instead of three, one-tenth aliquots were counted.

In Table 3 the results are summarized. While the controls had worm counts ranging from 52,682 to 232, 107 with a median count of 133,746, only two treated calves had burdens of 35 and 250 worms respectively. Moreover, diarrhoea ceased within 48 hours in all of the treated animals while two more untreated animals died before treatment could be instituted.

Resorantel was almost 100% effective. The reduction on the control median 133,746 (0.01) = 1,337 represents 99% efficacy. The maximum worm count of 250 (Calf 14, Table 3) in the treated group is but a fraction of this figure.

Table 3: EXPERIMENT 3: CONTROLLED ANTHELMINTIC TESTS IN CATTLE WITH NATURAL INFESTATIONS OF **P. MICROBOTHRIUM**

IMMATURES		ADULTS	
Calf No.	Small intestine: worms recovered post-mortem	Calf No.	Rumen: worms recovered post-mortem
	Undosed controls		
6	232,107	16	4,514
7	195,739	17	9,943
8	52,682	18	6,954
9	81,549	19	1,051
10	133,746	20	5,938
	Dosed with resorantel at 65 mg/kg per os		
11	0	21	1
12	0	22	0
13	35	23	1
14	250	24	1
15	0	25	0

(d) *Adult worms in cattle:*

Preliminary trials using faecal worm egg counts in infested cattle indicated that resorantel at 65 mg/Kg was highly effective.

At Tweespruit, O.F.S. a natural outbreak some weeks prior to treatment was used to assess the efficacy against adult worms in the rumen. An undosed control (Calf 16) was slaughtered and 4,514 worms recovered (Table 3). The weights of five calves were estimated with the aid of a weighband and they were treated. The five treated calves and an additional four undosed controls were transported to the Pretoria Abattoir and killed 10 to 11 days later. The *post-mortem* examination was similar to that described previously for adults in sheep, with one modification. Three one-fiftieth aliquots were counted, instead of total counts.

The controls had worm burdens varying from 1,051 to 9,943 with a median value of 5,938 (Table 3). No worms were recovered from two and the other three calves had but a single worm each.

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Experiment 5: *Fasciola* spp. in sheep

Twenty Merino sheep from the Tweespruit District, naturally infected with *F. hepatica* and *F. gigantica* were selected and eleven of them weighed. Six of them were dosed with resorantel at 65 mg/Kg and the other five at 130 mg/Kg. Nine undosed controls, killed on the day of treatment, had worm burdens ranging from 3 to 135 with a median of 65. Twenty-one days later the 11 treated sheep were killed. They were all infested regardless of the dose and worm burdens varied from 2 to 322 with a median count of 58.

DISCUSSION

The extremely high efficacy of resorantel against adult and immature *P. microbothrium* in cattle is a major advance in the treatment of this parasite.

In order to assess the results of anthelmintic trials Groeneveld & Reinecke[8] have developed a non-parametric statistical test. At the 90% confidence limit at least 5 controls are essential but 9 or more are advisable. In the treated group there should be at least 11 animals. Anthelmintics are classified as follows:—

Class A: More than 80% effective in more than 80% of the treated flock.

Class B: More than 60% effective in more than 60% of the treated flock.

Class C: More than 50% effective in more than 50% of the treated flock.

Class X: Ineffective.

Simulation studies have been carried out by C.J. Clark (Imperial Chemical Industries, Macclesfield, England). Based on the power curves obtained by simulation, he has convinced us that the following modifications to the non-parametric method of assessing results are fair and reasonable:

The worm burdens of the controls are regarded as being represented by the median rather than the lower limit of the median. Moreover, the power curves show that there is very little chance that a compound will cause 80% reduction (or less) in 80% (or less) of the treated animals if the calculation is based on the 75%:75% level. Thus:—

Class A is the control median 0.25 and one result of 11 treated animals may exceed this figure, and

Class B is the control median 0.4 and three results of 11 treated animals may exceed this figure, and

Class C is the control median 0.5 and four results of the 11 treated animals may exceed this figure.

The results in Table 2 show that against immature *P. microbothrium* in the 11 treated sheep only one result i.e. Sheep 29 with 129 worms exceeded the control median 103 (0.25) = 26. The adults were considerably more responsive to treatment. The control median 2,760 (0.25) = 690, and all 11 treated sheep had considerably less than this number. The compound is therefore classified as Class A. The number of cattle treated is inadequate to be able to make these calculations on a herd basis.

Groeneveld (Personal communication) has indicated that in critical trials with tapeworms all 11 treated sheep must be free for a Class A grading. One out of 11 for Class B and two out of 11 for Class C can still be infested after treatment. Even if 14 sheep are treated as is the case in these trials the fact that one animal (Sheep 3) was still positive means that the drug is effective in at least 60% of the flock (Class B).

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THE ANTIGENIC CLASSIFICATION AND DISTRIBUTION OF NATURALLY OCCURRING STRAINS OF BLUETONGUE VIRUS*

P. G. HOWELL**

SUMMARY

By *in vitro* serum virus neutralization tests 244 naturally occurring strains of bluetongue virus were classified into 16 immunological groups. A prototype strain was selected for each group and a complement-fixation test used to establish their common identity. Cross-neutralization tests between the 16 cloned strains of bluetongue virus and their homologous antisera established them as the prototypes of their respective immunological groups.

When related to outbreaks of disease amongst flocks of sheep or viraemia in apparently healthy cattle, there appeared to be a random distribution of the various antigenic types within the enzootic regions, although during the course of one widespread epizootic Type 2 strains appeared to be the most predominant.

Outside the enzootic regions of Southern Africa, in countries where the disease has in recent years made its first appearance, various antigenic types of virus have been indentified. The existence of homologous counterparts in Africa suggest that these strains originated from this part of the world.

The results are discussed in the light of the occurrence of disease in previously vaccinated animals and the prospects of effective control by prophylactic polyvalent immunization.

INTRODUCTION

The earlier literature on bluetongue suggests that reinfection of recovered sheep was not an unusual experience and it must be assumed that antigenic variants of this virus were in existence when the disease was first described[1,2]. The fact that recovered sheep could be reinfected experimentally was ascribed to either a short duration of immunity or the presence of some other infectious agent giving rise to a febrile reaction.

Later, Du Toit[3] was again faced with the problem of recurrent attacks of the disease in recovered and previously vaccinated sheep and the general acceptance of the explanation of a transient state of immunity gave rise to the practice of inoculating sheep twice yearly. It soon became apparent, however, that this procedure did not achieve the desired result and the disease continued to occur amongst sheep previously immunized with Theiler's monovalent vaccine.

With the expansion of the sheep farming industry, the problem became more acute and no alternatve explanation was forthcoming until Neitz[4] published the results of an extensive series of cross-protection tests in sheep. From this work it was conclusively demonstrated that a plurality of antigenically different strains of virus existed in nature. By the cross-protection test, however, it was not considered possible to group strains on the basis of common antigenic structure.

The first indication that tissue culture techniques might fulfil the requirements of a suitable host system was provided by the preliminary study of Haig, McKercher and Alexander[5], and subsequently confirmed by Fernandes[6]. In these papers it was shown that multiplication of bluetongues virus in cultures of primary lamb kidney monolayers was accompanied by a specific cytopathic effect, which could be inhibited by homologous serum antibody. A convenient system therefore became available for the rapid screening and classification by *in vitro*

* Based on a portion of a thesis submitted to the Faculty of Veterinary Science, University of Pretoria, in partial fulfilment of the requirements for the degree of D.V.Sc.

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serum-virus neutralization tests of samples of bluetongue virus, taken from affected animals in the field.

The later application of the plaque assay technique to the study of bluetongue virus[7], provided a more sensitive system for the study of the immunological differences between strains. By clone selection it was possible to prepare prototype strains of each immunological group and to study any possible cross-reactions between these strains, by plaque inhibition tests.

MATERIALS AND METHODS

Virus strains

(a) Field strains. During the past 20 years numerous specimens from outbreaks of bluetongue have been received by this laboratory for investigation. These specimens have been stored as infective blood in an oxalate-carbolic-glycerine (OCG) preservative at 10°C. Occasionally samples of spleen collected *post mortem* were stored in 50 per cent buffered glycerine.

(b) Prototype strains. Sixteen strains of virus each representing a particular immunological group were selected on the basis of preliminary cross-neutralization tests conducted on a group of 22 samples[8] and on the results of the screening of the above field specimens. All of these strains had undergone a similar process of isolation and adaptation to embryonated hens' eggs. For these experiments the lowest available egg passage level of each strain was used. The origin of these prototype strains is given in Table 1.

ISOLATION OF VIRUS FROM FIELD SAMPLES

The isolation and adaptation of bluetongue virus to multiplication in embryonated hens' eggs, has been described in detail elsewhere[9]. When specific mortality was observed in the inoculated eggs, the embryos were harvested and a suspension of the tissues freeze-dried and stored at −20°C.

Before undertaking neutralization tests, all the strains of virus successfully propagated in embryonated eggs were adapted to multiplication in tissue culture.

Primary lamb kidney monolayer cultures were inoculated with 0.2 ml of a 1 : 100 dilution of each reconstituted freeze-dried specimen. After 2 to 3 hours incubation at 37°C the cultures were washed twice in phosphate buffered saline (PBS) and fresh serum-free medium added to the cultures. These cultures were incubated at 37°C and examined daily for the development of cytopathic changes. When 75 to 100 per cent of the cells had been destroyed, the fluid was harvested and further passages conducted in primary lamb kidney cultures. The medium and cellular detritus from not less than two cultures of the second or third tissue culture passage were used as antigen for neutralization tests.

PREPARATION OF STOCK PROTOTYPE ANTIGEN

One ampoule of freeze-dried egg-adapted virus of each of the selected strains was reconstituted and serial tenfold dilutions prepared. Duplicate 60 mm petri plate cultures of L cells[10] were then seeded with each dilution and after adsorption, overlayed for

Table 1: ORIGIN AND IDENTIFICATION OF SELECTED PROTOTYPE STRAINS

Immunological Group	Sample	STRAIN IDENTIFICATION Year	Locality of Epizootic	Passage level used for plaque selection
1	Biggarsberg	1958	Vryheid — Natal	1
2	22/59	1959	Ermelo — Transvaal	3
3	Sample B	1943	Lanarca — Cyprus	1
4	Vaccine — Batch 603	1900	— — Cape Province	3
5	Mossop	1953	Machadodorp — Transvaal	1
6	Strathene	1958	Vryheid — Natal	1
7	Utrecht	1955	Utrecht — Natal	3
8	Camp	1937	Onderstepoort — Transvaal	1
9	University Farm	1942	Pretoria — Transvaal	1
10	Portugal	1956	— — Portugal	1
11	Nelspoort	1944	Nelspoort — Cape Province	1
12	Byenespoort*	1941	Pretoria — Transvaal	1
13	160/59	1959	Mt. Currie — Natal	1
14	87/59	1959	Ermelo — Transvaal	3
15	133/60*	1960	Onderstepoort — Transvaal	1
16	Pakistan	1960	Hazara — West Pakistan	1

*These strains are from cattle, the remainder were taken from sheep.

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plaque assay[7]. On the 6th day the plates were examined and one isolated individual plaque was aspirated from a plate infected with the highest infective dilution, showing no more than one to three dispersed plaques. This sample was seeded into a roller tube culture of primary lamb kidney cells .When advanced cytopathic changes were evident two further alternating plaque passages in L cells and primary lamb kidney monolayers was carried out with each strain. Reference antigens were prepared from cultures infected with the plaque purified viruses, which were freeze-dried and stored at −20°C.

ANTISERA

(a) Preinfection and convalescent phase sera. Blood was collected from all experimental animals before and 30 to 45 days after the inoculation of the field samples. The separated serum was stored at −20°C and inactivated at 56°C for 30 minutes immediately before use. Antisera for the preliminary *in vitro* screening of field strains were obtained from individual surviving donors each representing a provisional immunological group[8].
(b) Type specific antisera to the prototype strains were prepared by the hyperimmunization of young adult guinea pigs. These animals received two 5 ml doses of infective tissue culture fluid inoculated at an interval of 10 days. The guinea pigs were bled 30 days after the second injection.

NEUTRALIZATION TESTS

(a) The antigenic classification of virus strains from field samples.

From preliminary tests it was found that a final dilution of 1 : 20 of a convalescent sheep serum, would inhibit or delay the multiplication and resultant cytopathic changes produced by not more than 10,000 TC ID_{50} of homologous virus, during an incubation period of 6 to 8 days at 37°C. In each test therefore, a suitable dilution of antigen calculated to contain this concentration of virus was added in equal volume to 0.3 ml of undiluted serum representing each immunological type. Simultaneously each antigen was titrated by serial tenfold dilution in order to ascertain the actual concentration of virus used in the test. The serum-virus mixtures together with the antigen titration were held for 90 minutes at 10°C. Thereafter 0.2 ml of each mixture was inoculated into two roller tube cultures, incubated at 37°C and examined daily for the development of specific cytopathic changes.

(b) Standardization of antisera and plaque inhibition tests.

As a preliminary step in the cross-neutralization tests between the prototype strains, the potency of each serum pool was determined by preparing serial twofold dilutions of each type serum, commencing at 1 : 25 in Eagles' medium containing 1.1 grams of sodium bicarbonate per litre. An equal volume of 1 per cent agarose suspension was then added to each serum dilution, mixed well and placed in a waterbath at 44°C. A suitable dilution of virus, which on the basis of preliminary titration could be expected to produce between 40 and 60 plaques per plate, was then prepared and seeded onto nine petri dish cultures.

After adsorption for 1 hour at 25°C the antiserumagarose overlay was poured into duplicate petri plate cultures. Three control plates were included in which the serum was omitted from the overlay. These plates were used to determine the actual number of plaque forming units (PFU) of virus present in each test. On the 5th day of incubation 4 ml of overlay medium containing neutral red in a final concentration of 1 : 20,000 was added to each plate. Plaque counts were made on the 6th and 7th days. From these results the highest dilution of serum, which completely inhibited visible plaque formation by the homologous virus over a period of 6 days, was selected as the standard stock dilution for all tests.

In the final cross-neutralization test, petri dish cultures were again infected with a suitable dilution of each antigen. After adsorption, groups of three plates were overlayed with nutrient medium and agarose, containing the predetermined dilution of each of the type antisera. Plaque counts were made on the 7th day.

COMPLEMENT-FIXATION

The techniques of complement-fixation employed in this study has been described elsewhere[11]. A specific immune serum for this test was obtained from a sheep, 35 days after reacting to experimental infection with a Type 4 virus, which represented the oldest known laboratory strain isolated by Theiler in 1900. Before use this serum was inactivated at 54°C for 30 minutes.

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CELL CULTURES

(a) The tecnique for the production of primary lamb kidney monolayers in roller tubes was similar to that described by Youngner[12]. The cells were grown in a medium prepared according to the formula of Hanks and Wallace[13] to which lactalbumin hydrolysate was added to a final concentration of 0.5 per cent and yeastolate (Difco) to a final concentration of 0.1 per cent. For growth and maintenance, inactivated bovine serum was added to give a final concentration of 10 per cent. When virus was seeded into the cultures, the serum was omitted from the medium.

(b) A derivative of Earles' strain of mouse fibroblasts (NCTC clone 929 of strain L)[10] was cultured and petri dishes prepared as described elsewhere.

EXPERIMENTAL ANIMALS

Young adult sheep purchased from farmers in areas free of bluetongue were housed at the laboratory in insect free stables throughout the course of the investigation. The susceptibility of the stock was checked by complement-fixation tests undertaken before the commencement of an experiment.

RESULTS

Cross complement-fixation between the selected plaque purified prototype strains of virus

The fixation of complement by each of the 16 prototype strains with a common antibody, confirms the existence of a group reactive antigen in each of the strains. As indicated in Table 2, this *in vitro* reaction was specific by virtue of the fact that no complement was fixed by the preinfection serum sample, whereas a positive fixation was obtained with the antibody in the convalescent serum to Theiler's original strain of bluetongue virus. On the basis of this test, therefore, each of the selected plaque purified strains can be accepted as a member of the group of etiological agents of bluetongue.

Cross-neutralization between plaque purified prototype strains by plaque inhibition tests.

In the standardization procedure it was found that the antibody concentrations in the pools of hyperimmune guinea pig antisera were generally of a low order. It was observed that a final dilution of 1:50 to 1:100 was required in the agarose overlay to inhibit plaque formation by the estimated 50 PFU of each homologous serum-virus mixture.

Table 2: FIXATION OF COMPLEMENT BY PROTOTYPE STRAINS IN THE PRESENCE OF EXCESS ANTIBODY

Antigen No.	Estimated fixation of complement in Positive Serum: Antigen dilution of ud.	1 : 2	4	8	Negative Serum: Antigen dilution of ud.	1 : 2
1	4*	4	3	2	0	0
2	4	4	4	4	0	0
3	4	4	2	1	0	0
4	4	4	4	4	0	0
5	4	4	4	4	0	0
6	4	3	2	0	0	0
7	4	3	2	0	0	0
8	4	3	2	0	0	0
9	4	4	3	2	0	0
10	4	4	4	4	0	0
11	4	4	3	2	0	0
12	4	4	3	2	0	0
13	4	4	4	3	0	0
14	4	4	4	2	0	0
15	4	4	4	4	0	0
16	4	4	3	2	0	0
NTC	1	0	0	0	0	0
Pos. Mouse brain Control	4	4	4	4	0	0

*4 to 0 represents 0-100 per cent haemolysis.
NTC: Normal tissue culture.

The mean plaque count for each set of three plates in the final cross-neutralization tests with the 16 prototype strains is given in Table 3. In the reactions between homologous antigen and antiserum, there was either a marked reduction or a complete inhibition of plaque formation. Where plaques developed in spite of the prsence of homologous antibody, there was a noticeable reduction in both the size and number of plaques when compared with the controls.

On these plates seeded with heterologous mixtures of antigen and antibody, plaque formation was not affected. Where occasional low counts were obtained the mean plaque size was not reduced when compared with the controls. Since these differences in plaque counts were not reciprocal, they were considered to be due to non-specific causes and within the limits of experimental error.

Identification of field specimens

The immunological classification and

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Table 3: CROSS-NEUTRALIZATION BETWEEN PLAQUE PURIFIED STRAINS BY PLAQUE INHIBITION TESTS

Prototype Antigen No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Control Antigen Titration
1	0	31	43	36	34	34	35	40	43	38	36	34	38	39	47	37	40
2	70	0	65	54	41	69	69	49	66	69	68	90	72	59	68	59	37
3	15	18	0	17	17	13	19	12	13	15	13	14	14	15	13	20	22
4	14	17	11	0	20	21	15	24	23	17	20	26	19	25	31	24	23
5	31	21	27	25	0	30	31	21	26	23	23	25	14	19	44	17	50
6	50	64	42	47	62	0	75	51	70	49	56	61	54	53	47	72	86
7	24	26	26	27	32	33	0	34	43	31	28	18	27	28	33	45	34
8	5	11	16	21	13	16	15	0	16	15	15	18	15	14	18	13	19
9	11	13	11	11	15	11	8	11	3	16	16	14	21	15	25	20	20
10	13	13	13	17	13	14	10	12	10	0	9	10	11	10	11	9	8
11	19	23	14	18	17	16	17	13	16	12	0	11	18	13	15	16	9
12	61	65	63	57	62	66	76	69	49	53	66	16	49	77	64	79	49
13	19	22	25	25	37	30	29	19	32	22	23	25	2	21	30	27	36
14	10	6	10	12	11	12	15	19	11	18	9	19	16	0	30	19	21
15	6	11	9	17	19	16	18	10	15	11	14	9	17	18	0	13	12
16	34	38	51	27	40	49	25	31	46	45	40	48	34	40	42	0	34

distribution of the strains of virus recovered in South Africa is given in Table 4. From these results it would appear that apart from the predominance of a Type 2 virus in the Transvaal there is no definite pattern of distribution within the confines of localized epizootics on particular farms or over the whole country. The random distribution of the strains is particularly well illustrated by the identification of at least nine different immunological types of virus on the two adjoining farms Kolwani and Athole during the epizootic in 1959 which extended over a period of 34 days.

Table 4: SUMMARY OF THE ANTIGENIC CLASSIFICATION AND DISTRIBUTION OF STRAINS OF BLUE-TONGUE VIRUS ISOLATED IN SOUTH AFRICA FROM 1959—1965

Province—District	Farm	Year of isolation	No. of samples neutralized by provisional type antisera 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Transvaal																		
—Belfast	Bospoort	1959	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
		1960	1	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—
	Torburnlea	1959	—	—	—	—	—	—	—	2	—	—	—	—	—	—	—	—
	Doornkop	1959	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—Bethal	Kaallaagte	1959	—	1	—	—	—	—	—	—	—	1	—	—	—	—	—	—
—Carolina	Barneveld	1959	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—
	Frisgewaagd	1959	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Leeupan	1959	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Nederland	1959	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Strathea	1959	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—
—Delmas	—	1959	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—
—Ermelo	Athole	1959	1	2	—	—	—	1	—	—	—	1	—	—	—	1	—	—
	Boesmanspruit	1959	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Driehoek	1959	1	1	—	—	—	—	—	—	—	—	—	—	—	1	—	—
	Goedehoop	1959	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Highflats	1959	—	2	—	—	—	—	—	—	1	—	—	—	—	—	—	—
		1962	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—
	Kafferspruit	1959	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Kolwani	1959	1	14	1	—	5	—	—	3	5	2	—	—	—	3	—	—
		1961	—	3	—	—	—	1	—	2	—	—	—	—	—	—	2	—
		1962	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—
		1965	—	1	1	—	—	—	—	—	—	1	—	—	—	—	—	—

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Table 4: Continued

Province—District	Farm	Year of isolation	No. of samples neutralized by provisional type antisera 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	Langverwacht	1959	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Meadowbank	1959	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Moedig	1963	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—
	Remhoogte	1960	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—
	Saaiplaas	1965	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Tafelkop	1964	2	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—
	Welgevonden	1963	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—
	Winkelhaak	1959	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—
—Lydenburg	Oshoek	1959	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—Standerton	Paardfontein	1959	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—
—Wakkerstroom	—	1960	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Natal																		
—Amersfoort	Twyfelshoek	1962	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—
—Babanango	Hartskamp	1959	—	2	—	2	2	—	—	—	—	—	—	—	—	—	—	—
		1961	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Swartskop	1959	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—Dundee	Adelaide	1965	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Outfall	1965	1	—	1	—	—	—	—	—	—	—	1	1	—	—	—	—
—Estcourt	Clifton	1961	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—Kliprivier	Balbrogie	1959	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—
	Evenlode	1959	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—
—Lions River	New Forest	1959	—	—	—	—	—	—	—	—	2	—	—	—	—	—	—	—
	Woodlands	1965	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—
—Mt. Currie	Westlands	1959	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—

Table 4: Continued

Province—District	Farm	Year of isolation	No. of samples neutralized by provisional type antisera 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
—Newcastle	Gardenia	1965	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—Piet Retief	Lodewykslust	1969	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—
	Saaiplaas	1969	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—Umvoti	Buttops	1959	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—
—Utrecht	Klipspruit	1959	1	—	—	—	—	—	—	—	2	—	—	—	—	—	—	—
		1960	3	2	—	1	1	—	—	—	—	—	—	—	—	—	—	—
		1965	1	—	1	—	—	—	1	—	—	—	2	—	—	—	—	—
	Rooipoort	1962	—	1	1	—	—	1	—	—	—	—	—	—	—	—	—	—
	Sheepridge	1965	—	6	—	—	—	—	—	8	—	—	—	—	—	—	—	—
Cape Prov. (East)																		
—Albany	Cold Springs	1959	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Inverleith	1964	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Paardekraal	1965	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—
	Salem Park	1959	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Vaalkranz	1959	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—Aliwal North	Vineyard	1959	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—
—Bathurst	Ferndale	1965	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Govt. Farm	1965	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Steynsrust	1959	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—Bredasdorp	Zoetendals	1962	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—
—East London	Groothoek	1964	1	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—
—Graaff Reinet	Rheboksberg	1959	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—Humansdorp	Heathercliff	1965	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—King W. Town	Fort Cox	1959	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—Komgha	Glen Thorn	1959	—	1	—	—	—	1	—	—	—	—	—	—	—	—	—	—
	Simla Park	1959	3	—	—	—	—	3	—	—	—	—	—	—	—	—	—	—
		1960	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—

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Table 4: Continued

Province—District	Farm	Year of isolation	No. of samples neutralized by provisional type antisera 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	Ocean View	1961	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—
—Malmesbury	Klein Driefontein	1960	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
		1961	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—Port Elizabeth	Cypherfontein	1960	1	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—
—Somerset East	Bergvliet	1959	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Longhope	1961	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—
		1962	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—
		1964	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—
Orange Free State																		
—Bethlehem	Lucia	1959	—	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—Clocolan	Sunnyside	1965	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—Frankfort	Sandoog		—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—Heilbron	Christoffelina	1964	—	—	1	—	—	—	—	—	—	—	1	—	—	—	—	—
	Voorspoed	1963	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—
—Kroonstad	Doornspruit	1959	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Mariedale	1963	2	1	—	1	—	—	—	—	—	—	—	1	—	—	—	—
	Middenspruit	1959	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Verlangdedal	1965	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Welkom	1959	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—
—Ladybrand	Dieplaagte	1965	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—
—Petrus Steyn	Grootdam	1961	1	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—
	Katbos	1961	1	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—
		1963	—	—	—	—	—	—	1	—	—	—	1	—	—	—	—	—
	Ongegund	1961	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—
	Strydomspan	1965	—	—	2	1	—	—	—	—	—	1	—	—	—	—	—	—

The collection of samples from the field however did not provide a true reflection of the distribution or actual incidence of bluetongue during the period covered by this survey, since the collection of specimens was often determined by the enthusiasm of a few field officers or certain interested farmers.

From the data received with the specimens it was found that the majority of the samples had been obtained from animals over 1 year of age of which the majority had been previously vaccinated on more than one occasion (Table 5).

Table 5: RELATIONSHIP OF AGE AND THE IMMUNE STATUS OF SHEEP TO NATURAL INFECTION IN THE FIELD DURING THE 1959 EPIZOOTIC

Immunological Type	No. of Samples	No. of Affected Animals >1 Year	<1 Year	Unknown	Nature of Clinical Symptoms Severe	Mild	Fatal	Not recorded	Immune Status Vaccinated	Not Vaccinated	Not recorded
1	22	16	6	0	18	3	1	0	18	4	0
2	44	41	3	0	37	6	1	0	41	3	0
3	3	3	0	0	3	0	0	0	1	2	0
4	3	1	2	0	3	0	0	0	2	1	0
5	6	3	0	3	4	2	0	0	0	5	1
6	4	4	0	0	4	0	0	0	4	0	0
7	0	—	—	—	—	—	—	—	—	—	—
8	7	5	2	0	6	1	0	0	0	7	0
9	12	8	3	1	12	0	0	0	0	10	2
10	3	1	0	2	2	0	0	1	3	0	0
11	0	—	—	—	—	—	—	—	—	—	—
12	3	1	1	1	1	1	0	1	3	0	0
13	1	1	0	0	0	0	0	1	1	0	0
14	7	6	1	0	6	1	0	0	6	1	0
15	0	—	—	—	—	—	—	—	—	—	—
16	0	—	—	—	—	—	—	—	—	—	—

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The classification of the exotic samples of bluetongue virus is given in Table 6. Here it will be observed that with one exception all of the virus samples have their homologous counterparts in the enzootic regions of Southern Africa. The Type 16 strains would appear to have only a limited distribution at the present time, having been recovered more recently from clinically affected sheep in Israel and India[14] but not as yet in Africa.

DICUSSION

In the years preceding the 1959 epizootic a polyvalent egg attenuated vaccine containing four strains of virus, subsequently identified as representing Types 3, 4, 11 and 12 had been used extensively in the field. During the course of this survey strains representing these types were identified, the majority originating from previously immunized sheep. These incidents therefore represent genuine examples of immunological failure and an explanation for their occurrence is not possible in the absence of a serum sample taken at the time of illness of the donor, to determine the actual state of immunity. Many similar cases of immunological failure were reported amongst immunized horses suffering from horsesickness by McIntosh[21], who expressed the opinion that they were the result of minor antigenic differences within the type. Later it was shown that many immunological failures to this disease could be ascribed to the poor antigenicity of certain attenuated strains incorporated in the vaccine[22].

On the other hand in view of the fact that at least an additional twelve immunological types of bluetongue virus have been identified, over and above the four previously included in the vaccine, it is unreasonable to claim that the majority of animals from which samples were taken in this experiment, represent true vaccinal failures. While some degree of heterologous immunity may develop after infection by virulent virus[4], it would appear that this is not a property of the attenuated strains and the broad multiplicity of the antigenic types active in the enzootic areas is a more logical explanation for the high incidence of disease amongst the predominantly immunized sheep population. From these experimental results it can be concluded that a highly complex situation exists in nature, for which a potent and effective polyvalent vaccine would at this stage appear to offer the only practical method of control of the disease in the enzootic regions.

Table 6: ANTIGENIC CLASSIFICATION OF EXOTIC STRAINS OF BLUETONGUE VIRUS

Identification of Sample	Country of Origin	Year of Isolation	Species Affected	Immunological Type	Author
Sample D	Cyprus	1944	Sheep	3	Gambles (1949)[15]
Israel D—51	Israel	1951	Cattle	4	Komarov & Goldsmit (1951)[16]
Israel—64	Israel	1964	Sheep	4	Dafni (1966)[17]
California—8	United States of America	1952	Sheep	10	McKercher, McGowan & Saito (1954)[18]
Sheep 1—56	Portugal	1956	Sheep	10	Ribeiro & Noronha (1958)[19]
Cow 1—56	Portugal	1956	Cattle	10	„
241/59	West Pakistan	1959	Sheep	16	Sapre (1964)[20]
306—66/1	United Arab Republic (Egypt)	1966	Sheep	12	Bluetongue World Reference Centre
20—66/2	United Arab Republic (Egypt)	1966	Sheep	1	„
4/70	India	1969	Sheep	1	„
11/70	India	1969	Sheep	16	„

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Aldosterone	0.2	800
Prednisone	3.5	0.6
Prednisolone	4	0.6
Methylprednisolone	5	0
Triamcinolone	5	0
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Jl S. Afr. vet. med. Ass. 41 (3): 225-226 1970

RESEARCH NOTE

RECOVERY OF MERINO SHEEP FROM AN ANAEMIA INDUCED BY PARTIAL EXSANGUINATION

H. M. TERBLANCHE, L. P. NEETHLING, R. J. J. BRIEL AND J. M. M. BROWN

During the course of our studies on haemolytic and other anaemias in sheep[1] we found it necessary to study the response of sheep to therapeutic doses of iron. For this purpose we used two groups of sheep each containing nine clinically normal animals. These were bled once from the jugular vein to produce a net loss of whole blood of 44 and 47 per cent of the original blood volume respectively. Each animal of the group having the slightly higher blood loss (i.e. 47 per cent) received a single injection of Imposil 200 (Fisons Pest Control S.A. Pty. Ltd.) consisting of a 5 ml solution containing 10 per cent elemental iron as an iron dextran complex, 3 hours after bleeding. This group will be referred to as the treated group. The other group remained untreated and will be referred to as such. Both groups were maintained on a ration consisting of lucerne hay, teff hay and water *ad libitum* for the duration of this experiment. Each group consisted of six animals possessing the BB and three animals possessing the AB haemoglobin phenotypes.

Haemoglobin determinations were performed on whole blood by using the IL model M231 Haemoglobinmeter (courtesy of Protea Physical and Nuclear Instrumentation Pty. Ltd.) at various time intervals; once prior to the bleeding to provide base line figures and at various times thereafter. Thirty seven days after bleeding the haemoglobin concentrations of both groups seemed to approach the prebleeding baseline values once more. A second bleeding was performed on this day resulting in an average loss of 46 per cent in the treated and 43 per cent in the untreated group. No further iron injections were given to animals belonging to the treated group. Haemoglobin determinations were performed as before during a further period of 35 days.

During the course of the experiment one sheep was lost 2 days after the second bleeding from the treated group. Autopsy revealed the death to be due to anaemia, the carcass showing severe dehydration and cyanosis and degenerative changes in many organs.

The results which were obtained are tabulated in table 1 and presented graphically in figure 1.

Table 1: HAEMOGLOBIN VALUES OF UNTREATED AND TREATED GROUPS

Date	Mean Haemoglobin Concentration (g/100ml)	
	Untreated	Treated
9.3.70	11.9 ± 1.7	11.4 ± 1.6
9.3.70	8.3 ± 1.6	7.7 ± 1.5
10.3.70	7.3 ± 1.7	7.1 ± 1.4
11.3.70	7.3 ± 1.7	6.8 ± 1.2
12.3.70	7.3 ± 1.5	7.1 ± 1.1
13.3.70	7.4 ± 1.5	7.2 ± 1.1
16.3.70	8.5 ± 1.4	8.1 ± 1.2
17.3.70	8.6 ± 1.5	8.1 ± 1.2
23.3.70	10.0 ± 1.4	9.5 ± 1.2
26.3.70	10.4 ± 1.4	10.2 ± 1.5
31.3.70	10.6 ± 1.4	10.4 ± 1.2
7.4.70	10.8 ± 1.5	10.6 ± 1.3
14.4.70	11.1 ± 1.5	10.8 ± 1.3
14.4.70	7.0 ± 1.4	6.9 ± 1.3
15.4.70	6.5 ± 1.4	6.4 ± 1.1
16.4.70	6.7 ± 1.3	6.7 ± 1.3
17.4.70	6.8 ± 1.4	6.6 ± 1.1
20.4.70	8.0 ± 1.5	7.7 ± 1.2
21.4.70	7.5 ± 1.4	6.9 ± 1.1
22.4.70	7.6 ± 1.3	7.6 ± 1.2
27.4.70	7.9 ± 1.3	8.1 ± 1.4
30.4.70	8.4 ± 1.2	8.2 ± 1.3
5.5.70	9.3 ± 1.2	9.0 ± 1.2
12.5.70	10.1 ± 1.1	9.8 ± 1.1
18.5.70	11.1 ± 1.2	10.9 ± 1.5

(±) = standard deviation of the mean.

Although the coefficient of variation lies between 10 and 23 per cent for the various values given, it is quite clear that there are no significant differences between the treated and untreated groups. Both groups seem to need approximately 5 weeks to

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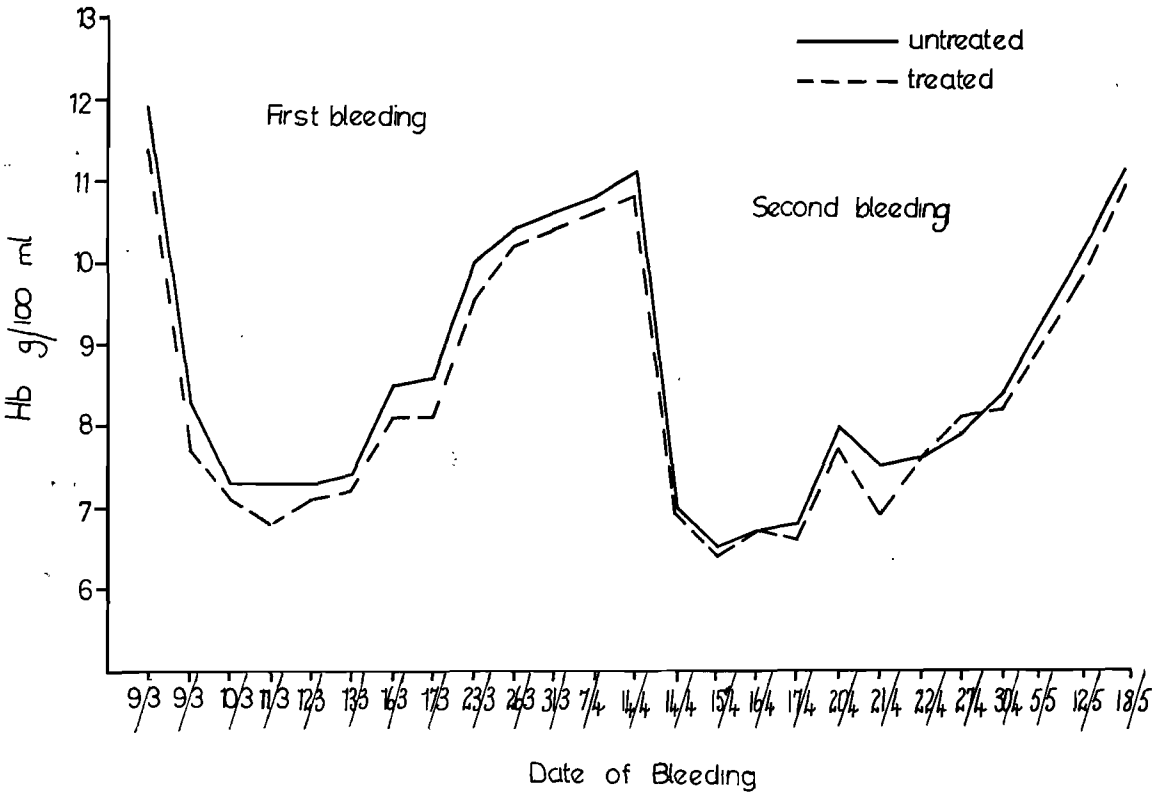
FIGURE I



recover from the induced anaemia. There is however a significant difference in the shape of the recovery curves for the first and second exsanquinations. The duration of the logarithmic phase of recovery is approximately three times longer after the second bleeding as compared to the first exsanquination, proceeding however at a much slower rate.

The fact that the treated group did not differ in their mode of recovery from the untreated group, is surprising especially after the second removal of blood.

It seems that either, the available iron pool in the sheep is large enough to cope with emergency situations in which a massive blood loss amounting to approximately 90 per cent of the blood volume spread over a period of eighty days or absorbtion of iron from the gut is significantly increased. Erythropoeiesis proceeds efficiently under these circumstances without apparent need for supplementation of the animals iron reserves, except from dietary sources.

The technical assistance of Mr. P. J. de Wet is gratefully acknowledged.

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RESEARCH NOTE

THE APPLICATION OF AN *IN VITRO* TEST FOR THYROID FUNCTION IN VETERINARY MEDICINE

H. M. TERBLANCHE, L. P. NEETHLING, R. J. J. BRIEL AND J. M. M. BROWN

Although dysfunction of the thyroid is well documented in animals, no practical clinical tests using radio-isotopes have been described by which the function of the thyroid can be routinely assessed. The availability of I^{125} labelled triiodothyronine in the form of a reagent kit, suggested to us its possible use in routine clinical work. The "Thyopac-3 kit" (The Radiochemical Centre Amersham England.) is used on a routine basis in many hospitals to carry out thyroid function tests in humans. The applicability of this kit to veterinary clinical work has to our knowledge not been studied.

The animals for this study, were readily available and apparently clinically normal in all respects. A search through the registers of the Onderstepoort Medicine clinic, has revealed a high incidence of thyroid dysfunction in dogs. Hereditary goitres are of importance in cattle in this country whilst endemic goitre is not unknown amongst the sheep and cattle flocks in many of the high rainfall areas. Abortion amongst Angora goats in the Cape Midlands, is recognised as a complex hereditary disturbance of general endocrine function. Studies on fitness in race horses are becoming increasingly important in equine physiology. It is for these reasons that we have largely concentrated on cattle, sheep, horses, dogs, and Angora goats.

The actual method of analysis was identical to that described by the manuafcturers of the isotope kit with the exception that plasma was used instead of serum and this was obtained by using heparin as anticoagulant. Counting of samples was performed with a Philips Automatic Well-type Scintillation Detector (Type PW 4003) equipped with a $1\frac{3}{4} \times 2''$ NaI/T1 crystal.

The experimental results which were obtained appear in Table 1 and a graphical presentation for those animals of most importance in practice, is given in figure 1.

It is evident that the method is applicable to the various animals used in this study as an indication of their thyroid activity. The capacity of the plasma to bind the labelled T_3 is a reflection of the prior saturation of the plasma proteins with endogenous T_4 (thyroxine) and is thus an indirect measure of the activity of the thyroid gland.

The variations in T_3 values obtained in this study between the various animals is due to specific differences in endogenous T_4, the levels of which vary from animal to animal and also from species to species.

Table 1: PLASMA T_3 VALUES OF VARIOUS CLINICALLY NORMAL ANIMALS

Animal	Mean T_3 value	Number of animals	S.D.
Cattle	104	52	10
Sheep	108	32	14
Angora goats	114	20	6
Horses	77	20	6
Dogs	77	20	4
Guinea Pigs	58	5	4
Rats	84	5	7
Goats	95	4	1
Rabbits	78	4	11

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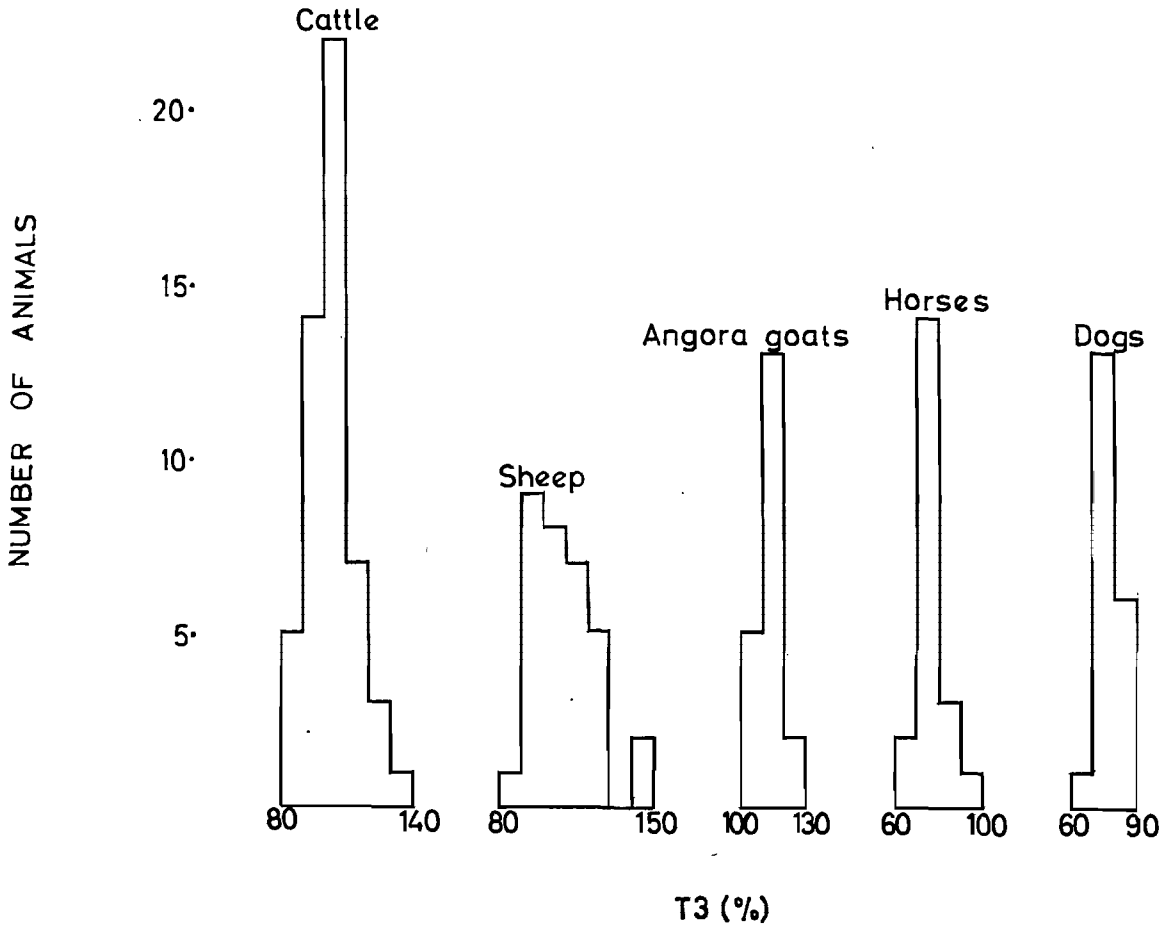
Figure I



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RESEARCH NOTE

A SIMPLE METHOD OF FEEDING TICKS ON MICE

G. EICHENBERGER*

The feeding of immature stages of ticks under laboratory conditions often presents a problem. Consideration of the feeding habits of many tick species in nature suggests that the readily available white mouse would be the host of choice. The method, described below, is both simple and efficient.

Switzerland the writer found the light aluminium hair-curler (so often used by women and available at almost every hairdresser or pharmacy) to be ideal as it is pliable enough to be adjusted to the body. The aluminium sheet is 1 mm thick and the holes 2.5 mm in diameter; the curler can easily be cut to the desired length and girth.

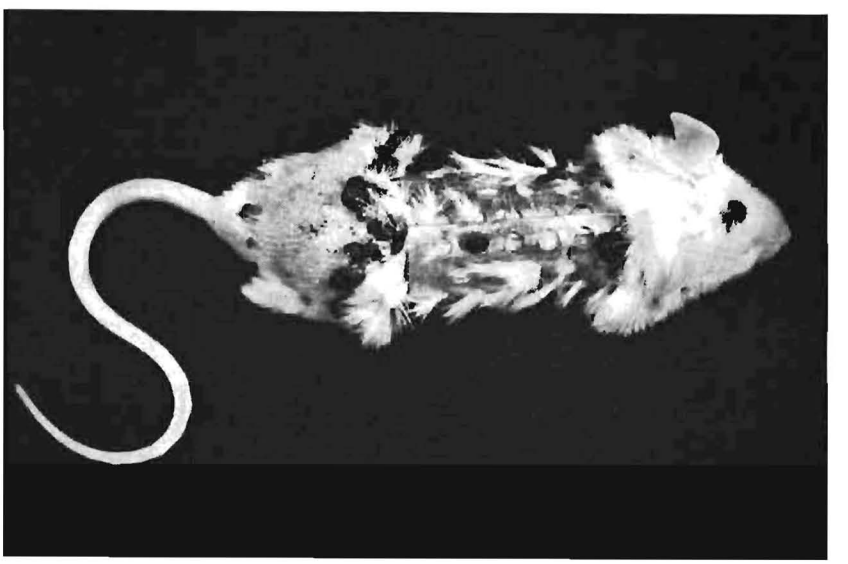

Fig. 1. — **H. marginatum rufipes** nymphae feeding on a corsetted mouse (15th day).

The body of a lightly narcotized mouse is encased in a cylinder made from a multi-perforated light metal sheet (Fig. 1) with the anterior end reaching to the shoulder and the posterior extremity to the pelvic girdle; the edges must be blunt so as not to cut or chafe the mouse. The cylinder should be adjusted to prevent the mouse from slipping out; by experience the skill will be acquired to fit the "corset" so as not to inconvenience the mouse too much. In

In fitting the corset to give stability, the long edges are made to overlap and to prevent it from revolving round the mouse, tufts of hair are drawn through some of the holes. This closely, but lightly applied corset, prevents the mouse scratching the body with the hind legs and from turning its head to nibble, but allows it sufficient freedom to run around and feed. Thus corsetted the mouse will live satisfactorily for a long time.

The corsetted mouse is placed into a

* Swiss Tropical Institute. 57 Socin Street, Basel, Switzerland.

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glass jar and the ticks applied. These may attach either to the exposed areas, beneath the metal sheet or at sites below the perforations. To assist the attachment of larvae, the exposed areas should be shaved. Petroleum jelly, smeared around on the internal surface of the jar just out of the mouse's reach will prevent ticks from escaping.

After 24 hours the infested mouse is transferred to a jar with a false floor of wire mesh (Fig. 2); the mesh should be large enough to allow engorged ticks to fall through and thus be out of reach so that the mouse cannot eat them. The jar is placed in a shallow receptacle containing water and its edge is smeared with petroleum jelly above the water level. This is essential to prevent engorged larvae and nymphae, which climb up the inside of the jar, from escaping to the exterior by trapping them in the water. The percentage recovery from the water outside the jar or from the debris within the jar varies considerably. The corsetted mouse is fed and watered daily. The jar is cleaned every 2 to 3 days or, when ticks are dropping, as frequently as it is deemed necessary.

To date the following immature stages of ixodid ticks have been reared on mice: as many as 100 larvae and 30 nymphae of *Ixodes ricinus*, up to 500 larvae of *I. pilosus* and in one attempt 30 nymphae of the two host tick, *Hyalomma marginatum rufipes*, have fed to repletion on individual mice.

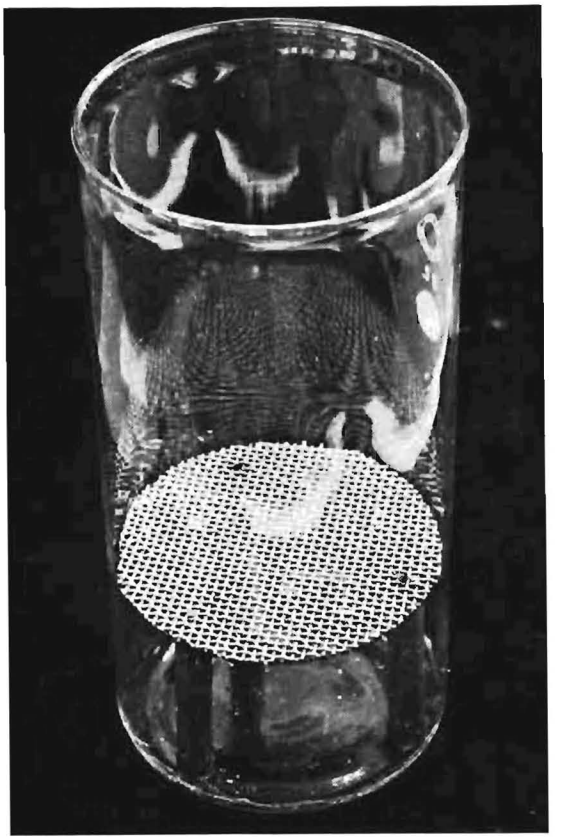

Fig. 2. — Glass jar with a false floor of wire mesh.

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Jl S. Afr. vet. med. Ass. 41 (3): 231-232 1970

CASE REPORT

POISONING OF CATTLE BY GANSKWEEK (*LASIOSPERMUM BIPINNATUM* (THUNB.) DRUCE)

A. E. FAIR*, R. C. TUSTIN** AND T. F. ADELAAR***

INTRODUCTION

During the winter of 1969 a visit was made by one of us (A.E.F.) to a farm in the Slabberts area (about 25 miles south-west of Bethlehem) after receiving a request from the owner to investigate an outbreak of acute poisoning involving 15 Friesland calves in a group of about 45, aged between 6 and 9 months. One animal had died and one was *in extremis* at the time of the examination.

The affected group of calves was allowed to graze for one hour a day in a wheat land which was in a rather poor condition. For the rest of the day they grazed the natural veld which was in good condition and consisted predominantly of dry *Themeda* sp. Another group of younger calves grazed on the same wheat land all day except for the hour mentioned previously; of these none were affected.

Before the older calves were allowed into the wheat land they congregated at its gate, in the vicinity of which a plant grew in abundance. As there was evidence that this plant had been grazed a specimen was taken for identification and it subsequently proved to be *Lasiospermum bipinnatum* (Thunb.) Druce. It was still green; the majority of the other vegetation apart from the wheat being brown and dry.

During the investigation several other plants including some *Senecio* spp. were observed and collected for identification.

CLINICAL SIGNS

The most outstanding clinical signs seen in the affected animals were those of severe colic, i.e. kicking at the abdomen, stamping of the forefeet, turning of the head towards the flank, stretching, walking backwards and holding the tail in an upright position. Recumbent animals either were motionless or lay kicking; some held their heads against the flanks. All of them had a rapid pulse. The muzzles were dry and cracked with the presence of a slightly caked bloody discharge in some of the calves. The faeces were hard and dry. The mucous membranes were normal except in the calf which was *in extremis* where the conjunctivae were oedematous, icteric and speckled with a few petechiae. This animal had a rectal temperature of 103°F and it was slaughtered and autopsied.

The following day all the calves in the particular group were affected. Several days after the initial signs the unpigmented areas of the skin developed marked lesions of photosensitisation.

PATHOLOGY

Macroscopic examination

The following lesions were seen in the slaughtered calf:— Mild icterus; ecchymoses in the lungs; severe epi- and endocardial petechiation; numerous petechiae in visceral and parietal peritoneum; oedema of the abomasal mucosa; dry, slightly bloody, mucous-covered faeces in the rectum; wall of the gall bladder markedly oedematous and a parboiled, mottled, friable liver.

Specimens of the liver and kidneys were taken for histopathological examination.

Microscopic examination

The liver showed the presence of a marked lysis necrosis in the periphery of the lobules which was in parts associated with

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haemorrhages. In addition, the rest of the hepatic parenchyma revealed cloudy swelling with early necrotic changes in rare odd cells. Cloudy swelling was present in the kidney.

A diagnosis of *L. bipinnatum* poisoning was made on the peripheral distribution and nature of the marked liver necrosis which are characteristic of subacute cases of this condition[1].

TREATMENT AND OUTCOME

Raw linseed oil was administerd to all the affected animals in order to alleviate the constipation and, with the exception of the two which died and the development of the solar dermatitis, recovery was uneventful.

DISCUSSION

The diagnosis of ganskweek poisoning in the slaughtered calf was based on the histopathology of the liver lesions, the presence of the plant in sufficient quantity to have caused poisoning in such a large number of animals and the fact that some of it had been eaten. It is of interest to note that this diagnosis was made on microscopical examination of the organ sections before the results of the plant identification had been made known. While there is no doubt that the slaughtered calf was suffering from ganskweek poisoning it is regrettable that no chemical pathological tests were done on the blood of some of the other sick animals in order to establish without any doubt that liver injury was present. This would have helped to confirm the diagnosis in these animals. The presence of abdominal pain in the absence of any evidence of enteritis and the development of photosensitisation do, however, indicate the possibility of liver injury. Adelaar, Terblanche, Smit and Naudé[1] who first established the toxicity of *L. bipinnatum* stated that, according to a farmer in the Eastern Cape, ingestion of the plant was a cause of "dikkop" (swollen head) in sheep. This is presumably due to photosensitisation. Apparently this is, therefore, also the case in cattle. The development of photosensitisation in this condition has not yet been experimentally confirmed as the cases in sheep produced by Adelaar *et al.*[1] were not exposed to sunlight. They also state that the plant is somewhat more resistant to frost than grass, with the result that in the late winter and early spring, just before the rains, there is relatively abundant ganskweek and sparse unpalatable natural pasture, which makes poisoning very likely. It is apparent that the group of older calves ate the plant which was still green and growing in the vicinity of the gate while waiting to be let into the wheat land. The group of younger calves did not enter the wheat field through this particular gate and were thus not affected.

As a differential diagnosis other forms of poisoning especially those associated with hepatic injury were considered, the most likely of these being *Senecio* spp. of which some were growing on the farm. However, poisoning by plants of this genus was unlikely because their numbers were inadequate to have caused poisoning simultaneously in 45 calves, the mortality rate is usually higher, the lesions are more progressive, photosensitisation is uncommon and the microscopic lesions are different from those encountered in this case.

ACKNOWLEDGEMENT

Drs. P. A. Basson and L. W. van den Heever are thanked for their helpful criticism of the manuscript.

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CASE REPORT

AORTIC RUPTURE IN A WARTHOG [*PHACOCHOERUS AETHIOPICUS AETHIOPICUS* (PALLAS)]

V. De Vos and C. A. W. J. van Niekerk*

SUMMARY

Sudden death in a free-living warthog *(Phacochoerus aethiopicus)* due to rupture of the aorta is described. An abscess caused by *Staphylococcus epidermidis* accounted for ulceration and weakening of the vessel.

CASE HISTORY

A warthog sow and her young one had become a familiar sight in the near vicinity of the Skukuza Rest Camp within the Kruger National Park. After a couple of weeks she was found ailing, and showed a tendency to lie down and a distinct reluctance to rise in seek of foodstuff. She died within two days of the first observation and was found in a recumbent position without any sign pointing to an act of violence or distress before death. The young one was observed to stay in the near vicinity of the dead sow. An immediate post mortem examination was carried out.

RESULTS

Post mortem examination

The post mortem examination revealed a large amount of clotted blood that had accumulated in the abdominal cavity. Closer inspection, however, showed the remains of a ruptured abscess extending from the posterior pole of the right kidney for 12 cm caudally, adjacent and adherent to the abdominal aorta and psoas muscular group. A tear of 3 cm on the ventral aspect of the abscess lining, or pyogenic membrane, was visible. On opening the membrane an ulcerative perforation of the aorta was evident. The opening in the vessel was irregular in shape and 7 mm in diameter.

Bacteriological report

A smear of the inner lining of the ruptured abscess revealed numerous Gram-positive cocci. The organism was subsequently isolated and identified as *Staphylococcus epidermidis*. The organism conformed to biochemical characteristics of this species, as laid down by Breed, Murray and Smith[1]. This diagnosis was later confirmed by the Veterinary Research Institute at Onderstepoort.

DISCUSSION

It is an accepted fact that abscesses and other localized infections in the tissues may involve the outer coats of an artery, producing an acute periarteritis, resulting in a weakening of the vessel to the point of rupture[2]. In this case the abscess caused an uloeration and subsequent weakening of the wall of the adjacent abdominal aorta. Rupture of the aorta caused sudden massive haemorrhage into the abdominal cavity through a tear in the abscess lining. It is impossible to assess whether rupture of the aorta took place before rupture of the abscess, or vice versa. Death, which obviously was caused by loss of blood, was sudden and apparently caused little or no anxiety towards the end.

Judging from the reduced mobility of the sow, pain was apparently associated with the abscess in the psoas muscle group.

Staphylococcus epidermis originally was isolated from small stitch-abscesses and other skin wounds[1]. It is common inhabitant of healthy skin and mucous membranes. In this case an internal abscess and ultimate death of a warthog are ascribed to the actions of this organism.

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* Veterinary Investigation Centre, Skukuza, Kruger National Park.

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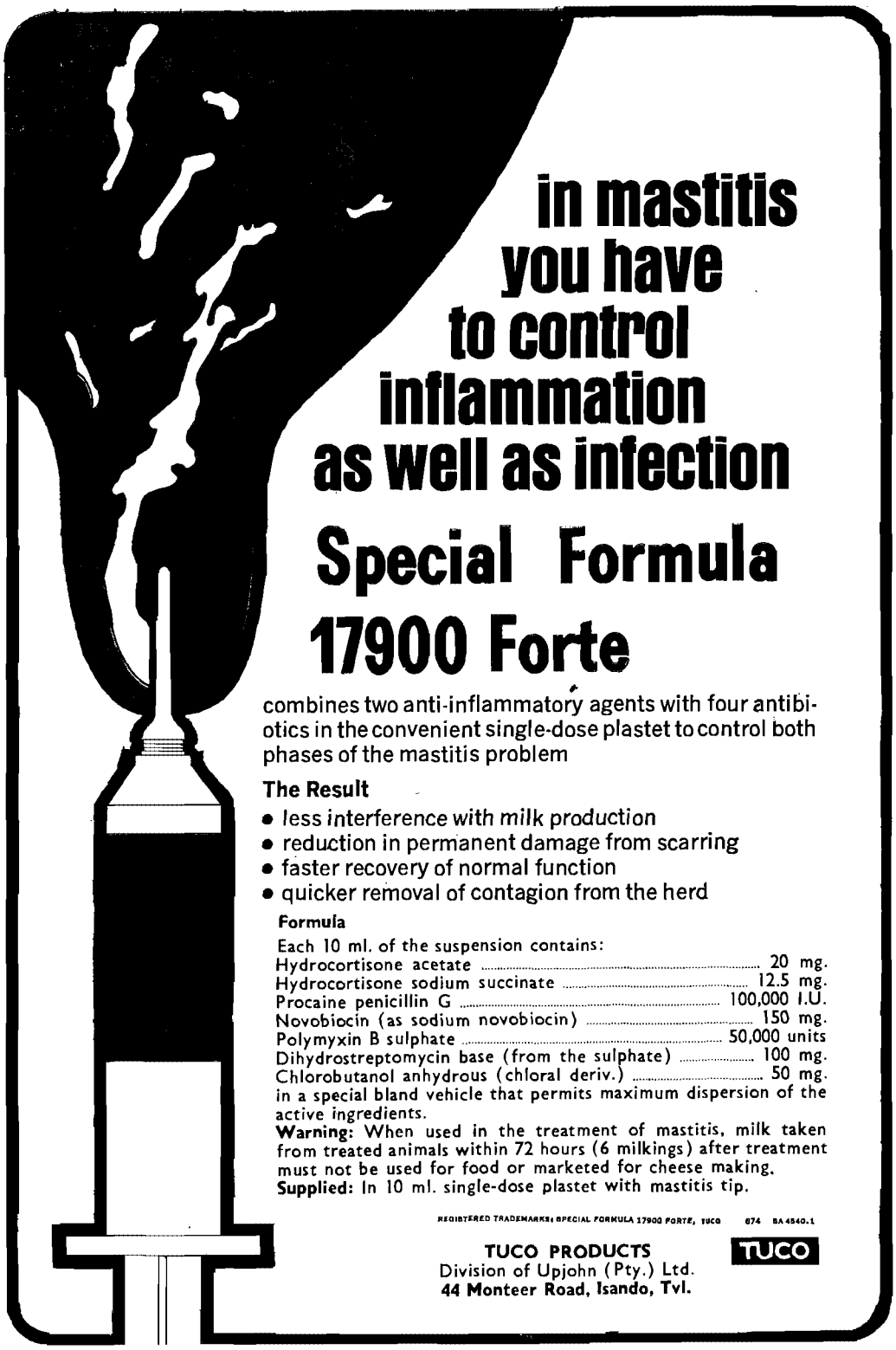

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Jl S. Afr. vet. med. Ass. 41 (3): 235-247 1970

EXPERIMENTAL EVIDENCE THAT LUPINOSIS OF SHEEP IS A MYCOTOXICOSIS CAUSED BY THE FUNGUS, *PHOMOPSIS LEPTOSTROMIFORMIS* (KÜHN) BUBÁK**

K. T. VAN WARMELO*, W. F. O. MARASAS*, T. F. ADELAAR**, T. S. KELLERMAN**, I. B. J. VAN RENSBURG** AND J. A. MINNE**.

SUMMARY

During October, 1969 a severe outbreak of lupinosis occurred in the Cape Province amongst a flock of sheep which had grazed on a field of sweet white lupines (*Lupinus albus* L. cult. Pflugs Gela). Typical lupinosis was induced in Merino sheep fed either stems, seeds or pods of lupine plants from this field. The toxic lupine plants were found to be heavily infected with the pathogenic fungus *Phomopsis leptostromiformis* (Kühn) Bubák. This fungus was readily isolated from infected, discoloured pods and seeds. Typical lupinosis was induced in sheep fed pure cultures of *P. leptostromiformis* grown on autoclaved white lupine seeds. It is concluded that lupinosis of sheep is caused by a hepatotoxin produced by the fungus *P. leptostromiformis*.

INTRODUCTION

Lupines (*Lupinus* spp.) are known to cause two distinct forms of poisoning in animals, viz. lupine or alkaloidal poisoning and lupinosis. The first of these is a nervous disorder caused by the alkaloids present in bitter lupines. The second is an icteric disease caused by a hepatotoxin (icterogen) of unknown structure and origin[1].

Lupinosis is a problem of long-standing importance but sporadic occurrence in Europe[1,2], Australia[1,3,4,5,6,7], New Zealand[8] and South Africa[9,10]. Detailed historical reviews of lupinosis have been given by Gardiner[1]; Hackbarth[2]; and Marsh, Glawson and Marsh[11]. Various possible aetiologic factors have been discussed[1,2,3,5,9,10,12,13,14,15]. The clinical symptomatology and histapathology of lupinosis in sheep, cattle and horses have been described[1,10,16,17,18,19].

Kühn[20] in 1880 first suggested that toxic metabolites of saprophytic fungi growing on the lupines may be the cause of lupinosis. He rejected the theory of Zurn[21] that fungi were directly responsible for the disease by developing in the animals after ingestion of the spores. This theory was untenable because the toxin could be extracted from the lupines by a process which killed the spores. Toxic lupines could, however, be rendered non-toxic by steaming at a pressure of one atmosphere for four hours[20]. In an attempt to determine the casual fungus, Kühn compared the mycoflora of toxic and non-toxic lupines. He found ten species of fungi on toxic and six on non-toxic lupines. A fungus which he described as a new species, *Cryptosporium leptostromiforme* Kühn[20], was found on the stems of toxic as well as non-toxic lupine plants. The toxicity of individual fungal species was not determined and the aetiologic role of fungi in lupinosis was thus not proved.

Gardiner[14] in 1966 proved that non-toxic lupine material could be rendered toxic by inoculating and incubating it with a mixed fungal suspesion from toxic lupines. The fungi responsible for toxin formation were not definitely established, although either *Cytospora* sp. or *Pleospora* sp. appeared to be a probable cause. Gardiner[1] concluded that one or more fungi were definitely involved in the development of lupinosis, but that the species of fungus responsible was unknown.

This paper reports on the occurrence of a field outbreak of lupinosis of sheep in South Africa, on the induction of lupinosis in sheep fed lupine material from this field, and on the experimental reproduction of lupinosis in sheep fed a pure culture of

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Phomopsis leptostromiformis (Kühn) Bubák, isolated from this toxic material and cultured on sterilized lupine seeds.

DESCRIPTION OF THE FIELD OUTBREAK OF LUPINOSIS IN SHEEP

During October, 1969 approximately 850 breeding ewes on the farm Burgersdrif, Hermon distric, Cape Province, were grazed on a field of sweet white lupines (*Lupinus albus* L. cult. Pflugs Gela), many of which were bearing pods in various stages of development. The lupine plants were approximately 9 inches tall and growing amongst a variety of grasses. Rain fell over this field on approximately the 12th and 5th day before the first mortalities occurred on October 19. Examination of the weather records for Langgewens Station, Malmesbury, the meteorological station which is closest to the farm (20 miles), revealed that the rainfall during October, 1969 was above average — 52.2 mm compared to the normal of 24.7 mm*. The records also showed that the maximum rainfall in 24 hours during the month (23.5 mm) occurred on October 8. The average daily maximum temperature for the month was 22.7°C compared with the normal of 23.7°C. The maximum temperature in 24 hours (36.6°C) occurred on October 5.

The ewes were immediately removed from this field after the first mortalities occurred and were fed lucerne hay. Mortalities increased on October 20 and 21 and then decreased. By October 25 a total of 530 ewes had died.

Examination of this field on October 25 revealed that the sheep had selectively grazed on the lupine pods containing seeds while the rest of the plant had been avoided. The external surface of the dry pods appeared black. Dark brown, water-soaked lesions were evident on some green pods. The discoloured pods were infected internally with a conspicuous, white fungal mycelium. Many of the seeds were also infected internally and were discoloured brown. The white mycelium was found only under areas showing an external discolouration.

ISOLATION OF THE CASUAL FUNGUS

The fungus was readily isolated in pure culture from pieces of infected pods and seeds placed directly on 1½% malt extract agar containing 100 mg/litre of sodium novobiocin. The same white fungus grew without the development of any contaminants from a total of 57 such platings. The complete dominance of this fungus on lupine pods from this field provided strong circumstantial evidence that it was responsible for the toxicosis.

CULTURAL CHARACTERISTICS OF THE CASUAL FUNGUS

A dense white surface mycelium is formed on 1½% malt extract agar. Irregular stromatic masses develop in the mycelial layer. These masses gradually develop into black, stromatic pycnidia. Mature conidia are exuded through an ostiole in a round, pink to orange spore mass. Sporulation can be induced within 21 days on sterilized lupine stems, 1½% malt extract agar, ¾% malt extract agar and corn meal agar, provided that the cultures are incubated at 16 - 18°C and are exposed to light.

Complete descriptions of the morphology and cultural characters of this fungus will be published elsewhere.

IDENTITY OF THE CAUSAL FUNGUS

A culture of the fungus isolated from *L. albus* was submitted to Dr. B. C. Sutton of the Commonwealth Mycological Institute, Kew, England, for identification. Dr. Sutton first suggested *Cryptosporium leptostromiforme* Kühn, as a possible name. In a later communication Dr. Sutton stated that he had compared the South African isolate with herbarium material of *Phomopsis rossiana* (Sacc.) Sacc. et D. Sacc.[22,23] on *Lupinus* sp. from Crete and found the specimens to be identical.

We examined the following collection of *Cryptosporium leptostromiforme* Kühn[20, 24] in the Mycological Herbarium, Pretoria: PRE 21698, on *Lupinus luteus* L., Krieger, Schädliche Pilze, Charlottenburg, Berlin, 16 Sept. 1899, Leg. Dr. O. Appel. The stromatic pycnidia and spores on the stem of this specimen were found to be identical with those of the South African isolate from *L. albus*.

C. leptostromiforme was transferred to the genus *Phomopsis* by Bubák (Exsicc. No.

* Weather Bureau Report for the Year 1969.

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660 in Lind[25]). This citation for the new combination *Phomopsis leptostromiformis* (Kühn) Bubák was also given by Grove[26]. It is, however, not clear whether the new combination was made for the first time in 1913 in Lind[25], or on Bubák's Exsiccatus No. 660 (Dr. B. C. Sutton, personal communication). This matter can only be cleared up by seeing Bubák's exsiccatus.

Dr. Sutton concluded that *P. rossiana* and *P. leptostromiformis* cannot be distinguished on the basis of the published descriptions and a decision as to their identity cannot be made without comparison of the type specimens. Although we have not seen the type of *P. leptostromiformis,* we have compared the South African fungus with authentic material of this species from Germany (Krieger, Schädliche Pilze). Moreover, the South African isolate was identified as *P. leptostromiformis* by Dr. J. A. von Arx of the Centraalbureau voor Schimmelcultures, Baarn, Netherlands. Therefore we conclude that the correct name of the fungus causing lupinosis in South Africa is *Phomopsis leptostromiformis* (Kühn) Bubák. The following tentative synonymy is suggested pending the examination of type material (if available):

Phomopsis leptostromiformis (Kühn) Bubák, Exs. No. 660, in Lind, *Danish fungi as represented in the herbarium of E. Rostrup,* p. 422, 1913.

Syn.: *Cryptosporium leptostromiforme* Kühn, *Ber. landw. Inst. Univ. Halle,* 2 : 121, 1880.

Phoma rossiana Sacc. apud Maire et Saccardo, *Annls mycol.* 1 : 222, 1903.

Phomopsis rossiana (Sacc.) Sacc. et D. Sacc., *Sylloge Fung.* 18 : 265, 1906.

Phomopsis leptostromiformis is known to occur on *Lupinus* spp. in Germany[20, 27], *L. albus* in Poland[28], *L. angustifolius* in Denmark[25], Germany[34] and Poland [28], *L. arboreus* and *L. polyphyllus* in England[26], and *L. luteus* in Denmark[25], Germany[34], Poland[28], Portugal[29] and the United States[30]. It causes a disease characterised by grey to light tan, bleached out, sunken, linear stem lesions containing black stromatic masses[20, 27, 28, 30, 31, 32, 34]. Young plants are killed by girdling of the stems[28, 30]. The fungus develops saprophytically on blue and white lupines in autumn and survives on woody pieces of lupine stem for at least three years[28, 34]. The spring pycnidiospores are the source of primary infection which is worst on dry sandy soil in hot weather[28]. High vitality of the plants is the most important factor in resistance[28].

Severe epiphytotics that killed more than half of the plants in a field before flowering have been reported in Germany[31, 32]. In Poland infection of all the plants in fields of *L. luteus* has been reported[28]. In the United States, however, reports indicate that less than 1% of the plants in a field are usually attacked[30].

In nature *P. leptostromiformis* is known to be pathogenic to *L. luteus*[28, 30, 34] and *L. angustifolius* var. *leucospermus*[34]. Pathogenicity has been proved by artificial inoculation [28, 30, 33, 34, 35]. Ostazeski and Wells[30] produced characteristic symptoms on plants of yellow and white, but not blue, lupines by inserting pieces of tooth-pick containing the fungus into the lower stems.

P. leptostromiformis is seed-borne and survives in seed of yellow lupine for at least two years[30]. Hot air treatments failed to eradicate the fungus from seed of yellow lupine even after the moisture content of the seed had been readjusted from an original 9.5 to 11, 13, 15 and 17 per cent[36].

TOXICITY TRIALS WITH FIELD MATERIAL

MATERIALS AND METHODS

Dry shelled pods, dry seeds and dry leafless white lupine stalks from the field where the outbreak had occurred were milled and dosed separately per stomach tube to sheep.

The sheep were clinically examined every day and chemical pathological determinations were done periodically on the blood[37, 38].

Sheep that died or were destroyed were necropsied and specimens for histopathological examinations were collected from various organs, fixed in 10% formalin, cut in a routine manner and stained with haematoxylin and eosin (HE). In special instances sections were stained either by the periodic acid Schiff (PAS), Schmorl's, Berlin Blue (BB) or Sudan Black (SB) methods.

The pod and seed material was tested for aflatoxin B and G according to the method of de Iongh *et al.*[39].

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EXPERIMENT I

MATERIALS AND METHODS

Shelled pods:

Milled dry shelled pods were dosed daily to four Merino wethers weighing between 18.5 kg and 35.0 kg, at the following rates:

Sheep 1 received 10 g/kg bodyweight for 12 days. The treatment was then suspended for 3 days and resumed for another 4 days. Then it was slaughtered for necropsy.
Sheep 2 received 7.5 g/kg bodyweight for 44 days when it was destroyed *in extremis*.
Sheep 3 received 5.0 g/kg bodyweight for 38 days when it died.
Sheep 4 received 2.5 g/kg bodyweight for 82 days when it was destroyed for necropsy.

RESULTS

Clinical signs

All the sheep developed a progressive inappetance and lethargy when the abnormalities in the blood chemistry became pronounced. Terminally in all cases except Sheep 4 there was a severe icterus (Fig. A and B).

Sheep 3 developed signs of ketosis a few hours before death. It wandered about in a soporific state pushing against obstacles in its path and making no attempt to avoid them until it became recumbent. It then suffered a few spasms and died.

Chemical pathology

The most significant chemical pathological changes occurred in the serum glutamic oxalacetic transaminase (SGOT) and total bilirubin (T.Br.) levels in the blood.

In the case of Sheep 1 the SGOT and T.Br. levels rose concurrently in the blood. A slight remission occurred when treatment was suspended but the levels rose again when it was resumed (Fig.A).

The serum of both Sheep 2 and Sheep 3 showed an increase in the SGOT content of the blood and a severe terminal icterus (Fig. B). Immediately before death the blood sugar levels of both sheep were higher than normal and at necropsy the urine of Sheep 3 tested positive for ketone bodies with Rothera's reagent.

The serum of Sheep 4 showed a slight but persistent increase in the level of SGOT but icterus did not develop.

Necropsy findings

Generalised icterus and atrophy of the liver with fatty degeneration and cirrhosis were the most important and constant features of the post mortem examinations. In all cases except that of Sheep 4 where the intoxication did not run its full course, impaction of the large intestine was present. Splenomegaly, ascites, hydrothorax and hydropericardium occurred in the majority of the more acute cases and in Sheep 4 the kidneys were pigmented brown.

Sheep 3 had an enteritis of the small intestine, enterorrhagia, and widespread fat necrosis.

Histopathological findings

The histopathological lesions in the livers of Sheep 1 and Sheep 2 were very similar, i.e. severe centrilobular fibrosis, which in some areas formed fibrous tracts often connecting the centres of adjoining lobules (Fig. 1). These bands of connective tissue disturbed the gross architecture and contained numerous pigment laden reticulo-endothelial cells (Fig. 2) in addition to, in some areas, islands of hepatocytes. The pigment granules that were yellowish brown in HE proved to consist of mucopolysaccharides (PAS), lipofuchsin (Schmorl's), lipoproteins (SB) and haemosiderin (BB).

Reticulo-endothelial cells were prominent and proliferation of bile ducts and bile duct epithelial cells (Fig. 3) was frequently observed. Megalocytosis, multinuceation; vesiculation of nuclei, (Fig. 11 & 12) karyopyknosis, and karyorrhexis (Fig. 9 & 10), that often resembled mitotic figures (Fig. 8), were noticed in a number of hepatocytes. Eosinophilic globules, often larger than the nucleus, occurred in the cytoplasm of some hepatocytes, (Fig. 4 & 7) and fat metamorphosis was present in a number of others.

The histopathology of the livers of Sheep 3 and Sheep 4 resembled the above except that fewer hepatocytes were affected and the fibrosis was more stellate in nature growing out from the central veins towards the portal tracts.

Haemosiderosis was seen in the spleens and periportal lymph nodes of all the sheep, while in the kidneys of Sheep 4 yellowish brown pigment granules were seen in the epithelial cells of the convoluted tubules.

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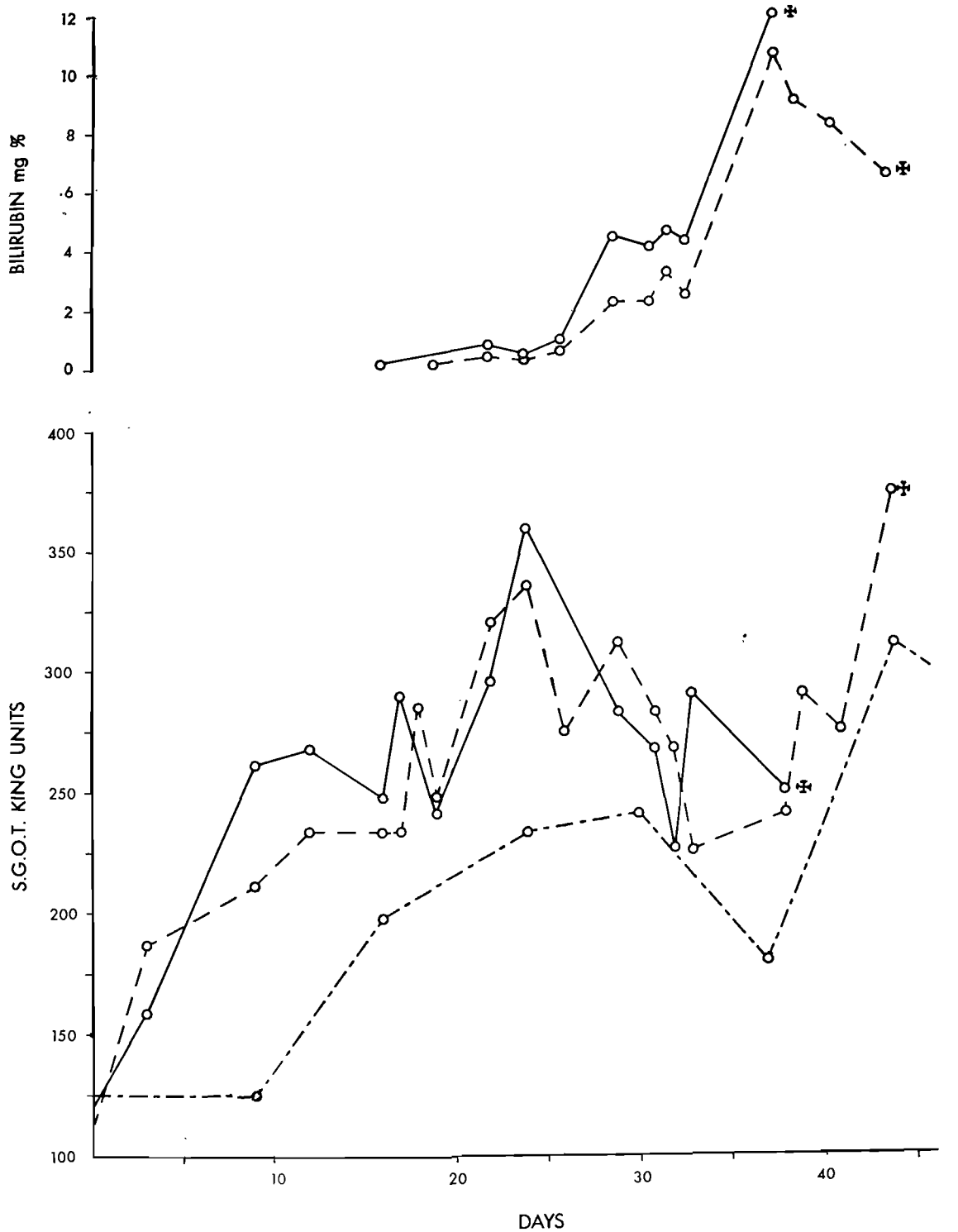

FIG. A Variations in the SGOT and plasma T. Br. levels of Sheep 1.

0— — — — —0 Sheep 2 0————————0 Sheep 3 0—— — —— —0 Sheep 4

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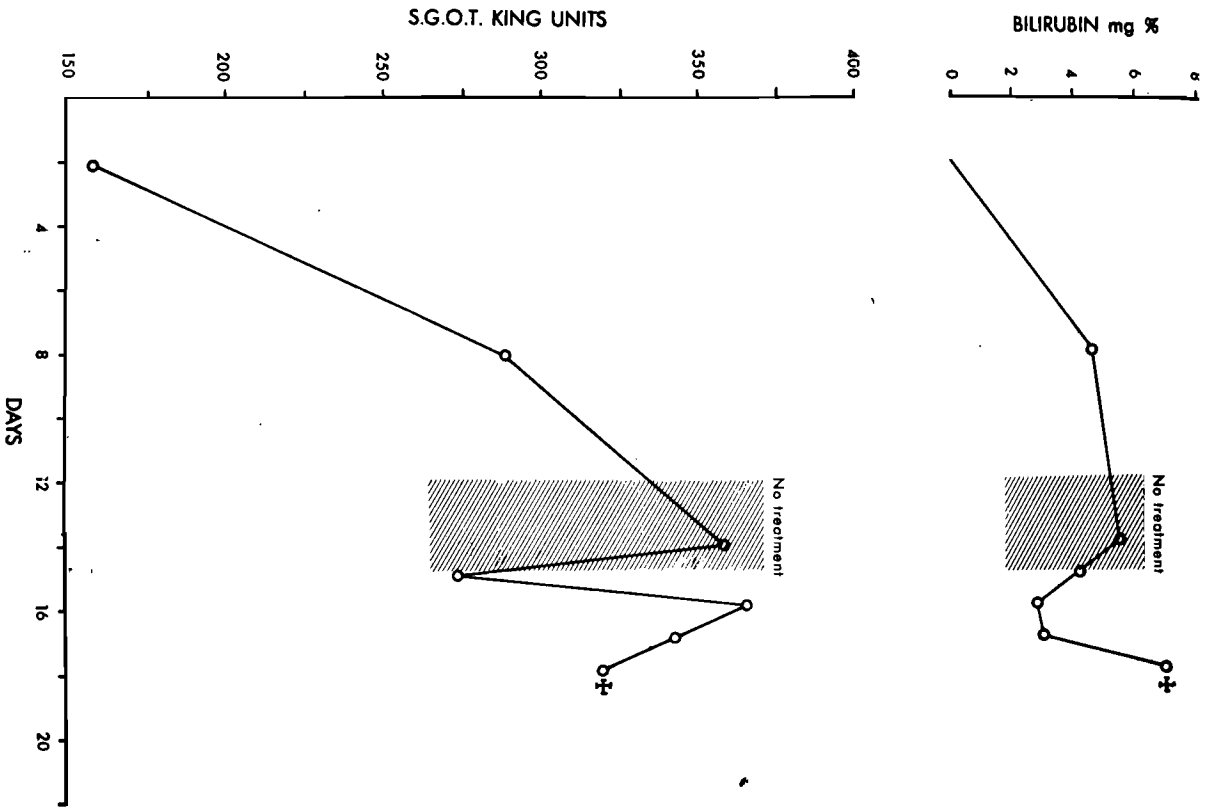

FIG. B Variations in the SGOT and plasma T. Br. levels of Sheep 2, Sheep 3 and Sheep 4.

Aflatoxin determination

The material was negative for aflatoxin B and G.

EXPERIMENT II

MATERIALS AND METHODS

Stalks:

Milled dry leafless stalks were dosed daily to Merino wethers weighing between 19.5 kg and 21.5 kg at the following rates:— Sheep 5 received 5 g/kg bodyweight for 58 days and 20 g/kg thereafter for 24 days when it was destroyed for necropsy.
Sheep 6 received 2.5 g/kg bodyweight for 58 days and 10 g/kg thereafter for 24 days when it was destroyed for necropsy.

RESULTS

Clinical signs

Sheep 5 developed progressive inappetance and listlessness, and finally mild icterus (Fig. C) but sheep 6 remained clinically normal.

Chemical pathology

No deviations from the normal occurred in the blood of Sheep 5 until the 51st day when a moderate rise in SGOT was observed. Thereafter the chemical pathological changes resembled those described in Experiment I. The blood of Sheep 6 showed a transient but sharp rise in SGOT when the dose was quadrupled but no icterus developed (Fig. C).

Necropsy findings

Anaemia, generalized icterus, severe atrophy and cirrhosis of the liver and a hydropericardium were found in Sheep 5. No lesions were seen in Sheep 6.

Histopathological findings

The histopathology of Sheep 5 resembled that described in Experiment I in all essential respects except that almost no fatty metamorphosis or hyaline droplet degeneration was seen in the hepatocytes.

The absence of significant macroscopic lesions at necropsy of Sheep 6 was confirmed by the histopathological examination that showed only a few isolated instances of pigmentation, fatty metamorphosis, hyaline

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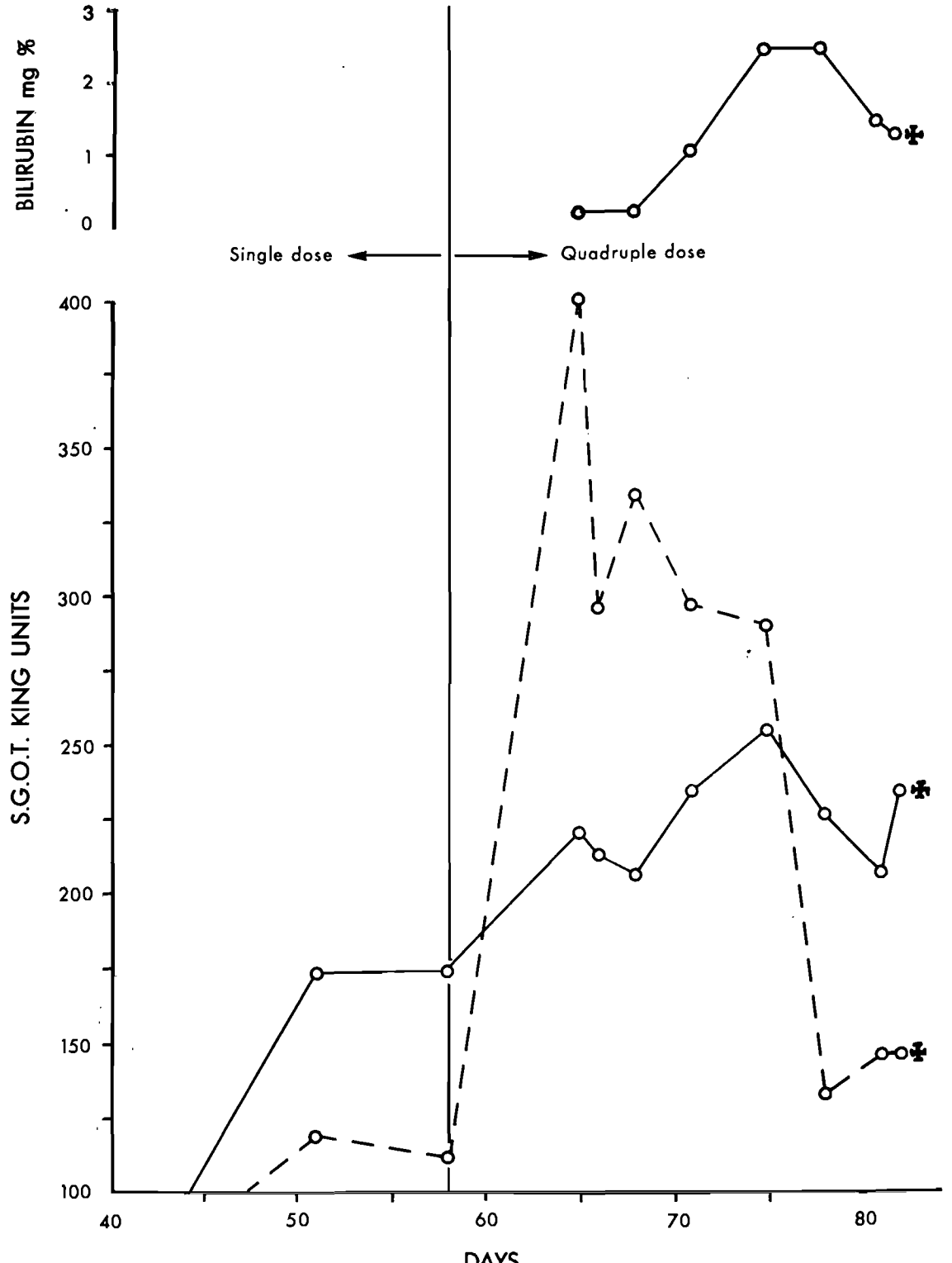

FIG. C Variation in the SGOT and plasma T.Br. levels of Sheep 5 and Sheep 6.

0——————0 Sheep 5 0— — — — —0 Sheep 6

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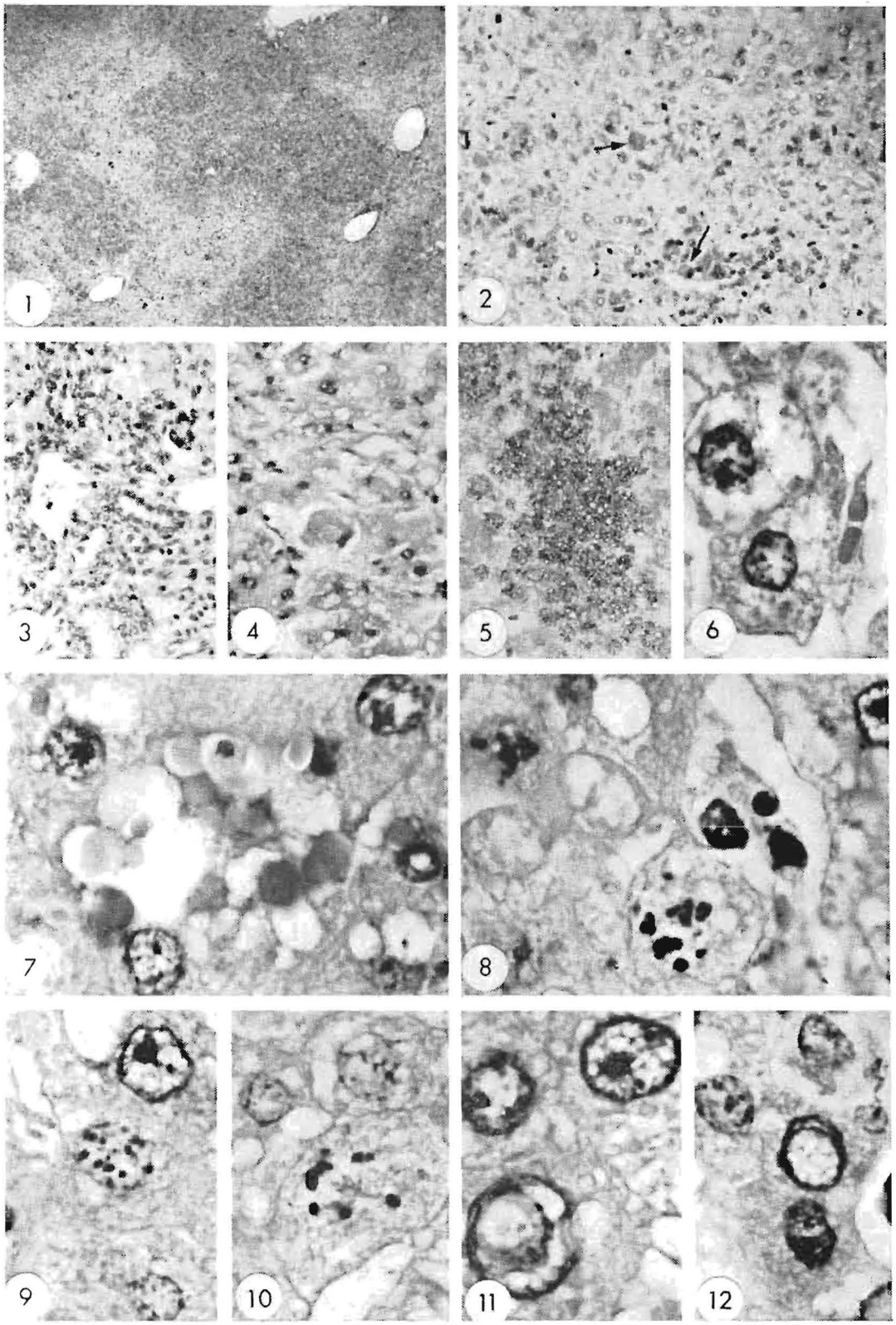

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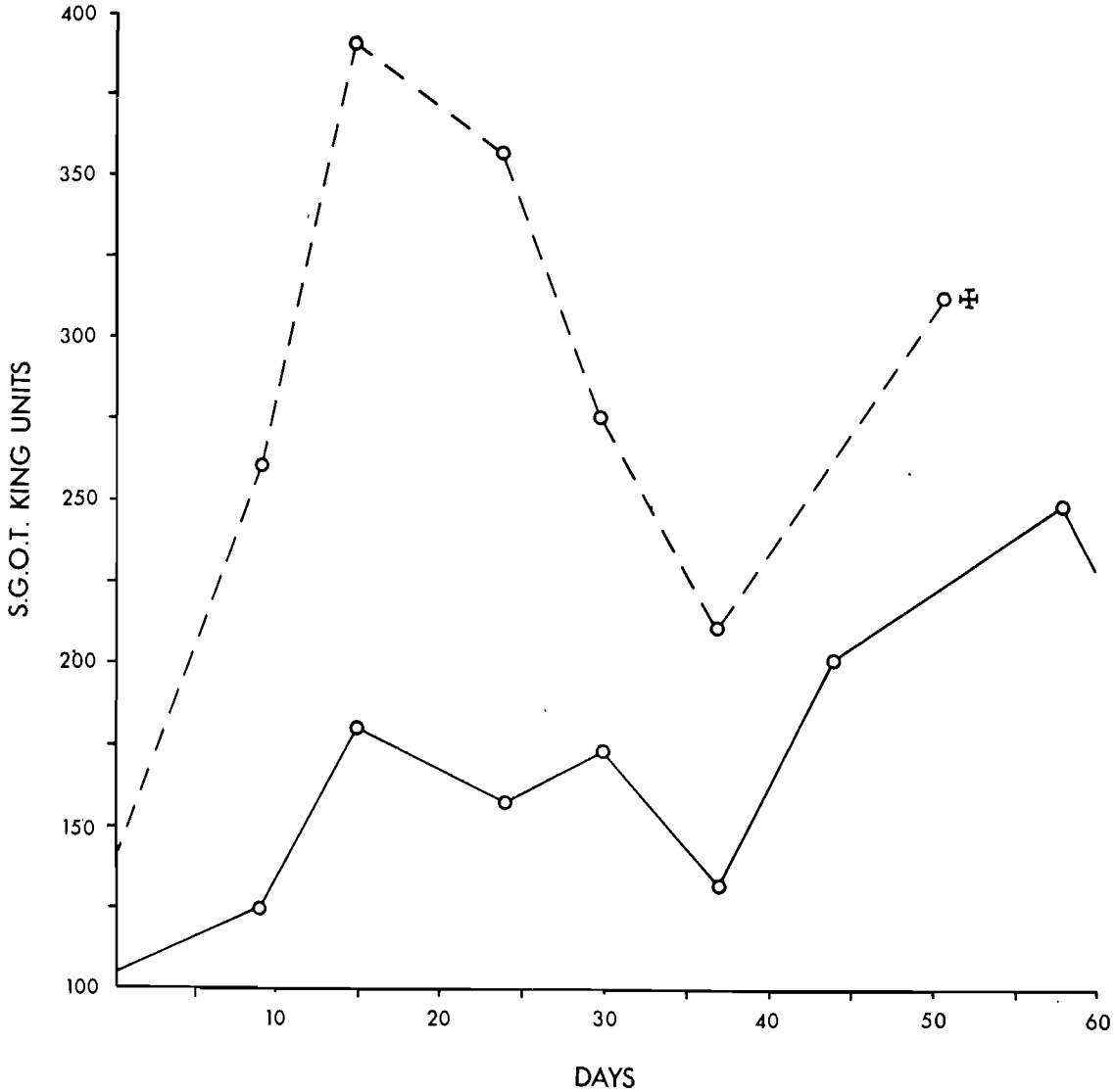

FIG. D Variations in the SGOT of Sheep 7 and Sheep 8.
0— — — — —0 Sheep 7
0————————0 Sheep 8

FIG. 1. Fibrotic tracts. X30. T. & E.
FIG. 2. Pigment accumulation. Variation i nsize of nuclei. X200. H. & E.
FIG. 3. Proliferation of bile ducts and bile duct epithelial cells. X200. H. & E.
FIG. 4. Eosinophilic globules and fatty metamorphosis. X350. H. & E.
FIG. 5. Spongy pigment laden cells. X200. Sudan Black.
FIG. 6. Degenerated and fat laden hepatocytes. X1200. H. & E.
FIG. 7. Eosinophilic globules. X1200. H. & E.
FIG. 8. Fatty degeneration, mitotic figure and karyorrhexis. X1200. H. & E.
FIG. 9. Karyorrhexis. X1200. H. & E.
FIG. 10. Karyorrhexis. X1200. H. & E.
FIG. 11. Vesiculated nucleus and variation in nuclear size. X1200. H. & E.
FIG. 12. Vesiculation of nucleus. X1200. H. & E.

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droplet degeneration, megalocytosis, and vesiculation of nuclei.

EXPERIMENT III

MATERIALS AND METHODS

Seeds:

Milled sweet lupine seeds were dosed daily to two Merino wethers weighing between 17.5 kg and 26.5 kg, at the following rates:—

Sheep 7 received 5 g/kg bodyweight for 51 days. On Day 52 it died.

Sheep 8 received 2.5 g/kg bodyweight for 81 days when it was destroyed for necropsy.

RESULTS

Clinical signs

Sheep 7 lost its appetite and became listless as the chemical pathological abnormalities increased, but did not develop icterus.

Sheep 8 remained clinically normal.

Chemical pathology

The blood of Sheep 7 showed a rise in the SGOT level while the SGOT level of Sheep 8 increased slightly at irregular intervals (Fig. D). The T.Br. level of both sheep remained normal (Fig. D).

Necropsy findings

Sheep 7 had a marked atrophy and cirrhosis of the liver together with rumenal stasis, serous atrophy of fat, hydrothorax, hydropericardium, oedema of the lungs and a severe lungworm infestation. The necropsy of Sheep 8 was negative.

Histopathological findings

The histopathological changes of Sheep 7 differed from the sheep used in Experiment I primarily in that in Sheep 7 the pigment containing reticulo-endothelial cells were swollen and spongy (Fig. 5).

In the liver of Sheep 8 pigmented cells and cells with vesicular nuclei were scarce and some proliferation of bile duct epithelial cells was noticed. The architecture of the liver was well preserved despite slight centrilobular necrosis.

Aflatoxin determination

The material was negative for aflatoxin B and G.

TOXICITY TRIALS WITH PURE CULTURES OF **P. LEPTOSTROMIFORMIS**

Pilot Trial

P. leptostromiformis was grown for two weeks at room temperature on *L. albus* seeds that had been autoclaved for 1½ hours at 15 p.s.i. The seeds on which the fungus had grown in pure culture were then minced, stored in a refrigerator at 4°C and dosed to Sheep 9 at 2.5g/kg bodyweight and to Sheep 10 at 5.0g/kg bodyweight daily, for 29 days. The only observable response in both cases was a slight rise in the SGOT level of the blood.

Dosing was withheld for a week and the trial repeated at the same daily dosage rate with a fresh culture grown for three weeks at room temperature on seeds autoclaved for 1½ hours at 15 p.s.i. on two consecutive days. Both sheep died within 14 days after showing typical clinical symptoms of lupinosis. The necropsy and histopathological findings are reported on in the main trial.

Two control sheep that were given uninoculated seeds from the same batch at the same dosage levels, remained normal.

The fungal culture material was tested and found to be negative for aflatoxin B and G[39].

MAIN TRIAL

Materials and Methods

L. albus seeds were autoclaved for three hours at 15 p.s.i. in 1-quart jars (200g seed in 150 ml distilled water/jar) and inoculated with 10 ml of a *P. leptostromiformis* spore suspesnion containing $1x10^6$ spores/ml. The jars were shaken to distribute the spores evenly and incubated in the dark at 25°C for 21 days. The contents of the jars were then minced in a meat mincer, care being taken that the material was not heated, and stored at 4°C in a refrigerator. Each day the required amount was withdrawn, weighed and blended with 2 litres of tapwater for dosing per stomach tube to sheep (Table 1).

Table 1: TOXICITY OF PURE CULTURES OF **P. LEPTOSTROMIFORMIS** TO MERINO SHEEP

No.	SHEEP Weight (kg)	Age (dentition)	DOSAGE DATA Daily dose (g/kg body wght.)	Days dosed	Day of death
11	27.5	2	2.5	5	10
12	24.5	2	2.5	6	6
13	23.0	2	5.0	5	6
14	35.5	6	5.0	5	6
15	25.0	2	7.5	5	5

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Uninoculated seeds from the same batch that were treated in the same manner but not incubated, were dosed to five control sheep.

The sheep were clinically examined every day and chemical pathological tests were done periodically on the blood[37, 38]. Sheep that died in the experiment were necropsied and the histopathological specimens were taken and prepared as before.

Results

The sheep in the control group remained normal and survived the experiment.

Clinical signs

All the treated sheep became depressed and developed an anorexia after about 36 hours. Seventy-two hours after the onset of treatment all the sheep had pronounced icterus and after Day 6, Sheep 11 showed rumenal stasis and a mild, almost inapparent bloat.

Chemical pathology

The most significant changes were sudden increases in the levels of SGOT and T.Br. in the blood (Table 2). In the final blood analysis of Sheep 13 done three days before death the SGOT level was normal while the T.Br. level was greatly increased.

Necropsy findings

Pilot trial:

The most significant lesions seen in the case of Sheep 9 were: icterus; generalised venous congestion; petechial and ecchymotic haemorrhages in the subcutaneous tissue, fascia, gut wall, epicardium, lymph nodes; severe fatty degeneration and suspected cirrhosis of the liver; impaction of the caecum and colon; splenomegaly; nephrosis; and congestion of the lungs. The urinary bladder was distended with dark reddish-brown urine.

Sheep 10 was too decomposed for examination.

Main trial:

The lesions in Sheep 11 - 15 resembled those described above. Icterus; severe acute fatty degeneration of the liver, but not cirrhosis; impaction of the caecum and colon; and oedema of the lungs, were the most constant features. Ascites, hydrothorax and hydropericardium, nephrosis, and splenomegaly were commonly present and in two of the cases catarrhal enteritis with enterorrhagia was seen.

Histopathological findings

Pilot trial:

The gross architecture of the liver was indistinct and the intralobular architecture was greatly disturbed. There was severe mid-zonal fat metamorphosis in the lobules, early centrilobular fibrosis and proliferation of bile ducts in the portal tracts.

Kupffer cells were prominent while several of these as well as some of the hepatocytes contained coarse yellowish brown granules. The straining characteristics of the pigment was similar to that described in Experiment I.

Eosinophilic globules, varying in size but often larger than the nuclei, were present in the cytoplasm of a minority of the hepatocytes. Megalocytosis and vesiculation of nuclei occurred fairly commonly.

Main trial:

The gross architecture of the liver was preserved but the intralobular architecture was disturbed by a massive fat metamor-

Table 2: EFFECT OF DOSING PURE CULTURES OF **P. LEPTOSTROMIFORMIS** ON BLOOD SGOT AND T.BR.

DAY	SHEEP No. 11		12		13		14		15	
	SGOT*	T.Br.**	SGOT	T.Br.	SGOT	T.Br.	SGOT	T.Br.	SGOT	T.Br.
1	139	0.0	173	0.0	125	0.0	166	0.0	152	0.0
2	200	0.0	132	0.0	112	0.0	159	0.0	206	0.0
4	187	0.8	212	1.6	152	3.2	247	4.8	226	2.8
8	274	7.6	—	—	—	—	—	—	—	—
9	296	9.4	—	—	—	—	—	—	—	—
10	342	5.1	—	—	—	—	—	—	—	—

* SGOT in King Units.

** T.Br. in mg%.

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phosis (Fig. 6) that distended the hepatocytes and frequently distorted the nuclei. The central vein, moreover, was compressed and difficult to distinguish in many lobules.

In the cases of Sheep 11-14 a small proportion of the reticulo-endothelium cells and of hepatocytes in the central areas of the liver lobules contained pigment while in the case of Sheep 15 many more cells were affected.

A small percentage of hepatocytes contained eosinophilic globules or had vesicular nuclei.

The lesions in the other organs resembled those described in Experiment I (i.e. nephrosis and haemosiderosis of spleen and lymph nodes).

DISCUSSION

Lupinosis of sheep has been studied since the latter part of the previous century. Since 1880 the fungus *P. leptostromiformis* has also been known to cause a serious disease of lupines. Although this fungus was actually described as a new species in one of the first papers dealing with lupinosis[20], the relationship between the fungal disease of lupines and the icteric disease of sheep have remained obscure until now.

The histopathological lesions seen in the livers of sheep that died in the field outbreak was similar to those of the trials described in this paper, and by Gardiner[16]. We therefore believe that the same toxic principle was present in both the field and pure culture material, and that the aetiology of the disease known as lupinosis has now been elucidated. Differences in the disease produced in these experiments are due to the relative acuteness or chronicity of the intoxication. The field material produced chronic intoxication whilst that of the pilot and main trails was either sub-acute or acute. It appeared that the pods were more toxic than the seeds or stalks.

Our findings confirmed chemically that the toxic principle of lupinosis is not aflatoxin[40]. The macro- and microscopic lesions of the disease, however, do resemble those of aflatoxicosis in sheep although Gardiner has reported that in mice the lesions differ[41]. J. D. Smit (Veterinary Research Institute, Onderstepoort, personal communication 1970) described the most significant macroscopic lesions of experimental aflatoxicosis in sheep, as generalised icterus, and fatty degeneration of the liver. Microscopically fatty degeneration, pigmentation and bile duct proliferation is present in the liver. Difficulty may also be experienced in differentiating between the histopathological lesions of chronic lupinosis and those of chronic enzootic icterus and seneciosis.

ACKNOWLEDGEMENTS

We are grateful to:

1. Dr. N. P. J. Kriek and Dr. J. de Wet, Regional Veterinary Investigation Centre, Stellenbosch, for their assistance with the field investigation and for establishing the toxicity of the material by a preliminary feeding trial.
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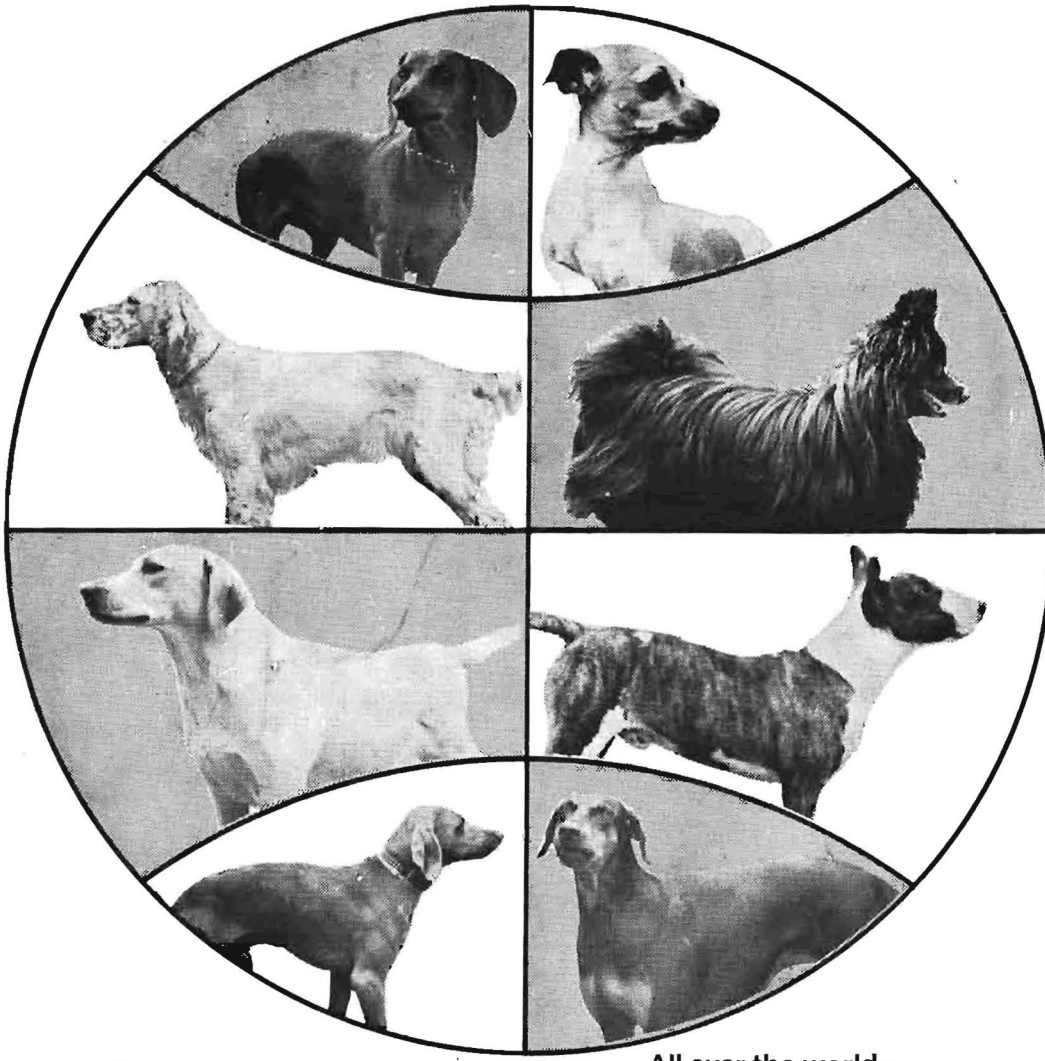

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ABSTRACTS FROM THE ONDERSTEPOORT JOURNAL OF VETERINARY RESEARCH

ANNA VERSTER

A taxonomic revision of the genus *Taenia* Linnaeus, 1758 *s.str. Onderstepoort J. vet. Res.* (1969), 36 (1), 3 - 58.

The genus *Taenia* Linnaeus, 1758 *sensu strictu* is revised. Besides the type species, *Taenia solium* Linnaeus, 1758, the valid species are: *T. acinonyxi; T. brachyacantha; T. crassiceps; T. crocutae; T. endothoracicus; T. gonyamai; T. hyaenae*; *T. hydatigena; T. ingwei; T. laticollis; T. macrocystis; T. martis; T. multiceps; T. mustelae; T. omissa; T. ovis*; *T. parenchymatosa; T. parva; T. pisiformis; T. polyacantha; T. rileyi; T. regis; T. saginata; T. selousi; T. serialis; T. taeniaeformis; T. taxidiensis*; *T. twitchelli.* "*T. laticollis*" of Skinker (1935) and Joyeux (1945) is renamed, *T. pseudolaticollis. T. brauni* is considered a subspecies of *T. serialis* and *T. krabbei* a subspecies of *T. ovis.* Invalid species and *species inquirendae* are also listed.

BASSON, P.A., MORGENTHAL, J.C., BILBROUGH, R.B., MARAIS, J.L., KRUGER, S.P. & VAN DER MERWE, J.L. de B.

"Grootlamsiekte", a specific syndrome of prolonged gestation in sheep caused by a shrub, *Salsola tuberculata* (Fenzl ex Moq) Schinz var. *tomentosa* C.A. Smith ex Aellen. *Onderstepoort J. vet Res.* (1969), 36 (1), 59-104.

A specific syndrome of prolonged gestation in sheep in South West Africa was studied and eventually reproduced by artificial feeding of the shrub, *S. tuberculata* var. *tomentosa.* It was determined that the main period of insult occurred during the last 50 days of pregnancy. The trend of both progesterone and cortisol levels resembled those of normal ewes except for a considerable delay during this 50-day period. Apart from the abnormal length of gestation and concomitant features such as retarded udder development, no signs of toxicosis were manifested by the ewes. The most significant features in the postmature lambs were progressive hypophysial, adrenal and thymic atrophy; hypertrophy of the female genitalia, polyfollicularity of the ovaries and Leydig cell hypoplasia; long haircoat, erupted incisors and pigmentation especially of the kidneys and lymph nodes, but no abnormal anatomical features were found. In rats, rations containing the shrub prolonged both gestation and the dioestrous phase of the oestrous cycle. The active ingredient of the plant is not an oestrogenic or anti-oestrogenic substance. It was successfully extracted with alcoholic compounds.

MCCULLY, R.M., KRUGER, S.P., BASSON, P.A., EBEDES, H. & VAN NIEKERK, J.W.

Strongylidoses: Delafondiasis in the zebra. *Onderstepoort J. vet. Res.* (1969), 36, (1), 105 - 128.

Post mortem examinations on 125 zebras (*Equus burchelli* (Gray, 1824)) from the Kruger and Etosha National Parks revealed nodular and cystlike lesions of parasitic thrombophlebitis within the intrahepatic branches of the portal vein of most of the adults. These lesions contained either the larvae of the fifth stage of *Delafondia vulgaris* (Looss, 1900) Skrjabin, 1933. The lesion was usually a combination of thrombosis, its organization and host response to the parasite. The fifth stage specimens were larger than sexually mature *D. vulgaris* present in the caecum and ventral colon. Though larger, none of the females in the liver contained ova in their uteri.

Somewhat similar lesions due to fifth stage *D. vulgaris* were rarely observed in the pulmonary artery. Fourth stage larvae and the fifth stage of the parasite were found in the anterior mesenteric arteries and their branches of many of the zebras. Though enlarged and having thickened walls, the lumens were narrowed and none of the arteries appeared to have true aneurysms.

These findings in zebras lend support to the contention of a previous investigator that it is the time factor and not the environment of the larva that determines its moults. It was obvious that some of the larvae of *D. vulgaris* migrate into the liver. Whether it is in the course of a normal migratory pattern or an aberrant one was not determined. Those which become trapped in the lesions are at a dead end. The authors refer to the disease by the derivative from the generic name of the nematode, viz. "Delafondiasis".

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McCULLY, R.M. & KRUGER, S.P.

Observations on bilharziasis of domestic ruminants in South Africa. *Onderstepoort J. vet. Res.* (1969), 36 (1), 129-162.

Bliharziasis of 100 sheep nad 14 cattle caused by *Schistosoma mattheei* Veglia & Le Roux, 1929, was studied in detail from the histopathological aspect. The ovine cases included natural as well as experimentally infested animals. The most significant changes resulted from the presence of schistosome ova and the dead schistosomes in the branches of the intrahepatic portal vein. The host reaction to the ova is of a granulomatous nature which is interpreted as a type of delayed hypersensitivity reaction. In a few cases there was an even more marked sensitivity superimposed on this. It was characterized by concentrations of eosinophiles around miracidial — containing ova as well as shells of ova present in the centre of mature granulomas. This was accompanied by necrosis of adjacent liver cord cells and necrosis of masses of eosinophiles. This appears to be analogous to the toxaemic form of human bilharziasis.

In both sheep and cattle, particularly the latter, the host response to dead adult schistosomes in the intrahepatic branches of the portal vein was striking. The initial thrombosis was followed by a granulomatous response to remove the parasite, and a localized lymphoid proliferation which destroyed the wall of the vein and remained after the schistosome had been removed.

The Hoeppli phenomenon occurred in response to ova in both sheep and cattle, being more pronounced and more frequently present in cattle. The conclusion is drawn by the authors that cattle are less affected by bilharziasis than sheep and goats.

LETTER TO THE EDITOR

Sir,

re: SHORR'S TRICHROME STAIN* FOR VAGINAL SMEARS

I would like to clear up an apparent misunderstanding regarding the use of the above stain for examination of vaginal smears from bitches.

The original modifications of Massons trichrome stain as described by Shorr 1, 2, involved a number of solutions and somewhat lengthy staining procedure. These were followed by a final modification describing a single differential stain for use as a "diagnostic index and guide for therapeutic use of reproductive hormones" (Shorr 3,). In this stain all the original components were incorporated with the exception of haematoxylin and the staining procedure considerably simplified.

The staining procedure which has been found to give satisfactory results at this laboratory is as follows:—

1. Cleanse the vulva with a swab soaked in disinfectant.
2. Introduce a pipette almost vertically as far as the posterior vagina.
3. Squeeze and release the bulb to collect the fluid (if no fluid is found—for instance if the bitch is in an-oestrus a small quantity of saline may be introduced to suspend the cells.
4. Apply one drop of vaginal fluid to a clean dry miscroscope slide.
5. Smear fluid over half the slide.
6. Air dry and fix in 70% alcohol.
7. Stain in Shorr's trichrome stain for one minute.
8. Wash in 70% alcohol and dry.
9. Examine using oil immersion.

A definite differentiation is obtained between keratinised cells staining orange red and non-keratinised, green, other elements such as erythrocytes, leucocytes and bacteria are also differentiated. The method is particularly applicable to general clinical use as the time involved in making, staining and examining a vaginal smear is now reduced to ±10 minutes.

The indications for examining vaginal smears are:—

a) detection of the different phases of the sexual cycle.
b) to determine the best time for mating.
c) diagnosis and treatment of hormonal insufficiency.
d) diagnosis of endometritis.

P. W. THOROLD (Dr.),
Glaxo-Allenburys (S.A.) (Pty.) Ltd.

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Dr. LESLIE (PAT) STONIER

Leslie Stonier was born in Cape Town on April 6, 1901. Later his parents moved to the Transvaal. He received his schooling at Premier Mine and matriculated at the Pretoria Boys' High School in 1920. He taught for a short while before undertaking his veterinary studies at Onderstepoort, eventually obtaining his B.V.Sc. from the University of South Africa in 1927. His first appointment was that of GVO (Government Veterinary Officer) at Vryburg. In 1929 he married Dorothy White, a nurse from Premier Mine. In 1933 he was transferred to the Allerton Veterinary Laboratory and in 1935 the family moved to Kimberley. During World War II he served with the Union Defence Force.

Pat Stronier was a loyal member of St. Cyprians Cathedral and the Diocese of Kimberley and Kuruman. He served on countless church committees and sang in the Cathedral Choir for about thirty years. He was a member of the Rotary Club and served as Scribe for several years.

He remained in Government service for a year after retirement in 1964 and was subsequently employed by the Kimberley City Council in meat hygiene control.

Pat Stonier was one of the first veterinarians to contract Rift Valley fever in South Africa and this resulted in impaired vision due to retinal detachment. He was, however, in excellent health before he succumbed to a massive coronary thrombosis a week before his 69th birthday.

To his widow, two sons, and grandchildren we extend our sincere sympathy. Their loss is shared by us all.

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Dr. DOUGLAS ERIC TRUTER

Die Truter-familie, die Afdeling Veeartsenydiens en die veeartsenykundige professie het 'n baie gevoelige verlies gely deur die skielike heengaan van dr. D. E. Truter op 11 April, 1970.

Hy is op 2 November 1920 te Beaufort-Wes gebore en het sy skoolopleiding aan die Paarlse Gimnasium ontvang. Na matrikulasie in 1937 is hy na die Universiteit Pretoria waar hy in 1942 die graad B.V.Sc. verwerf het. Hy het in 1943 sy loopbaan in die Staatsdiens begin en was Staatsveearts op Estcourt, Vryheid, Nongoma, Armoedsvlakte, Onderstepoort, Rustenburg en Kaapstad. In 1949 is hy getroud met Veronine Justiene Schoeman, van Rustenburg. In 1960 is ons kollega as Senior Staatsveearts, Heidelberg (Tvl.) aangestel, en hy is in 1963 na Pretoria as Eerste Staatsveearts verplaas. In 1964 is hy bevorder tot Assistent Hoof in beheer van die O.V.S.-streek, met Bloemfontein as hoofkwartier. In 1965 is hy in dieselfde hoedanigheid verplaas na Pretoria om die Transvaalstreek te behartig.

Hy het nooit eie belang eerste gestel nie. Die belange van sy personeel en dié Diens het sy primêre aandag gekry, en hy was 'n geliefde leier. Sy toegewydheid is vir ons almal 'n voorbeeld.

Ons innige simpatie gaan uit na Veronine en hul kinders, Estelle, Anita, Linda en André.

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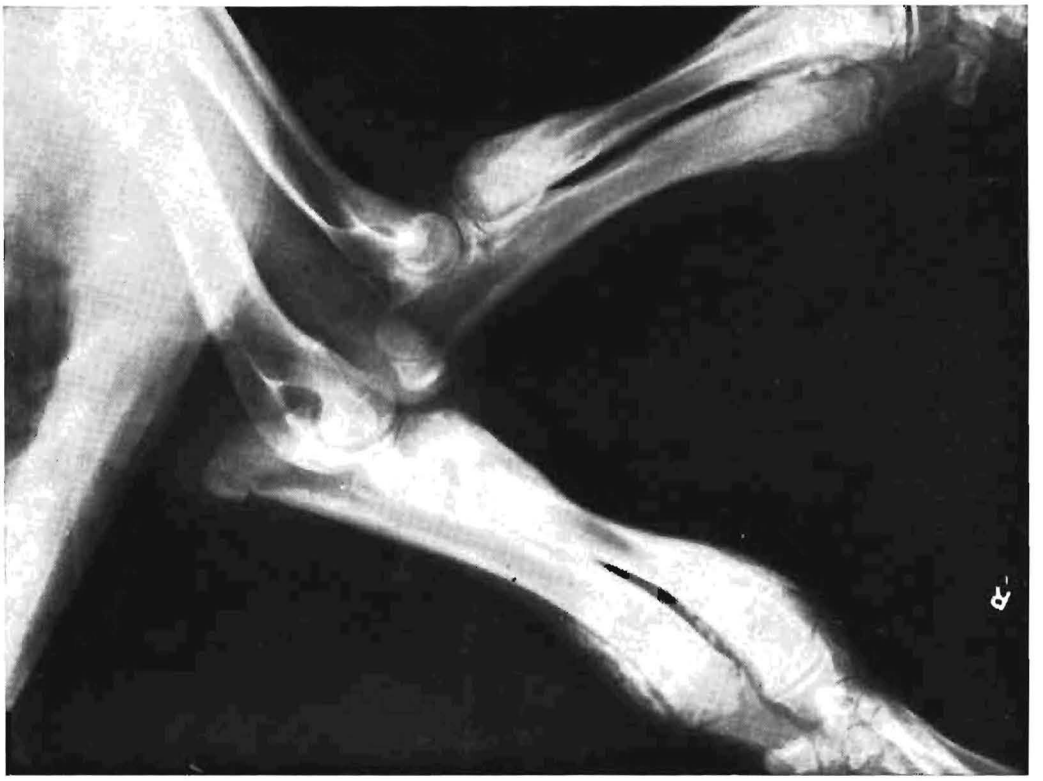

DIETARY HYPERTROPHIC OSTEODYSTROPHY IN THE YOUNG DOG — A VERY STRANGE "RICKETS"

Some two to four clinical cases of this metabolic skeletal disease are presented annually at outpatients clinic, Faculty Veterinary Science, Onderstepoort. The patient is a young dog, usually between four and six months old, of either sex, and to date, often a Boxer or Boxer-cross, although the disease has occurred in a Dalmatian and also the giant breeds. The owner's complaint mentions depression, lameness and reluctance to stand, often accompanied by signs of pain. Appetite may be capricious, poor or absent. Questioning of the owner usually, but not always, elicits the information that the dog's diet has for some time been fortified with calcium and/or vitamin D. The rectal temperature may vary between normal and 106° F and obvious pain is usually elicited by handling and flexion of carpal and tarsal joints. The distal metaphyseal regions of the radius, ulna and tibia are moderately to markedly enlarged, bone-hard, often warm or even hot to the touch, and painful on pressure. Over-extension and/or abduction of the foot may be present in the heavier breeds.

Radiography is essential to correct diagnosis, and the characteristic findings are illustrated above in the plate. The distal metaphyses of the radius and ulna are enlarged and noticeably radiopaque. Increased ulnar metaphyseal diameter, compared with that of the radius, further indicates the activity of bone growth. Distinct radiopaque soft tissue calcification, peripheral to the periosteum, is visualised at the distal ulnar and radial metaphyses. Epiphyses and growth plates appear normal.

Rapid clinical improvement has occurred with cage rest and the discontinuation of further mineral-vitamin supplementation. Severe pain can be promptly relieved by short-term corticosteroid therapy, while we have occasionally administered antibiotics for persistent pyrexia. Recovery is heralded by improved habitus, appeite and locomotion. The unsightly joint deformity disappears slowly after a variable time. Riser[1] states that the remainder of the skeleton appears to be radiographically normal and that he has found metastatic calcification of the endocard, aorta and kidneys at autopsy in this disease.

REFERENCE:

1. Riser, W.H. & Shirer, Jane F. 1965 **J. Amer. vet. radiol. Soc. 6 : 50.**

Submitted by R. K. Loveday, Department of Medicine, Faculty of Veterinary Science, Onderstepoort.

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