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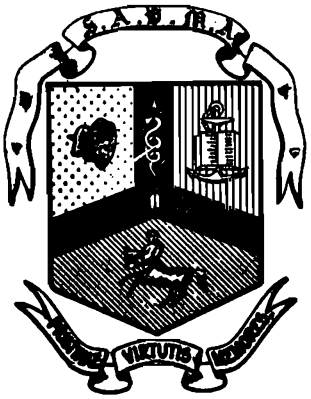

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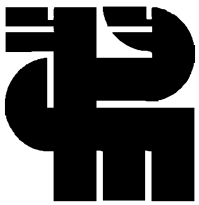

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EDITORIAL

THE TEACHING OF ANATOMY

Anatomy is often regarded as a fossilised subject, well suited to fossilising students, petrifying them literally and figuratively.

The teaching of this subject has special problems of its own; more has probably been written about these problems than on those concerning other subjects of the veterinary curriculum. Many of them arise from the strictly academic approach, which demands that each structure be described in detail, moreover, that students be set the task of memorising such detail, regardless of the ultimate object in view.

A few practical facts pertaining to pedagogics too frequently are forgotten. Verbal description of structures that in their complexity defy all geometrical concepts is difficult enough, far more difficult is the task of translating such description into accurate three-dimensional mental images, and infinitely more so of compounding a multitude of such images into an understandable whole. The neophyte, pummelled into docile, sponge-like absorbing of facts — and periodic regurgitation thereof — by years of brainwashing at school, has an unenviable task ahead of him. This does not imply that anyone can "succeed in Anatomy without really trying", or that "Anatomy in Ten Easy Lessons" is either possible or desirable. Good old-fashioned mental drill is still the pivot in the mechanics of the learning process. The effort, however, must be directed clearly towards an ultimate goal. This does NOT consist of the ability to describe verbally the multitudinous structures of the body. Surely it is expecting too much of the student after one or two years of study to memorise facts written by the expert with the dissections and literature at hand. Still more unreasonable is it to expect the student to retain such facts for more than a few days after the final examination.

The ultimate goal should be the ability to find one's way about the body with as full appreciation as possible of the significance of the features encountered. All teaching and all examining should be aimed unequivocally at this objective.

Ability to find one's way about the body depends on visual and tactile recognition of structures in their topographic relationship, initially in the dissected cadaver, eventually on the living body. It also implies the ability to expose structures by dissection. However much time may be saved by making use of demonstration dissections, each student must do sufficient dissecting himself until his ability is developed fully. Neither teacher nor text can do this for him. The minutiae of structural detail in any operative field are too many in number to be memorised; they vary from individual to individual in any case. Hence the importance of development of this ability.

Appreciation of the significance of anatomical structure forms the key to professional training in this subject. It allows the development of sound associations of ideas, indispensable in any pedagogic system, wherewith to transform discrete data into an intelligible and meaningful whole. Already within the confines of anatomy in the narrower sense, basic patterns and principles of structure become apparent. What is in practice a thorny problem in the veterinary curriculum, namely, the study of more than one species, can be turned to great advantage to demonstrate such patterns and principles forcibly and clearly. Adherence to the comparative approach, in order to emphasise fundamental similarities rather than detailed differences, offers the veterinary student deeper insight into and a more intelligent grasp of the subject. This is materially enhanced by demonstrating how fundamental differences are the outcome of adaptations to the particular animal's way of life. (Once having adopted this procedure, the teacher must be prepared for some difficult questions posed by an eager student body: he will make a number of rather surprising discoveries himself.)

In these respects teaching should not be confined to comparative anatomy in the strict veterinary sense: the whole field of general comparative anatomy is available, and can be turned to good account. By drawing freely from phylogeny, principles of evolution may be brought home, not merely for the sake of theoretical interest, but also to make the student aware of the adaptation of different breeds of domestic stock to different surroundings, an awareness of vital importance in modern global thinking with special reference to the development of emergent areas.

Ontology comes to one's aid in discerning simple, fundamental features in seemingly complex structures, such as the omentum, particularly as it occurs in ruminants.

Transcending strictly morphological thought by physiological concepts is a necessity. Form has no meaning when divorced from function, neither has phylogenetic and ontogenetic development of form. (Teleological principles are useful teaching dodges, provided their artificiality be clearly expounded.) Dynamic interpretation of apparently static structure should be the constant aim.

So much for the underlying theoretical principles, which should always carry a practical superstructure. In fact, the student must be shown how the theoretical principle has been derived from practical observation. If well aligned, these principles form a natural setting for applied considerations. The clinical applications are obvious and need no further expatiation. Although the anatomy class is not the place to teach formal pathology, medicine or surgery, it is important to give brief and clear, albeit elementary indications of the clinical importance of appropriate anatomical facts to provide sound motivation for their study and so erect the anatomical pylon of the bridge to the paraclinical and clinical subjects.

Rather than have students memorise figures concerning weights and sizes of organs, which are very variable anyway, it is more valuable to have them develop an almost unconscious appreciation of normal sizes and colours — they must get their "eye in" — and of normal textures and consistencies, i.e. the "feel" thereof. This can be attained by repeated intelligent observation of fresh specimens. In this respect, too, the veterinary student has a considerable advantage, which must be exploited to the full. On similar principles topographical knowledge must be developed, rather than have students stuffing their heads full of figures: "from the so-manieth rib to the so-manieth". A few key points will suffice.

The comparative study of bones and organs with the object of well-reasoned species identification is extremly useful. The practical applications find their place in meat inspection. Of even greater value is the development of powers of accurate observation and logical deduction, whilst making due allowances for biological variation.

A most important field of application of anatomical knowledge is that of zootechnics, in which anatomical and physiological knowledge is integrated and applied to enhancement of production. Closer liaison between anatomy and zootechnics offers tremendous opportunities to further the study of both. Conformation in terms of anatomical structure is critically considered in terms of optimal physiology and thus affords practical exercise in the dynamic interpretation of static structure. With the help of everyday knowledge of mechanics, the kinetics of a particular structure must be scrutinised more closely in its reciprocal action on other structures and consideration must be given how they contribute to body function as a whole during the various animal activities, as well as to the efficiency thereof. Immediately each component part of the structure gains greater significance and is seen in a new light. Of even greater importance is the development of fuller appreciation of norms of conformation and movement as points of reference to be used later in clinical observation. Predisposition to various pathological states may then be deducted almost automatically.

No set methods and procedures can be prescribed: in these respects "The Ideal Anatomy Course" does not exist. Rather, the details should be left to the ingenuity and drive of the teacher and the receptiveness of the student body, under the dictates of local circumstances and expediencies, provided these do not curb the teaching. In the final analysis all depends

on the ability of teacher and student. The potentialities of the latter are in the main underrated and merely await suitable challenge and stimulation along proper pedagogic lines.

The proper development of the abovementioned abilities and insight takes time, time to assimilate and time to mature. In terms of time most of our curricula are based on the false premises that a single or at best a few fleeting exposures are sufficient for the average student mind to absorb a fact. No one seems to dare ask how is it possible for a student to assimilate properly within one evening all that has been taught during a full day's programme. The answer is that it is not, and that it is not done. Consequently the student has to cram for the examination and "pull the chain" afterwards. In this he is aided and abetted by systems of teaching and examination that tend to develop and test memory potential to the exclusion of all other abilities. Meanwhile the roster is raised to the position of an immutable demigod — whose trappings at most may be swapped around — to whom we, in helplessly hypnotic trance sacrifice the student mind. No wonder that accusations are made that universities tend to turn out awkwardly stuffed carpet bags instead of intelligent, competent beings.

FIRST THEILER MEMORIAL LECTURE

The Faculty of Veterinary Science, University of Pretoria possesses a fund for the promotion of veterinary education in South Africa. Among the objects of this fund is the sponsoring of lectures by prominent scientists whose experience will contribute to the knowledge of the veterinary profession in this country. These lectures are designated "Theiler Memorial Lectures."

The first of these lectures was delivered by Professor C. Rimington, F.R.S., Professor of Chemical Pathology, University College Hospital Medical School, London at the University of Pretoria on Tuesday the 24th September, 1963.

PHOTOSENSITIZATION SYNDROMES DUE TO PORPHYRINS IN ANIMALS AND MAN

Mr. Rector of the University of Pretoria, Professor Jansen, Professor du Toit, Ladies and Gentlemen.

It is with deep humility that I stand before you to deliver this first Theiler Memorial Lecture. Sir Arnold Theiler was a very great Scientist and a great human personality to whose memory it is a privilege and honour to pay tribute. I had the great good fortune to know him and to work beside him at Onderstepoort during my young and formative years and time has only made me realise more clearly how much I owe to his inspiration, example and encouragement. Sir Arnold could be a strict disciplinarian both as regards habits of work and, most important of all, as regards habits of scientific thought. Lessons so learned are a priceless equipment for later life.

It was in 1931 that we first met before I sailed for South Africa to take up a special research assignment on the chemistry of poisonous plants. I knew little about the problem and nothing about the conditions in South Africa or the prevalence of diseases in stock due to plant poisoning and it was with some trepidation that I went to see Sir Arnold at his home overlooking the lake of Lucerne in his native Switzerland — for it was not until the following year that he returned to Onderstepoort.

He and Lady Theiler received me most kindly after which Sir Arnold secured me firmly by drawing up a chair in front of mine in a corner of the room. There then began an exposition of what was known about the chemistry and pathology of the most important plant poisoning diseases which left me — a little bewildered, perhaps — but thoroughly impatient to reach Onderstepoort and to get started on the job without more waste of time!

The facts of a man's life and even his professional achievements are easily recounted. What is more difficult is to convey an impression of the man himself. In Sir Arnold Theiler's case, that quality which stands out most vividly in my memory is his great humanity. He would talk with the same sincerity and interest to a backveld farmer as he would to the most eminent scientist who visited him from overseas. Such an attitude springs from a great heart which knows humility before God and man. To him, Science was the pursuit of Truth, not for honours or rewards but for its own sake.

It has been said that Theiler put South Africa on the scientific map of the world and the Institute which he founded and directed for many years is indeed revered wherever Veterinary Science is taught and practiced. His interests were wide, as they had to be in a young and

growing country, covering bacteriology, parasitology, pathology and biochemistry but I like to think that he was particularly intrigued by problems of plant poisoning. Among the latter, Tribulosis ovium with its symptoms of photosensitization and icterus was peculiarly baffling and I think I could not do better, in choosing a subject for the scientific part of this Memorial Lecture, than to describe the work which he saw unfolding at Onderstepoort as a result of the efforts of Quin, Roets and myself and which is still going on today towards elucidation of the Geel-dikkop syndrome. Following this, I shall tell the story of the discovery of the first living animal cases of Congenital Porphyria, another disease characterized by photosensitization, in which Sir Arnold was implicated and upon which I worked in collaboration with Fourie and Roets. The South African cases have made history. Finally, I will tell of the discovery of yet another type of erythropoietic porphyria with photosensitization, discovered in man for the first time in London.

Tribulosis ovium, or Geel-dikkop, meaning "Yellow thick-head", as it is commonly known amongst farmers has probably existed for a very long time. Information about it was collected by Hutcheon in his Report of the Colonial Veterinary Surgeon for 1886 in which he described it as a derangement of the liver causing a deeply jaundiced condition of the whole body and with effusions of serous fluid under the skin, especially about the head. Some farmers considered it to be a form of plant poisoning, but many other views were expressed.

Sir Arnold Theiler's classic paper on Geel-dikkop appeared in 1918 in the volume of his 7th and 8th Reports as Director of Veterinary Research which was published by the Government Printer in 1920. In it he summarised what was known concerning the disease and gave a detailed description of its symptomatology and pathology. He also recorded the first successful attempt to reproduce the disease by allowing animals to graze exclusively upon the plant *Tribulus terrestris*. These experiments were performed on the farm Bergrivier at Luckhoff in the Fauresmith district while the plants were in the luxuriant flowering state. Cases occurred after 10 to 16 days of feeding with a morbidity of 60% and mortality of 45%. In a discussion of the pathogenesis of Geel-dikkop, Theiler concluded that the toxic substance derived from the plant acted on the skin of the head of affected animals as well as on the liver. He was careful to note, however, that in some instances the skin lesions developed before there was any generalized icterus and also that sunlight appeared to play some role. It might, he suggested, convert an inactive toxic substance into an active one or give rise to an ulcerative dermatitis in the same way as after the feeding of buck-wheat which contains a photosensitizing pigment. The influence of the sun was not, however, firmly established.

This brilliant work of Sir Arnold Theiler's upon geel-dikkop marked a turning point in the investigation of the disease but another 15 years was to elapse before the complexities of the syndrome were finally disentangled.

Following up the clue of a possible photosensitizing factor, Quin investigated the effects on sheep of feeding the St. John's Wort, *Hypericum* species, or of injecting fluorescent dyes such as eosin, rose bengal, acriflavin and haematophorphyrin. Skin lesions closely resembling those of geel-dikkop were produced in each case but there was no icterus. Only one plant, *Lippia rehmanni* provoked both transitory photosensitization and transitory icterus. Recourse was then had to surgical obstruction of the bile flow. This, of course, produced an intense jaundice but most animals also exhibited marked photosensitization between the 5th and 14th days. At post-mortem, the bile ducts were seen to be grossly dilated with bile, a picture quite different from that seen in the livers of sheep dying from geel-dikkop.

It was at this time that I joined Quin in the investigation and our first aim was to isolate and identify the photosensitizing factor from the blood of sheep suffering from the natural disease. In this we were successful.. We suspected that a porphyrin might be responsible but were somewhat surprised when we isolated a chlorophyll porphyrin, phylloerythrin, from the blood. Phylloerythrin was well known as a constituent of sheeps' faeces, having been isolated from this source by Marchlewski in 1904. We found it to be present in quantity also in sheep's bile. This afforded the key to the situation. When for

any reason the biliary excretory function of the animal is impaired, all constituents of the bile, phylloerythrin included, find their way into the general and peripheral circulation where any photo-active material would cause lesions in skin exposed to sunlight. It remained to be proved that phylloerythrin was indeed photo-active and to discover how it arose from chlorophyll; whether in the plant before ingestion or in the animal body after feeding.

We prepared phylloerythrin chemically from chlorophyll and found it on intravenous injection to be a highly potent photosensitizing agent. Some years later Quin and Riemerschmid extended this finding by showing that the action spectrum corresponded to the absorption spectrum of the pigment. That phylloerythrin arose by the action of the ruminal micro-organisms upon chlorophyll and that it was absorbed from the small intestine into the portal system, was demonstrated by analyses of the contents of the alimentary tract which Quin, Roets and I carried out and by isolation of the ruminal protozoa.

As a result of our work, we were able to put forward the hypothesis that in geel-dikkop the primary lesion is a toxic inhibition of biliary secretion, the photosensitization being a purely incidental consequence of this interference with hepatic function. Photosensitization of ruminants caused in this way has been termed 'hepatogenous photosensitization' and it is interesting to record that as soon as details of our work reached New Zealand, the scientists working there on Facial Eczema of sheep were able to show that in this disease, also, phylloerythrin is the actual photosensitizing agent.

The next step was to elucidate the nature of the factor causing the primary liver injury. The toxicity of *Tribulus* is fluctuating so Quin, Roets and I selected the plant *Lippia rehmanni* for the further study. Although not generally grazed by stock, it caused a typical geel-dikkop syndrome when dosed to sheep. From it we isolated a triterpene acid which was similarly active and which we named Icterogenin. The quantity present fluctuated with the season and was greatest in fresh green leaves. Icterogenin causes a hepatogenous photosensitization with jaundice but it also inhibits the movements of the alimentary canal. My colleague, Dr. West, has shown that it has anti-serotonin activity.

For further study of its hepatotoxic action, a small laboratory animal was required which would react in the same way as the sheep. Unfortunately, administration by mouth or by injection to rats, guinea pigs and rabbits was unsatisfactory and the difficulty was only overcome in 1960 when a group working with me in my laboratory in London found that we could use rabbits provided with an external biliary fistula and produce intrahepatic biliary stasis by administering icterogenin intraperitoneally at the time of operation. By this time the chemical structure of icterogenin had been fully elucidated by Barton and De Mayo and another closely related triterpene acid, rehmannic acid had been isolated from *Lippia rehmanni*.

Our team studied first the biliary excretion by normal rabbits of bilirubin, bromsulphthalein, coproporphyrin and phylloerythrin given as loading doses by intravenous injection. It was quickly obvious that the efficiency of the liver for concentrating and excreting phylloerythrin is much less than that for bilirubin — a point to which I shall return later.

We next repeated these experiments with rabbits to which icterogenin had been given some 12 hours previously. Marked retention was observed of all the test substances and again phylloerythrin retention was the most protracted.

In a complimentary investigation, Reese and I have recently studied the action of icterogenin on the liver of the rat by ultraviolet fluorescence microscopy. This elegant technique was perfected by Hanzon in a study of fluorescein excretion. The liver of the anaesthetised animal is exposed and illuminated by incident ultraviolet light, the natural fluorescence of the tissue being sufficient for recognition of the cells, bile capillaries, etc. Coproporphyrin or phylloerythrin was injected through a cannula lying in the jugular vein and within a few seconds the characteristic bright red fluorescence of the porphyrin could be seen in the blood flowing through the sinusoids.

Coproporphyrin was taken up by the liver cells, as indicated by their bright red fluorescence, within about 2 minutes and after 6 to 15 minutes its secretion into the biliary canaliculi caused them to stand out as brilliant thin red lines.

The affinity of the liver cells for phylloerythrin, on the contrary, was visibly less and only after 20 to 30 minutes did faint red fluorescence appear in the bile canaliculi.

These observations on the liver of the surviving rabbit and rat offer a plausible explanation of the fact noted by Sir Arnold Theiler and by the New Zealand workers on facial eczema that in hepatogenous photosensitization, skin lesions may appear before jaundice is noticeable. The excretory efficiency of the liver for phylloerythrin is so much less than it is for bilirubin. Prior administration of icterogenin to our rats abolished the capacity of the hepatic cells to take up phylloerythrin from the sinusoidal blood. Using sporidesmin the chemical agent responsible for hepatogenous photosensitization in facial eczema we were able to detect two stages of intoxication, the first inhibiting canalicular secretion but not uptake by the hepatic cells. After more severe intoxication, the latter activity is also lost.

By what means does icterogenin inhibit biliary excretion? This problem is not yet solved although some pertinent information has been obtained. In as yet unpublished work from my laboratory, Heikel has shown that icterogenin and related triterpenes, shortly to be mentioned, which have a similar pharmacological action, all depress oxidative phosphorylation of isolated liver cell mitochondria. Conventional histology of the livers of icterogenin-poisoned animals reveals little abnormality but Goldfischer, Arias, Essner and Novikoff, using the electron microscope, have detected changes in the morphology of the canaliculi such as vesiculation and reduction in the numbers of the microvilli. Marked changes also occur in the enzymes alkaline phosphatase, Adenosine-triphosphatase and nucleoside diphosphatase, the former associated with the cellular and intracellular membranes, the latter with the Golgi apparatus.

It is conceivable that icterogenin acts upon some specific enzyme or surface within the hepatic cell in such a way as to inhibit biliary secretion. If such was the case, much might be learned from a comparison of pharmacological activity and chemical structure within the triterpene series, just as the essential configuration of the active centre in choline esterase has been revealed by study of a series of inhibitors of this enzyme.

To this end, Dr. Brown undertook in my laboratory and at Onderstepoort the preparation and testing for icterogenic activity of 16 selected triterpenes belonging to the oleanane or 24-noroleanane series. These included 22β-angeloyloxyoleanolic acid, discovered as a minor constituent in extracts of *Lippia rehmanni* by Anderson, de Kock and Enslin and also prepared by them synthetically from rehmannic acid. It proved to be by far the most potent icterogenic material yet investigated.

The results of this study, just published in the Proceedings of the Royal Society by Brown, myself and Sawyer, are not easy to describe without an extensive preliminary chemical background but in summary it may be said that a surprisingly high degree of structural specificity was found to be associated with icterogenic action, supporting the view that a specific enzyme or surface structure participates in biliary secretion. Thus, it was shown that for inhibition to occur 2 hydroxyl groups are essential, the one at position 22 being esterified by angelic acid whilst the other could be either at position 3 or 24.

When placed at position 3, two geometrically different isomers become possible, depending upon whether the group is placed equatorially or axially with respect to the pentacyclic ring structure. Both isomers were prepared and tested, the equatorial one being highly active, the axial one having no icterogenic activity whatsoever. The pharmacological specificity thus extends as far as the spatial orientation of the relevant parts of the molecule.

All this work has been done with materials derived from *Lippia rehmanni* and its relevance to *Tribulus* poisoning must be considered. *Tribulus* is rich in saponins, glycosides of triterpenes, but so far no material similar to icterogenin has been isolated from them. Work is still proceeding on the problem under Dr. Enslin, but in the meantime Dr. Brown has shown at Onderstepoort that an entirely new factor may enter into the development of Tribulosis ovium. This is the abundance of selenium in the pasturage. The discovery may throw a new light upon the distribution and occurrence of the disease and will certainly open a new chapter in its investigation. I can not, however, at the present time do more

than indicate the latest and quite unexpected turn which events have taken.

In my introductory remarks to this lecture, I mentioned that Sir Arnold Theiler had also been implicated in the discovery in South Africa of the first living cases of Congenital Porphyria in animals. The scene was Onderstepoort. Sir Arnold had called a few of us to have a look at some specimens of chocolate-coloured bovine bone sent up by a Veterinary Surgeon in Swaziland at the instigation of Dr. Fourie. Sir Arnold briefly recounted their history and called for comments. The only similar material in human pathology was the pigmented bones and teeth of the very rare genetic abnormality 'Congenital Porphyria' or Günther's disease. In this, pigmentation is due to uroporphyrin and I asked Sir Arnold for some bone to carry out chemical tests. Uroporphyrin was easily extracted and characterised as the crystalline octamethyl ester so there could be no doubt that this was indeed a bovine case of congenital porphyria.

Dr. Fourie and I equipped ourselves as a miniature travelling laboratory and drove down to the farm in Swaziland. There we found several living animals with the disease, one of which we killed bringing back practically everything but the hide to be worked up chemically at Onderstepoort. This went on during the next 3 or 4 months and in the meantime the bull and some of the females were acquired by Onderstepoort for controlled breeding experiments. Unfortunately, the breeding records at the farm were not adequate to establish the mode of inheritance. Two bulls had been used, both derived from the same Shorthorn stock and careful inspection of their pedigrees revealed a blood relationship. The transmission of the disease as a Mendelian recessive character was finally established with a very high degree of probability by the selective breeding experiments which Fourie carried out at Onderstepoort.

Shortly after the discovery of congenital porphyria in the grade Shorthorn herd, another case was found, this time in a Friesland cow, Cedara Ria 4th, by Fourie and Rimington and 2 cases in Frieslands by Flight. The latter were sired by a pedigree bull 'Kamnatie Charles' out of his own daughters and some blood relationship between him and the Cedara case seemed possible. They did indeed prove to have common ancestry in a bull Albert 1306^{H} who was probably a carrier. Kamnatie Charles inherited Albert 1306^{H} blood on his mother's side through both sire and dam. In the case of Cedara Ria 4th his blood came through the paternal side. Her mother was descended from an unregistered Friesland-type cow 'Angel' of the Stellenbosch-Elsenburg College of Agriculture where records show that she was unthrifty and was eventually destroyed. She may have been a carrier or a case of congenital porphyria. It will be seen that in no case was a recessive mode of inheritance of the disease as yet unequivocal. Final proof of the Mendelian recessive character came through cases found in England in 1957 by Amoroso, Loosmore, Rimington and Tooth. Together with cases reported from America, Jamaica and Denmark, a total of 49 bovine cases are now known, in all of which inheritance is compatible with Mendelian recessive transmission.

As in the human disease, photosensitisation is one of the most marked symptoms in bovines. The biochemical error lies, in this case, in the bone marrow where excessive quantities of uroporphyrin and coproporphyrin belonging to the isomeric series I are produced. This is an abnormal isomer which the body cannot use and the pigments are either excreted or deposited in the teeth and bones. The visible discolouration in the teeth led Fourie to name the disease in cattle "pink tooth".

As early as 1936 when we were investigating our first living case, I put forward an enzymic theory of haemopoiesis which suggested that in the condensation of precursor molecules to form the macrocyclic porphyrin ring, one of the two isomeric possibilities was favoured by enzymic catalysis, and served for haemoglobin and cytochrome production, etc., the other, formed in relative traces as a by-product, being excreted in urine and faeces. In congenital porphyria, it was assumed that catalysis has broken down and more or less equal quantities of the two isomers then result from the biosynthesis. Modern work has established the essential correctness of this hypothesis. There is a factor, uroporphyrinogen isomerase, which has to be present for formation of the physiological III series isomers to be possible. In its absence, the unutilizable I series

porphyrins are formed. One can transform a normal red cell haemolyzate by simple heating to 65° into a system indistinguishable from that prepared from congenital porphyria blood. The uroporphyrinogen isomerase is in this case destroyed by heat; in congenital porphyria it is partially or completely lacking from birth.

Coproporphyrin, and especially uroporphyrin, are very potent photosensitizers. In congenital porphyria they are present in quantity in the superficial tissues and cause very severe lesions when these are exposed to sunlight.

Although other hepatic forms of porphyria have been known for some time, congenital porphyria in man and bovines was the only form, due to a haemopoietic disturbance, familiar to medicine until 1961. In this year I and colleagues in London described a young man who since childhood had suffered from hypersensitivity to sunlight. He reacted with erythema and delayed oedema but had none of the scars or mutilations seen in congenital porphyria. His urinary porphyrins were grossly raised. Examination of his blood revealed nearly 100 times the normal amount of protoporphyrin and coproporphyrin in his red blood cells, which were brilliantly fluorescent under the ultra-violet fluorescence microscope. The protoporphyrin, which dominated the picture, belonged to the normal isomeric series, so there was no isomer defect as in congenital porphyria and difference from this disease was further emphasised by the complete lack of any increase in uroporphyrin, either of series I or III. It was evident that we had encountered a new porphyria syndrome and to it we gave the name 'Erythropoeitic Protoporphyria'.

It has since been recognised in America and Sweden and we ourselves have recorded it in a further 9 cases in 3 families. It appears to be inherited as a Mendelian dominant character and will probably prove to be much less rare than congenital porphyria. The photosensitivity differs symptomatically from that of congenital porphyria and this we can only attribute to the different porphyrin involved and perhaps also to its different and rather specialised location, namely for the most part within the red cell.

In this lecture, I have attempted to survey topics in which Sir Arnold Theiler was interested and with which he was particularly concerned. The study of Tribulosis ovium has led to the recognition of hepatogenous photosensitisation in ruminant animals. The primary hepatic lesion may be brought about by different agencies but the mechanism of the photosensitisation is always the same, the accumulation of the chlorophyll porphyrin, phylloerythrin, in the plasma as a result of its inadequate concentration and excretion by the liver. Experiment has shown that the efficiency of the liver for the biliary excretion of phylloerythrin is normally much lower than that for bilirubin, which explains the observation made by Sir Arnold Theiler and others that photosensitisation may be marked before jaundice appears. Chemical studies of the triterpenes have given us a valuable tool for the further study of the mechanism of biliary secretion and with the discovery of the role of selenium in Tribulus poisoning, a new and wider view of the pathogenesis of geeldikkop is opening up.

The work started at Onderstepoort on the historic cases of bovine congenital porphyria has been followed by further reports of this disease in cattle from different parts of the world and by the recognition of a new form of erythropoietic porphyria in man.

Sir Arnold and Lady Theiler were with me and my wife in Norway only some few days before his tragically sudden death in London where he had travelled to attend the 2nd International Microbiological Congress of which he was Vice-President. We had planned to meet again there. He was tired after many scientific visits in Europe and several times remarked upon the peacefulness of our beautiful surroundings.

In remembering Sir Arnold Theiler for his personal qualities and for what he has left in achievement and inspiration, let us be certain that he would rejoice with us today to know that his Onderstepoort Veterinary Research Laboratory still worthily carries on the high traditions of its Founder.

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TOXAPHENE* AND LINDANE RESISTANCE IN *RHIPICEPHALUS APPENDICULATUS*, THE BROWN EAR TICK OF EQUATORIAL AND SOUTHERN AFRICA

by

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SUMMARY

A number of *in vitro* and *in vivo* tests have been carried out, which indicate that strains of *Rhipicephalus appendiculatus*, the brown ear tick of Africa, have developed resistance to toxaphene and gamma-BHC. In most cases this appears to have been induced by sustained treatment of the strain with toxaphene-based dips, but in many cases gamma-BHC treatments had been carried out before or between periods of toxaphene treatments, and it is suggested that gamma-BHC treatments assisted in the selection process. The results of this work and that of other workers quoted, indicate that there is linkage between toxaphene and gamma-BHC resistance in several species of ticks.

The toxaphene and gamma-BHC resistant strains were not cross-resistant to the organophosphorus insecticides Delnav* and Supona.+

INTRODUCTION

The brown ear tick, *Rhipicephalus appendiculatus* (Neumann), is a serious parasite of cattle in the wetter areas of equatorial and southern Africa. It is a three-host tick which also parasitizes goats, sheep, horses, mules, donkeys, dogs and numerous species of wild animals. According to Hoogstraal[1] it reaches its northern limits in southern Sudan, in Ethiopia and between the Congo and West Africa. In South Africa its distribution south of Pretoria is confined mainly to the eastern sector of the country and it is here, in the coastal areas of the Eastern Cape Province that some of the most abundant infestations of this tick are found.

The chief environmental requirements of *R.appendiculatus* are adequate rainfall and vegetative cover since this tick has a high humidity demand. In South Africa, a minimum annual rainfall of 15 in. per annum will support this tick provided there is sufficient vegetational cover[2]. In the higher temperatures of Kenya, 20 in. or more is necessary with equivalent vegetation[3].

The importance of *R.appendiculatus* as a parasite rests on two aspects of its parasitism, namely the favoured attachment site of the adult on the ears of cattle, and its transmission of *Theileria* spp. the causal organisms of East Coast Fever and related diseases. Larvae and nymphae of the tick may become infected with one of these organisms after feeding on an infected beast and transmit the infection to their next bovine host.

Adult ticks attaching within the ear are difficult to control, because it is possible for cattle to pass through either a dip bath or spray-race without getting the insides of the ears totally wetted and it is only by hand-dressing with the wash or an appropriate grease or oil that proper

*Toxaphene and Delnav. Registered Trade Markes of Hercules Power Company, Wilmingtong, U.S.A.
+Supona. Registered Trade Mark of Shell.

control of severe ear tick infestations can be gained. Contributing to this difficulty is the conformation of the ear with its protective fringe of hair and its bare inner surface which does not retain insecticide to kill or affect newly infesting ticks. The residual protection in the ears is therefore never very good.

Being a quick-feeding tick in the adult stage, *R.appendiculatus* can only be well controlled by a good residual insecticide applied at weekly intervals. It is possible for an adult female to attach and engorge within five days and to transmit a theilerial infection after three days attachment. In theory, therefore, at least four days residual protection is required under a weekly treatment routine and two days under a five-day interval treatment routine, to prevent disease transmission. Under conditions of heavy challenge no known insecticide will give four days protection against this tick and five-day interval treatment may be resorted to in areas, such as the Kenya highlands, where *Theileria parva* is a threat and the tick challenge may be heavy.

Several insecticides, notably arsenic and gamma-BHC, will give a good immediate kill of *R.appendiculatus*, but of those in common use today only toxaphene has, in addition, the residual properties needed to control this tick adequately. Toxaphene is used widely in Africa, chiefly for the control of multi-host ticks, but it is also effective against single host ticks of the genus *Boophilus*, where these ticks are not resistant.

While the one-host ticks, of which *Boophilus* is the only economically important genus, have become resistant to many types of insecticide, the only recorded resistance amongst two-host ticks is that of *Rhipicephalus evertsi* to toxaphene in several parts of Africa[4]. Among the three-host ticks there is only one recorded instance of resistance, that of the brown dog tick, *R.sanguineus*, to chlordane in New Jersey, U.S.A.[5]

The bionomics of one, two, and three host ticks make it likely that, other things, such as treatment practices, being equal, one-host ticks will become resistant to a particular insecticide more quickly than two-host ticks, which in turn will become resistant more quickly than three-host ticks[6]. This very broad generalization has turned out to be true in the field of cattle tick control, the final step of three-host tick resistance having been accomplished by the toxaphene/gamma BHC resistant strains of *R.appendiculatus* reported here.

Materials and Methods

(i) *Test procedures*

Comparative laboratory tests on larvae, nymphae and adults of a number of strains have been carried out using immersion methods of application. In addition an *in vivo* spraying trial was carried out on two of the strains. The details of the methods used in these tests are given below:—

a) Larvae.

Larvae, 14 to 21-days old, bred from engorged females in the laboratory are immersed in a series of concentrations of insecticidal wash. During the immersion period of 10 mins. the larvae are held between two filter papers immersed in the wash in a petri dish. The larvae are then taken from the wash, the excess moisture is removed by drying on filter paper and the larvae are stored at 80% r.h. and 26 to 27°C (80°F) for 17 to 18 hours after which mortality is assessed. The details of the technique are described by Shaw (in press).

The larval mortality counts for each concentration are based on at least two replicates. The data obtained are analysed by a Ferranti Mercury Computer using a probit analysis programme.

b) Nymphae and Adults

Engorged nymphal and adult female ticks are manually removed from untreated animals for *in vitro* tests. After immersion for 60 seconds in the concentration under test in a beaker, the nymphae or adults are dried on blotting paper whilst exposed to a stream of warm air. They are then placed in individual glass tubes and incubated at 26 to 27°C (80°F) and 80% + r.h. Observations are made of egg laying and larval hatching with the adult ticks and of adult emergence with the nymphae.

c) *Field sprayings*

Groups of two cattle are thoroughly hand-sprayed with the respective treatments employed after initial tick counts have been made. The pre-and post-treatment tick numbers are ascertained by counting the number of live adult *R.appendiculatus* on the inner and outer surface of both ears of each of the cattle in the group. The ticks are also classified according to their degree of engorgement.

(ii) *Sources of tick strains*

The tick strains used in these comparative tests were obtained from several sources. Those named Riverdale, Brooklyn, Wiltonside, Tayside, Caprice and Ferndale were collected from properties of these names in the East London district of the Eastern Cape Province, South Africa. That named Hekpoort was collected on a property in the Hekpoort district of the Transvaal, South Africa. The WRL strain was obtained from the Wellcome Research Laboratories, Kabete, Kenya. All these strains, except the last, have been subjected to routine insecticidal treatments in the field over a number of years. Their histories will be discussed later. The WRL strain has been bred at the Wellcome Laboratories for several years, but was originally obtained from the East African Veterinary Research Organisation, Maguga, Kenya and has had occasional infusions of additional numbers from this source. It is assumed that it has never been exposed to insecticidal treatments and certainly not to any of the more recent synthetic insecticides.

Larvae of all these strains except the Caprice and Ferndale strains have been tested for susceptibility to toxaphene, gamma-BHC and Delnav.

Engorged nymphae of the Riverdale and WRL strains have been tested for susceptibility to toxaphene and gamma-BHC and engorged females of the Riverdale, Brooklyn, Tayside, Caprice, Ferndale and WRL strains tested for susceptibility to toxaphene and gamma-BHC and in the case of the Riverdale and WRL strains to Delnav and Supona.

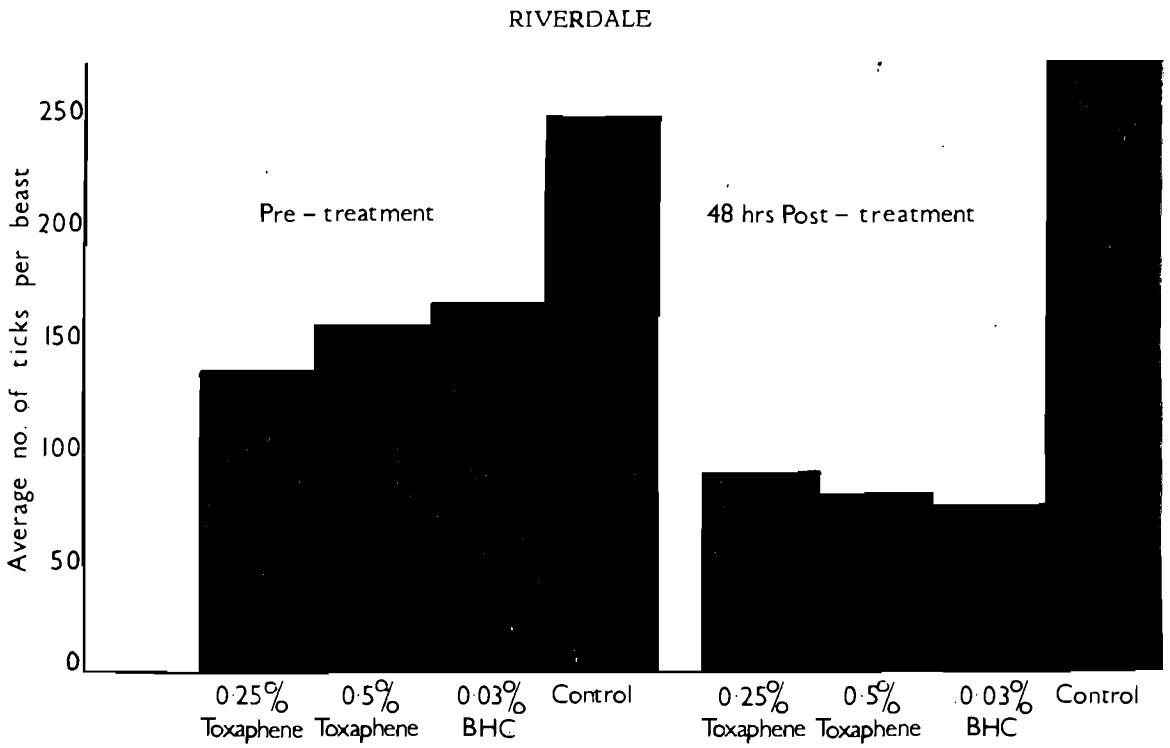

Fig. 1. — A comparison of the counts of *R. appendiculatus* adults on the ears of groups of cattle at Riverdale before and 48 hours after treatment with 0.25% toxaphane, 0.5% toxaphene and 0.03% gamma-BHC.

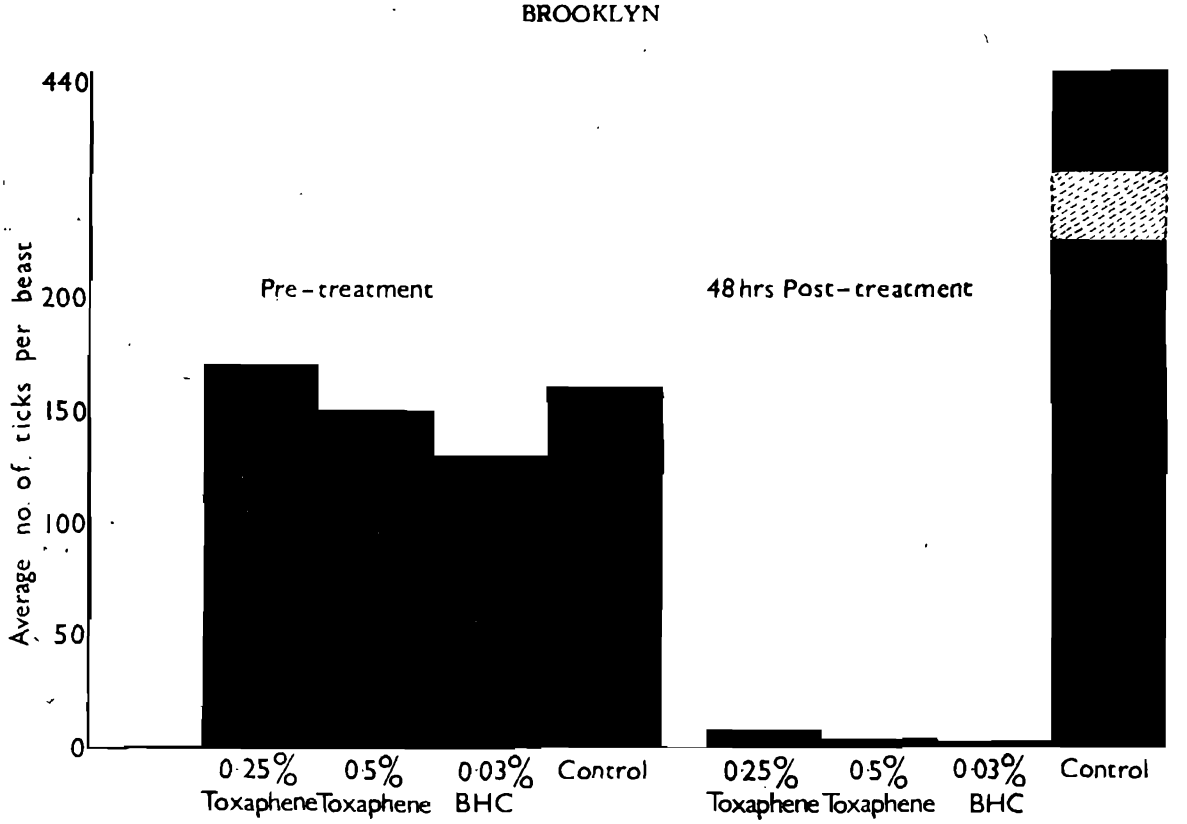

Fig. 2. — A comparison of the counts of *R. appendiculatus* adults on the ears of groups of cattle at Brooklyn before and 48 hours after treatment with 0.25% toxaphene, 0.5% toxaphene and 0.03% gamma-BHC.

(iii) *Insecticides*

The insecticidal materials used in these tests were as follows:—

1. A 75% w/v water miscible concentrate of toxaphene: Octachloro-camphene.
2. A. 20% w/v water miscible concentrate of gamma-BHC (lindane): gamma-1,2,3,4,5, 6-hexachloro cyclohexane.
3. A 30% w/v water miscible concentrate of Delnav (dioxathion): 1,4-dioxan-2,3-diyl bis-(0 0-diethyl phosphorodithioate).
4. A 20% w/v water miscible concentrate of Supona (chlorfenvinphos): 2-chloro-1-(2,4-dichlorophenyl)-vinyl diethyl phosphate.

Results

a) In vitro tests

(i) *Larvae*

The larval results are expressed as the LC 50 (estimate of dose giving 50% kill of the population) in terms of the percentage concentration of the insecticide in the wash. Also given are the 95% confidence limits of the LC 50 and the slope of the log. dose/probit mortality regression line. The figures for the other strains are compared with those for the susceptible WRL strain, and factors of resistance compared with this strain, together with their significance, are given in Tables 1–3. All the other strains tested were less susceptible than the WRL strain to the two chlorinated hydrocarbon materials, toxaphene and gamma-BHC, but there was no sig-

nificant difference in the susceptibility of any of the strains, apart from that from Tayside, to the organophosphorus compound, Delnav.

Larvae from Hekpoort, Tayside and Wiltonside all showed considerable resistance to toxaphene, but there was a marked difference in the heterogeneity of the population in these three strains as indicated by differences in the slopes of the regression lines. Larvae from Riverdale were less markedly resistant to toxaphene and this contradicts the evidence from Riverdale engorged nymphs and engorged females and the field sprayings. The Riverdale larvae used in this test were the progeny of a very few females, which were possibly not a representative sample of the population. The Brooklyn larvae were also less highly resistant to toxaphene.

Larvae of all the strains showed low level resistance to gamma-BHC, least in the case of the Brooklyn and Hekpoort larvae, where heterogeneity was low, and of the Tayside larvae.

(ii) *Engorged nymphae*

The engorged nymphal results in terms of the percentage failure of adult emergence are expressed as the percentage mortality at each concentration and these are given in tabular form in Tables 4–5.

Engorged nymphae of the Riverdale strain were resistant to both toxaphene and gamma-BHC in comparison with those of the susceptible WRL strain.

(iii) *Engorged females*

The engorged female results in terms of the percentage failure to lay viable eggs are expressed as the percentage mortality at each concentration and these are also given in tabular form in Tables 6–7.

Engorged females of the Tayside and Caprice strains were both highly resistant to toxaphene and gamma-BHC, as were those of the Riverdale and Ferndale strains to a lesser degree, but the resistance in the Brooklyn strain, as with its larvae, does not appear to be of a high level. There is little difference between the W.R.L. and Riverdale strains in their susceptibility to the organophosphorus compounds Delnav and Supona, the latter exhibiting a very high level of activity against both strains.

Tables 1–3. *Larvae.* A comparison of the susceptibility of larvae of six strains of *R.appendiculatus* to toxaphene (Table 1), gamma-BHC (Table 2), and Delnav (Table 3).

TABLE 1.—TOXAPHENE

Strain.	LC 50 (%)	95% Fiducial Limits.	Slope.	Factor of Resistance	Significance at $P \leqslant 0.05$.
WRL	0.039	0.052–0.031	1.10		
Brooklyn	0.12	0.15 –0.094	1.10	3.1	Significant
Riversdale	0.097	0.13 –0.059	1.04	2.5	Significant
Hekpoort	0.56	–0.20	1.00	14	Significant
Tayside	0.31	0.47 –0.19	0.51	8	Significant
Wiltonside	0.43	2.14 –0.28	0.56	11	Significant

TABLE 2.—GAMMA-BHC

Strain.	LC 50 (%)	95% Fiducial Limits.	Slope.	Factor of Resistance	Significance at $P \leqslant 0.05$.
WRL	0.00017	0.00021–0.00012	4.20		
Brooklyn	0.00037	0.00039–0.00035	3.80	2.2	Significant
Riverdale	0.00062	0.00095–0.00041	0.51	3.6	Significant
Hekpoort	0.00031	0.00036–0.00026	6.10	1.8	Significant
Tayside	0.00034	0.00049–0.00017	0.90	2.0	Not Significant
Wiltonside	0.00049	0.00081–0.00021	1.05	2.9	Significant

TABLE 3.—DELNAV

Strain.	LC 50 (%)	95% Fiducial Limits.	Slope.	Factor of Resistance	Significance at $P \leqslant 0.05$.
WRL	0.00049	0.00058–0.00041	6.3		
Brooklyn	0.00040	0.00044–0.00036	6.3		
Riverdale	0.00043	0.00045–0.00041	5.1		
Hekpoort	0.00041	0.00055–0.00029	3.5		
Tayside	0.00073	0.00084–0.00067	4.1	1.5	Significant
Wiltonside	0.00042	0.00044–0.00040	5.9		

TABLES 4–5.—ENGORGED NYMPHAE

A comparison of the percentage failure of adult emergence of engorged nymphae of two strains of *R. appendiculatus* when exposed to a series of concentrations of toxaphene (Table 4) and gamma-BHC (Table 5)

TABLE 4.—TOXAPHENE

Percentage Concentration	WRL strain		Riverdale strain	
	No. treated	% mortality	No. treated	% mortality
0.05	25	100	250	55
0.10	25	100	150	37
0.125			100	44
0.2			100	75
0.25	25	100	150	35
0.5			200	53
2.0			30	73
Control	20	0	540	12

TABLE 5.—GAMMA-BHC

Percentage Concentration	WRL strain		Riverdale strain	
	No. treated	% mortality	No. treated	% mortality
0.001	25	88		
0.002	25	100		
0.005	25	100		
0.006			100	20
0.01	27	100		
0.015			100	47
0.02	27	100		
0.03			150	53
0.06			50	62
0.12			50	83
Control	32	6	540	12

TABLES 6–8.—ENGORGED FEMALES

A comparison of the percentage failure to lay viable eggs of engorged females of six strains of *R.appendiculatus* when exposed to a series of concentrations of toxaphene (Table 6), gamma-BHC (Table 7) and of two of these strains when exposed to Delnav and Supona (Table 8)

TABLE 6.—TOXAPHENE

Percentage Concentration.	WRL		RIVERDALE		BROOKLYN		TAYSIDE		CAPRICE		FERNDALE	
	No. treated.	% mortality.	No. treated.	% mortality.	No. treated.	% mortality.	No. treated.	% mortality.	No. treated.	% mortality.	No. treated.	% mortality.
0.01	15	7										
0.02	25	4										
0.05	26	42										
0.10	25	40										
0.25	25	80	70	23	50	60	20	0	20	0	50	32
0.50			70	41	50	74	20	50	20	5	50	62
1.0			70	43	50	98	20	45	20	5	50	64
2.0			58	45	50	94	20	45			50	80
4.0			64	50	50	100					40	83
6.0			62	53							50	86
8.0			62	71							50	88
Control	15	0	107	2	20	0	20	5	10	0	112	0

TABLE 7.—GAMMA-BHC

Percentage Concentration.	WRL		RIVERDALE		BROOKLYN		TAYSIDE		CAPRICE		FERNDALE	
	No. treated.	% mortality.	No. treated.	% mortality.	No. treated.	% mortality.	No. treated.	% mortality.	No. treated.	% mortality.	No. treated.	% mortality.
0.001	15	0										
0.002	22	32										
0.005	22	86										
0.01	22	96										
0.03			50	46	50	64	34	56	20	10	51	51
0.06			49	53	50	96	34	70	20	25	51	84
0.12			50	52	20	100	34	68	20	30	50	84
0.24			50	48	30	90	34	79			50	94
Control	15	0	107	2	26	0	20	5	10	0	112	0

TABLE 8.—DELNAV AND SUPONA.

Percentage Concentration	DELNAV				SUPONA			
	WRL		RIVERDALE		WRL		RIVERDALE	
	No. treated	% mortality	No. treated	% mortality	No. treated	% mortality	No. treated	% mortality
0.001					17	94		
0.00125							50	86
0.002					12	100		
0.0025							50	90
0.005	12	0	43	2	17	100	76	97
0.01	17	18	80	13	17	100	46	100
0.02	17	47	92	7				
0.05	17	77	92	40				
0.1	17	94	44	70				
0.2			50	88				
Control	15	0	107	2	15	0	107	2

b) Field sprayings

The levels of resistance noted in the *in vitro* tests on the Riverdale and Brooklyn strains, high in the Riverdale strain and low in the Brooklyn strain, are confirmed by the detailed observations carried out after the *in vivo* spraying of infested cattle on the two properties. The degree of control exercised by 0.25% and 0.5% toxaphene and 0.03% gamma-BHC, assessed by examinations of the ears 48 hrs. after treatment, was poor on the Riverdale property and good on the Brooklyn property. The results of these detailed observations are expressed in the histograms, Fig. 1 & 2. These show the levels of infestation of adult *R.appendiculatus* in the ears of groups of two cattle before treatment and 48 hours after treatment. On the Riverdale property all the treatments resulted in a reduction in infestation compared with the control group, but the effect was well below the level expected from toxaphene or gamma-BHC at these concentrations against susceptible ticks and would be totally unsatisfactory. All the treatments, however, gave good control over the Brooklyn strain.

At Riverdale, treatment with 0.25% toxaphene, 0.5% toxaphene and 0.03% gamma-BHC gave reductions in tick numbers of 34%, 49% and 55% respectively compared with pre-treat ment counts. Of the ticks present at the 48hrs. count, 18%, 16% and 17%, respectively were partially or fully engorged, indicating that they had probably been present at the time of treatment.

The corresponding figures for the Brooklyn treatments are (i) reduction in numbers: 97%, 98% and 98%, (ii) partially or fully engorged ticks present: one to two ticks per group.

Discussion

There is a good deal of evidence from other tick species, notably *Boophilus decoloratus*[7], *Boophilus microplus*[8], and *Rhipicephalus evertsi*[4] that resistance, where present in a strain, occurs in both larvae and adults. This appears to be true of the two strains of *R.appendiculatus* tested here as larvae and engorged females, the Riverdale and Tayside strains, both of which are resistant to toxaphene and gamma-BHC in each of these stages. In addition, the Riverdale nymphae are resistant to both these insecticides. It is, therefore, reasonable to assume that both these strains together with the Hekpoort and Wiltonside strains, which were only tested as larvae, and the Caprice and Ferndale strains, which were only tested as engorged females, are resistant in all three stages to toxaphene and gamma-BHC. The Brooklyn strain is less resistant to toxaphene and gamma-BHC than any of these.

The difference between the Riverdale and Brooklyn ticks, in the laboratory, is confirmed by the field sprayings in which toxaphene at 0.25% and 0.5% and gamma-BHC at 0.03% barely checked the Riverdale ticks, but exercised good control over the Brooklyn ticks. The low level resistance of the Brooklyn ticks noted in the laboratory was barely discernible in the field. Resistance may, therefore, be building up in the field for some time before it is noticed in terms of poor practical tick control.

There was little difference between the susceptibilities to Delnav of the larvae of any of the strains tested, except that from Tayside, where a composite Delnav/toxaphene or gamma BHC wash had been used for five years. There was a slight difference between the susceptibilities of the engorged females of the Riverdale and WRL strains to Delnav, but not to Supona. This indicates that the toxaphene and gamma-BHC resistant strains are not specifically cross-resistant to either of these organo-phosphorus types.

gamma-BHC was introduced into South Africa in 1947, but its use was curtailed by resistance to it in the *Boophilus* genus and by the introduction of toxaphene in 1951. Both these insecticides have, however, been used extensively for tick control throughout Africa since their introduction, although in recent years the use of gamma-BHC has declined and that of toxaphene increased.

The history of insecticide usage on these properties is known fairly accurately in some cases. The Riverdale property has used a wide variety of insecticides since 1946 when the use of arsenic was discontinued following *Boophilus* resistance to it. These include gamma-BHC, DDT, toxaphene, Sevin, and Delnav, with extensive pe-

riods of use of both gamma-BHC and toxaphene. Toxaphene has been used continuously at Tayside either alone or in a composite wash since 1955, with a short break in 1959–60 when a Delnav/gamma-BHC dip was used. At Hekpoort a toxaphene spray has been in use for five years, with gross tick infestations controlled by gamma-BHC or dieldrin greases. Prior to that, hand spraying of gamma-BHC was used on the property. At Brooklyn the principal dip used for a number of years was arsenic with occasional additions of gamma-BHC.

It is impossible to determine with certainty how the resistance to toxaphene and gamma-BHC was induced in these strains. The use of gamma-BHC usually preceded that of toxaphene and was often discontinued within one to three years because of *Boophilus* resistance to it. gamma-BHC may, however, have begun the selection process, which was continued by the later use of toxaphene until toxaphene resistance linked with gamma-BHC resistance developed. It is very unlikely that these two resistances are unrelated, although there does not appear to be very good correlation between the levels of resistance to toxaphene and gamma-BHC noted in the larvae of the various strains. This, however, does not apply to the nymphal and engorged female figures and is probably due to inadequate sampling of the adult population used in production of larvae for these tests.

There are previous reports of species acquiring resistance to toxaphene and gamma-BHC following the use of these insecticides in the field. Norris & Stone[9] reported toxaphene resistance in *Boophilus microplus*, but pointed out that this resistance was noted after a history of gamma-BHC resistance. They suggest that the toxaphene resistance was cross-resistance induced by gamma-BHC selection.

Whitehead[7] reported gamma-BHC and toxaphene resistance in two strains of *Boophilus decoloratus*, one (Ferndale ticks) selected with gamma-BHC and the other (Frankenwald 1958 ticks) selected with toxaphene. Whitehead & Baker[4] reporting toxaphene, gamma-BHC and dieldrin resistance in *Rhipicephalus evertsi* following four years of toxaphene use on the property concerned, Tayside, suggest that the use of gamma-BHC for two years, six years before the introduction of toxaphene "may have contributed to the selection of a population resistant to the chlorinated cyclic hydrocarbon group of insecticides". The evidence of these reports and of the present instance of *Rhipicephalus appendiculatus*, suggest that in all these species toxaphene and gamma-BHC resistance are completely or partially linked.

Engorged females of some of the strains mentioned here were also exposed to dieldrin. There was considerable variation in the response indicating the probability of resistance, but since it was not possible to expose the susceptible WRL strain to dieldrin, the results of these tests are not given. Dieldrin may have been used periodically on several of these properties as an ear and tail grease for controlling severe multi-host tick infestation, but so far as is known it has not been used as a dip or spray.

It was not possible to explore the resistance spectrum of these strains beyond the two organophosphates, Delnav and Supona. The susceptibility of all the strains tested, apart from the Tayside strain, to both these materials suggests that the organo-phosphorus group will prove a useful source of insecticides for brown tick control, provided materials with sufficiently good residual properties can be found. Resistance to organo-phosphorus insecticides, which exists in *B.microplus*[10] does not appear to involve universal cross-resistance within the group and this absence of a specific resistance mechanism effective against all organo-phosphates gives rise to the hope that the group will yield a succession of effective insecticides.

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CANINE BABESIOSIS: A REPORT ON THE PATHOLOGY OF THREE CASES WITH SPECIAL REFERENCE TO THE 'CEREBRAL' FORM

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SUMMARY

The literature on cerebral babesiosis in the dog is summarised and the macroscopic and microscopic pathology of two such cases are described. The distribution of accumulated parasitized erythrocytes in vessels of the brain, eyes and periorbital tissues is given. In addition to these previously described sites, clumping of parasitized red blood cells is reported for the first time in the spleen, kidney, skeletal musculature, intestine and lymph nodes. Bilateral thrombosis of the ophthalmic veins, necrosis of the reaction centers of the lymphoid tissues, localized necrosis of the skeletal muscles and lesions in the brain are recorded. The possible pathogenesis of the lesions is discussed.

It is suggested that cerebral babesiosis in the dog be defined as an atypical manifestation of babesiosis which is characterized by the following: nervous symptoms; sludging of parasitized erythrocytes in the smaller vessels and capillaries of the brain and referable lesions.

INTRODUCTION

In spite of the recognition of canine babesiosis as a disease entity for more than half a century and despite an otherwise voluminous literature, only a few brief reports on the so-called 'cerebral' form of the disease are available. The object of this article is to describe the macroscopic and microscopic appearance of lesions observed in the brain and other organs in two such cases of babesiosis in dogs and an additional case which is not regarded as a cerebral form.

Parant[1] in France, described an acute fatal case of babesiosis in a dog which had shown nervous symptoms. This report appears to be the first record of cerebral babesiosis in the literature. Other French workers[2, 3, 4] later mentioned the occurrence of nervous symptoms in babesiosis in dogs.

Purchase[5] described three cases of cerebral babesiosis in dogs, which he encountered over a period of five years in Kenya. A hyper-acute case reported by Piercy[6] also from Kenya, died suddenly without showing any symptoms whatsoever. This case was regarded primarily as cerebral babesiosis. Numerous parasites and haemorrhages were found in the brain. In an article on atypical symptomatology of *Babesia canis* infection in South Africa, Malherbe and Parkin[7], described cases showing nervous symptoms.

In these references there is little said about the histopathology of the brain other than calling to attention the accumulation of parasitized erythrocytes in the capillaries.

Reusse[8], drew attention to the histopathology of the brain in 3 cases showing nervous symptoms. They were the only ones out of 21 experimental cases of canine babesiosis to show symptoms referable to the central nervous system.

MATERIAL AND METHODS

Specimens of various organs from two cases were collected for histopathological study and fixed in 10% formalin. Brains from the cases reported here were fixed in a large volume of 10% formalin. Coronal cuts were made and the slices of fixed brain were examined closely for gross lesions. Suspicious areas were selected and embedded in paraffin wax. Sections were cut at 3 μ thickness and stained with haematoxylin-eosin as well as Giemsa.

History

Case A

A three year old Boxer dog was presented for post mortem with a history of sudden death. No gross pathological changes indicative of babesiosis were recognized. The diagnosis, however, was made on examining bloodsmears which revealed a severe parasitaemia with the typical pear-shaped organisms.

Case B

A three year old French Poodle, showing violent convulsions, was examined by a private practitioner. Numerous *Babesia canis* were present in a blood smear taken from this animal. The patient died thirty minutes after treatment for babesiosis and specimens of kidney, liver, spleen and brain were subsequently submitted for examination in 10% formalin.

Case C

An Irish Terrier cross of between two and three months of age died after an illness lasting five days. The owner noticed loss of appetite, vomition, depression and languor. No convulsions were noticed. In films made from the peripheral blood large numbers of *Babesia canis* were seen.

Pathology

Macroscopic

Case A

On external examination of the carcass the only significant change observed was a marked bilateral chemosis. General congestion and cyanosis without any apparent signs of anaemia or icterus were noticed. At autopsy the following were observed in the thorax: a mild degree of pulmonary oedema and emphysema; slight hydropericardium; subepi- and subendocardial petechiae and haemorrhages on the base of the pnlmonary artery. In the abdominal cavity the only striking changes were enterorrhagia and a mild splenomegaly. In addition the spleen showed elevated roundish haemorrhagic areas about 0.1 to 1.0 cm. in diameter. On the cut surface these areas had a roughly pyramidal shape with the base facing the capsule. Advanced decomposition of the liver was apparent.

With the exception of the chemosis, the most striking feature of the post mortem was found in the skeletal musculature, *viz.* bilateral focal disseminated haemorrhagic areas. The muscles mainly affected were the flexors of the fore and hind legs: teres major, coracobrachialis, triceps, biceps brachii and the quadriceps group — more particularly the rectus femoris. In some areas these haemorrhages were confluent, forming large haemorrhagic patches in association with oedema of the intermuscular connective tissue. Haemorrhages were also present in the diaphragm and intercostal muscles.

The brain was very congested with haemorrhagic foci in the cortical grey matter of the cingulate gyrus, g. proreus, g. suprasylvius (post.), sulcus cruciatus, s. sylvius, the anterior and posterior fissurae rhinalis and bilaterally in the pyriform lobes. On the cut surface of the fixed brain large roughly bilaterally symmetrical haemorrhages were also observed in the dorsolateral area of the caudate nucleus (Fig. 2a).

Case B

Numerous haemorrhagic areas were seen in the cerebral cortex, their distribution being from the frontal to occipital poles of both hemispheres. These areas were mainly confined to the sulci, affecting the cortical grey matter of opposing gyri and extending into the depth of the sulci (Fig. 1e). The following sulci were affected: presylvius (deep end), coronalis suprasplenialis, suprasylvius med. and ectosylvius. On the cut surface haemorrhagic areas were also found in the region of the dentate nucleus. Intense congestion of the meningeal vessels was present.

Case C

On post mortem examination the typical picture of babesiosis was seen to be present with anaemia, jaundice and a tumor splenis as prominent features. No macroscopic changes were seen in the brain.

Fig. 1.

(A) Vein in caudate nucleus with cell infiltration in V.R. space. H.E. x 190.

(B) Large vein in deep white substance of cerebellum showing pavementing by parasitized erythrocytes. H.E. x 480.

(C) Skeletal muscle with hyaline necrosis, myolysis, haemorrhage and oedema. (Case A.) H.E. x 90.

(D) Small artery in caudate nucleus with leucostasis and haemorrhage into the V.R. space. Small haemorrhages in the brain substance and oedema of the brain can also be seen. H.E. x 190.

(E) Cerebral cortex of Case B showing two haemorrhagic areas in the cortical grey matter, extending into the depth of the sulci.

(F) Ophthalmic vein with an agglutination thrombus. H.E. x 25.

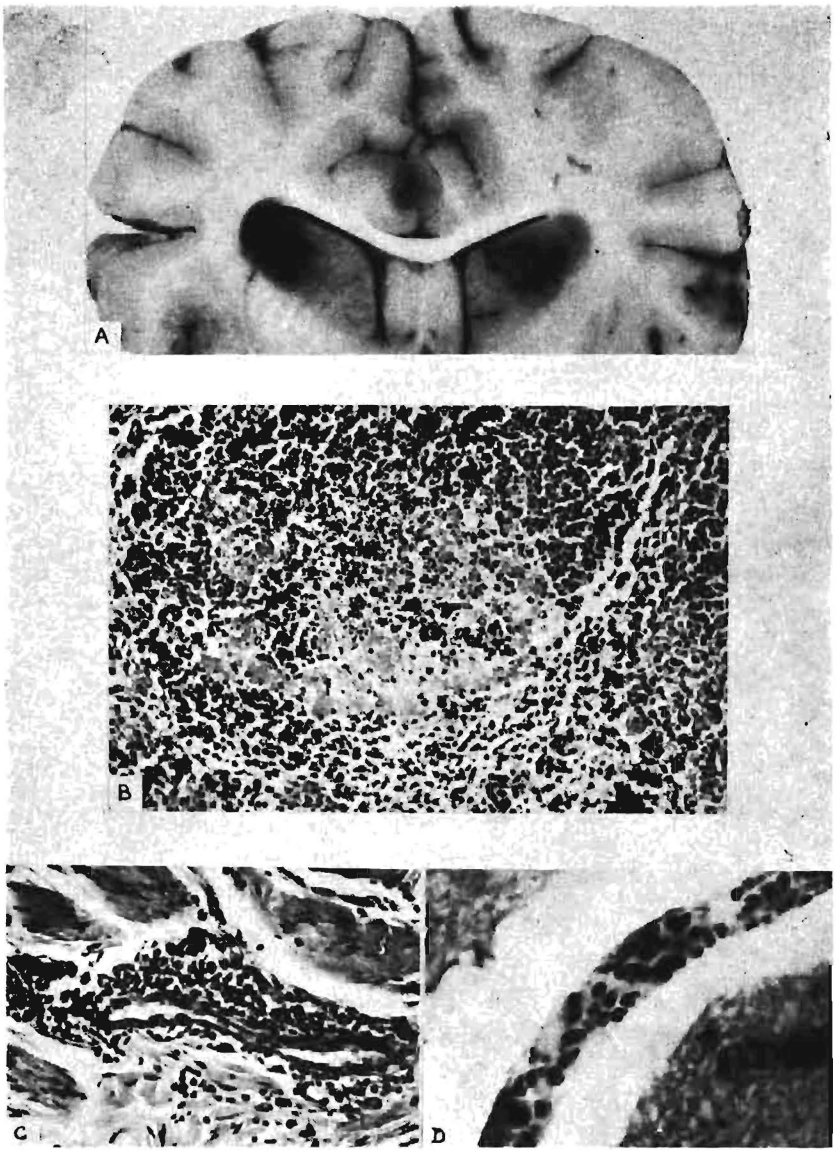

Fig. 2.

(A) Bilateral symmetrical haemorrhagic areas in the caudate nucleus of case A. A smaller lesion can be seen in the g. cinguli.

(B) Spleen. P.A.S.positive 'fibrinoid' material at periphery of lymph follicle and karyorrhexis of white pulp. H.E. x 190.

(C) Episcleral vein showing perivascular infiltration. H.E. x 190.

(D) Capillary in midbrain of Case C with numerous parasites. Giemsa stain. x 1200.

Microscopic

Brain: Cases A, B and C

The bilateral haemorrhagic areas in the caudate nucleus in case A consisted microscopically of numerous closely grouped perivascular haemorrhages from small vessels and capillaries. Irregular vacuolation of the neuropil was present and the majority of the vacuoles contained a finely granular pink staining material. The white matter of the internal capsule, adjoining the caudate nucleus, showed more advanced cavitation than the grey substance of the nucleus. Many of the vessels, particularly towards the periphery of the haemorrhagic lesions, from which no haemorrhage had taken place, showed an accumulation of an eosinophilic substance in the Virchow-Robins (V.R.) spaces and the adjoining brain. Pronounced congestion and oedema were present throughout the brain and meninges. All the perivascular spaces were enlarged.

Most of the capillaries and small veins, throughout the brain were tightly packed with parasitized erythrocytes. The arteries similarly contained large numbers of parasitized erythrocytes but appeared less distended than the veins. Many of the parasites were extracellular. Within the haemorrhagic foci, the extravascular erythrocytes were seldom parasitized. In the V.R. spaces of the vessels running through the haemorrhagic areas the erythrocytes were mostly free of piroplasms although a few aggregations of free organisms were seen. This seemed rather remarkable in view of the heavy parasitaemia within the lumen of such blood vessels. A slight infiltration of polymorphonuclear cells and round cells was observed in the V.R. spaces of some vessels and in the adjoining oedematous brain substance (Fig. 1a). Some of these cells were macrophages containing lipid droplets. Leucostasis was present in many of the vessels within these lesions (Fig. 1d). The perivascular cell infiltration and leucostasis were confined to the haemorrhagic lesions.

The neurones in the haemorrhagic and lytic areas exhibited what is frequently regarded as anoxic degenerative changes viz. shrinkage, pycnosis, cytoplasmic basophilia and hyperchromasia. Necrosis of cytons and glial cells was also present. Some of the glial cells throughout the brain, frequently showed an increase of cytoplasm. This has been regarded as indicative of the oedematous state of this organ. In the walls of some of the vessels and V.R. spaces, within the affected areas of the brain, proteinaceous globules of various sizes and shapes were noticed which stained mildly P.A.S. positive and homogeneously light or medium purplish in the H.E. sections. In some of the larger veins there was a definite pavementing of the endothelium by parasitized red cells (Fig. 1b). Both parasitized erythrocytes and extracellular organisms showed a strong tendency to adhere to each other, forming irregular aggregations. Erythrocytes towards the centre of the lumen in such vessels contained parasites only occasionally. Pavemented vessels also contained an accumulation of oedematous fluid in the perivascular spaces.

Similar microscopic lesions were present in the cerebral cortex in the areas mentioned under the macroscopic pathology, with all the layers of the cerebral cortex being affected. Some neurones in the central grey matter were necrotic and small haemorrhages and cavitation were seen in the superior colliculi.

In case B the general microscopic appearance of the lesions in the cerebral cortex and region of the dentate nucleus resembled that of case A fairly closely. The vacuolation and oedema, however, were not so prominent; the former being more particularly confined to around some of the smaller vessels from which no haemorrhage had occurred.

Blockage of capillaries, throughout the brain, by large numbers of parasitized erythrocytes (Fig. 2d) and the presence of proteinaceous material in some V.R. spaces were the only significant histopathological features seen in case C. None of the other lesions, as seen in the brains of cases A and B were observed. In this case, the brain was unfortunately the only organ to be submitted for comparative study.

Skeletal muscles: Case A

Apart from diffuse haemorrhage and marked oedema a very scanty infiltration of polymorphonuclear cells was also present in the haemorrha-

gic foci in the skeletal muscles of this case. The muscle fibres showed a widespread irregular type of Zenker's hyaline necrosis, fragmentation and vacuolation. Many fragments of fibres appeared lytic (myolysis), being reduced in volume and replaced by a finely granulo-reticular, somewhat basophilic substance (Fig. 1c). Their nuclei were pycnotic, but the lining of the sarcolemma in most of the lytic areas was still intact. Aggregations of parasitized cells were mainly confined to medium arteries and some capillaries. Erythrocytes within the capillaries were not as heavily parasitized as in the brain.

Spleen: Cases A and B

In case A, parasitized eythrocytes were occasionally found throughout the red pulp of the spleen but were most apparent in the engorged central arteries of the splenic corpuscles, where almost every red cell was seen to be invaded. Some of the trabecular and follicular arteries presented the same phenomenon to a minor degree but the veins on the contrary, contained only a few parasites. At the periphery of most of the Malpighian bodies the presence of eosinophilic 'fibrinoid', P.A.S.-positive material containing entrapped erythrocytes, was very striking. This material appeared to have originated from within the capillaries supplying these Malpighian bodies. Marked karyorrhexis (Fig. 2b) of the white pulp surrounded this fibrinoid material. The pyramidal shaped haemorrhagic areas seen at autopsy, resembled haemorrhagic infarcts histologically. Serial sections of these areas, however, revealed no evidence of thrombi. None of these lesions seen in case A were noticed in the second case.

Lymph nodes: Case A

Congestion, haemorrhages, mild oedema, accumulation of macrophages in the sinusoids and depletion of lymphocytes with concomitant karyorrhexis in the reaction centres were evident in these structures. Only a few vessels showed distention by parasitized cells. Some erythrophagocytosis was observed in both the lymph nodes and spleen.

Eyes: Case A

In the eyes, conjunctivae, periorbital tissues and ocular muscles every vessel was seen to be severely distended with parasitized red cells. This was particularly marked in the retinae, laminae choriocapillares and ciliary bodies. Advanced conjunctival and periorbital oedema was present together with a diffuse infiltration of polymorphs, some round cells and macrophages which tended to accumulate around certain vessels (Fig. 2c). Bilateral thrombosis of the ophthalmic veins was a notable feature, practically every erythrocyte within the thrombus being parasitized (Fig. 1f). This was the only locality where thrombi were encountered. A homogeneously intense eosinophilia of the ocular muscles with nuclear pycnosis and disappearance of cross striations was apparent.

Other organs: Cases A and B

Plugging of the capillaries, small arteries and veins was also noticed in all the various layers of the gut, the myocardium and kidney in the first case. No other significant pathological changes were obvious in the intestine. Congestion, slight diffuse oedema and mild hydropic degenerative changes were seen in the myocardium. Many endothelial nuclei were seen to be pycnotic and a small number of capillaries contained a midly basophilic substance within their lumena resembling the proteinaceous material described in certain areas of the brain. Leucostasis was present in some of the vessels. Occasional vessels in the cortex of the kidney contained clumped parasitized erythrocytes whereas most of the small medullary vessels and arteriolae rectae, especially in the inner zone of the medulla, appeared markedly affected. Fairly severe congestion and oedema were present in the lungs of case A but no clogging of any vessels by parasitized cells could be detected either in this organ or in the liver and kidneys of case B. The livers in both instances showed very intense congestion and dilatation of the sinusoids; advanced decomposition, however, rendered both organs unsuitable for finer histological study.

Discussion

Purchase[5] stated that canine cerebral babesiosis is probably analogous to cerebral malaria in man. Malherbe and Parkin[7] and Malherbe[9] also regarded the changes and manifestations

encountered in atypical forms of babesiosis as closely parallel to those observed in malaria, particularly the malignant tertian form. In this form of the disease the 'internal sporulation and localized blockage of capillaries by *P. falciparum* in different organs, such as the brain, heart, intestine and spleen by parasitized corpuscles, which adhere to one another and to the capillary epithelium', is held responsible for the diverse symptomatology[10].

In addition to the plugging of capillaries in the brain by parasitized erythrocytes, there are also other similarities in the pathology of the two diseases viz. pavementing of vessels with cells containing parasites, damage to the endothelial wall, small perivascular haemorrhages and foci of rarefaction of cerebral substance with slight, rare round cell accumulations. In canine babesiosis no mention has previously been made of vessel plugging by parasitized erythrocytes in organs other than the brain except by Lavier and Fombeure[11] who observed it in the retinal capillaries and ciliary body. The occurrence of clumped parasitized red blood cells in organs other than the brain in case A is therefore another aspect that points to similarity between atypical forms of canine babesiosis and malignant tertian malaria in man. Pigment similar to the malaria pigment and lesions resembling malarial granulomas (Dürck's nodules) were absent. Actual thrombosis is rare in cerebral malaria[12] and judging from the limited information present on cerebral babesiosis, this appears to be equally true for this condition.

The exact pathogenesis of the lesions in cerebral babesiosis is still obscure. Reusse[8] regarded the earlier theory of capillary blockage by parasitized cells being the possible explanation of the nervous symptoms in cerebral babesiosis as misleading. According to him histopathological changes in the brain develop rarely and then only in the final stages of canine babesiosis. On this, Innes and Saunders[13] commented that if vascular occlusion were to be a factor in the pathogenesis, then cerebral damage would have to be more serious than so far recorded. The lesions observed in the brains of two of the three cases (A and B) which are presented, could obviously explain the death and nervous symptoms in canine cerebral bebesiosis.

In the cases under review the mere plugging of capillaries by parasitized erythrocytes does not appear to be the sole mechanism involved in the pathogenesis of the lesions observed. Most of the capillaries throughout the brain contained such cells without necessarily showing lesions other than oedema. Haemorrhagic, degenerative and necrotic areas were, however, confined to areas which are usually regarded as the most vulnerable to anoxia, such as the depths of the sulci and the caudate and dentate nuclei. It is of interest to note that terminal arteries which supply the cerebral or cerebellar cortex in the depths of the sulci are areas included by Lindenberg[14] in his hypothesis of selectivity of vascular lesions as the sequel to local pressure which some arterial systems undergo during sudden and considerable increase of intracranial pressure in man.

A severe sudden onset of parasitaemia, even without apparent accompanying anaemia, would evidently result in hypoxia with subsequent dilatation of the vessels, decreased bloodflow, endothelial damage and eventual oedema, haemorrhages and degeneration of the brain. The accumulation of parasites and parasitized erythrocytes and a possible local multiplication of the piroplasms, could well be explained by this sequence of events. Retarded flow of blood would cause the parasitized cells to move towards the periphery[17]*, thus pavementing and gradually blocking the smaller vessels. Partial stasis of bloodflow and the increase in viscosity would also enhance sludging of the blood[15]. Gilles and Maegraith[16] incriminated as the basic disturbance giving rise to stasis and subsequent anoxic changes, one by which fluid escaping through abnormally permeable vessel walls would leave the erythrocytes concentrated and compressed. Agglutination due to an antigen — antibody reaction could also possibly explain the clumping of parasitized cells and extracellular parasites.

*In sections of the pampiniform plexus from a case with a heavy parasitaemia it has recently been observed that parasitized cells settle at the level of the leucocytes on top of the unparasitized erythrocytes. This could possibly point to a lower specific gravity of the parasitized cells and would explain the phenomenon of pavementing. Further experimental work is being undertaken to substantiate this observation.

Some of the phenomena mentioned above could lead to an increased volume of the brain and thus to an elevation of the intracranial pressure. This in turn would interfere with the blood supply of certain areas of the brain and may help to explain the occurrence of lesions in the more vulnerable areas such as the depths of the sulci, the caudate and dentate nuclei (vide cases A and B where the lesions resembled complete haemorrhagic infarcts). Moreover, agglutination thrombi such as those observed in the ophthalmic veins of case A, may be formed.

Sludging of parasitized cells within the capillaries and small blood vessels in various organs including the brain has also recently been observed in several cases of babesiosis in bovines[17]. These cases presented a normal course of the disease without evidence of nervous symptoms or brain lesions. Gilles and Maegraith[16] noticed in their laboratory-induced cases of canine babesiosis blockage of small vessels with parasitized cells and their layering along the endothelium in the brain. These cases were not regarded as cerebral babesiosis[9]. Symptoms and lesions referable to the nervous system were also absent in our case C. The validity of a diagnosis of 'cerebral' babesiosis based on the mere presence of accumulated parasitized erythrocytes in the capillaries of the brain, unaccompanied by nervous symptoms or brain lesions, thus appears to be questionable. Sludging of parasitized red blood cells in these instances apparently occurs terminally during the normal course of the disease with insufficient time before death for development of lesions.

The confinement of piroplasms to the brain in some cerebral forms has been described by Smit[18] and Malherbe and Parkin[9]. However, in at least one of the cases described by Purchase[5], in the case reported by Piercy[6], in two out of three cases examined by Malherbe and Parkin[9] and in both cases described here, parasites were present in the peripheral circulation. This evidence casts serious doubt on the concept of cerebral babesiosis being an atypical manifestation of babesiosis where piroplasms are confined solely to the brain. In view of all this it is suggested that cerebral babesiosis be defined as an atypical manifestation of the disease which is clinically accompanied by nervous symptoms and histopathologically characterised by sludging of parasitised erythrocytes and referable lesions in the brain. Peripheral bloodsmears in these cases may be either positive or negative for piroplasms. Two of the three artificially produced cases studied by Reusse[8] developed ataxia four weeks and four months respectively after recovery. No parasites were present in the brains. Foci of demyelination and gliosis with proliferation of blood vessels in the internal capsule were present in one case. Slight diffuse gliosis of the white substance, proliferation of the blood vessels and focal proliferation of endothelium were seen in the other. These rather non-specific changes, unless sequelae to damage of the nervous tissue during the acute phase of the disease, are probably unrelated to babesiosis. Reusse's third case, during the acute phase of the disease, showed sudden incoordination of the hind legs progressing to complete paralysis within three days. The lesions described in this case are reminiscent of our cases A and B. No mention, however, is made of the occurrence of parasitized erythrocytes in the blood vessels of the brain.

Lesions in skeletal musculature have not been described previously. The lower tonus in the flexor muscles could promote sludging of blood leading to the ensueing sequelae (vide case A). Unlike the flexors, the extensors with the higher tonus had relatively few lesions. Rheumatoid muscular pains, particularly in the region of the back and loins, were reported in atypical forms of babesiosis by Cuille and Darraspen[4]. According to Malherbe[9], Brown[9a], noticed masseteric myalgia which caused dogs to scream with pain when their heads were touched or their mouths opened. Brown also regarded rheumatic manifestations, chiefly in the legs, with lameness and even paraplegia as prominent and frequent in atypical babesiosis. The lesions encountered in the skeletal muscles of case A may very well account for the symptoms described by these authors.

As stated earlier serial sections revealed no thrombi in any vessels of the spleen. The presence of such thrombi cannot however be excluded with any certainty. These lesions were otherwise microscopically identical to haemorrhagic infarcts. Apart from these, the only other splenic lesions were confined to the white pulp which has a capillary supply where blocking can

more readily occur than in the red pulp with its network of sinusoids. Necrosis of the lymph follicles has also been noticed in ordinary forms of piroplasmosis in dogs and cattle[17].

Basically the lesions within the eyes, spleen and lymph nodes could be explained in the same manner; anoxia and sludging being the primary underlying factors. Maegraith[19], in discussing the pathogenesis of malaria indicated the presence of a substance(-s) in the serum of malarial patients which depressed the respiratory activity of liver mitochondria. This was regarded as evidence for the activity of either non-specific substances released during the infection or of a toxin liberated by the malarial parasites. It is felt that these possibilities should not be disregarded in the pathogenesis of babesiosis.

These prelimanary observations and remarks on cerebral babesiosis are at present being followed up by a more intensive study on the pathology and pathogenesis of babesiosis and its various atypical manifestations.

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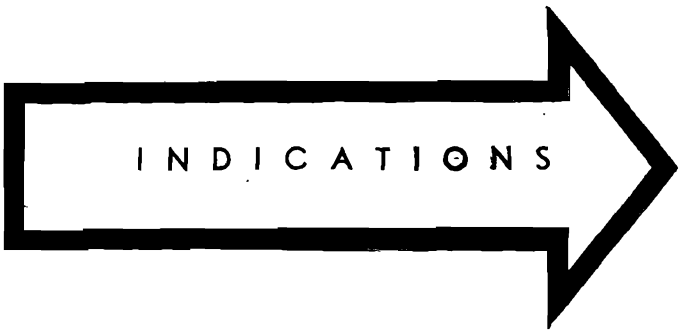

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PRACTICAL ASPECTS OF A.I. IN DOGS I: COLLECTION OF SEMEN

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SUMMARY

By means of artificial vaginas which were constructed from rubber hosing, 22 ejaculates were collected from 14 dogs representing a variety of breeds. The collections in these cases were done for the sole purpose of inseminating bitches where normal service proved unsuccessful or was contra-indicated.

Only the first two fractions of the ejaculate were collected, giving a mean volume of 4.76 ml. Over 80 per cent of the dogs accepted the vagina immediately, while the rest were guided to acceptance within 10–15 minutes. In all cases a good semen sample was obtained.

INTRODUCTION

Although the first artificial insemination in dogs was done nearly 300 years ago by the Abbe Spallanzani, this method as an aid to breeding received very little attention until ten years ago.[1, 2, 3].

Different methods to obtain reliable semen samples were developed. Bartlett[3] states that as early as 1863 Eckard succeeded in obtaining semen from dogs by electrical stimulation. This technique was refined and again propagated by Christensen and Dougherty[4] in 1955. Masturbation and digital manipulation also proved successful[1, 8]. The most widely advocated method, with or without the presence of a teaser bitch, is the use of an artificial vagina[1, 3, 5–7].

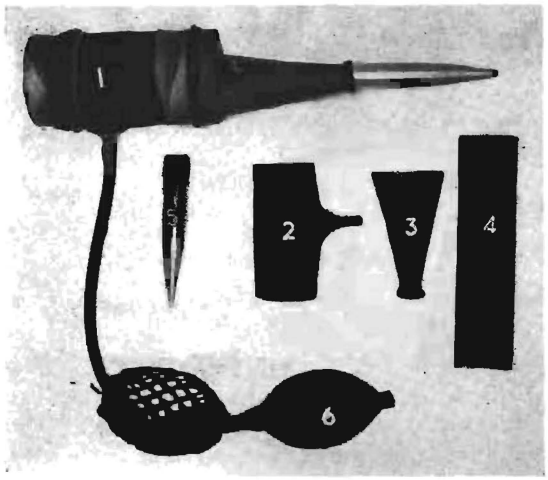
Excellent work has been done on the process of ejaculation, semen morphology, biochemical analysis, dilution, storage and transportation[3, 5, 6, 8–23]. Unfortunately in very little of this work was observation followed through to successful conception and birth; where it was done, the cases were few in number or the conception rates low. The oestrus cycle of the bitch deserves closer investigation. Only by pinpointing the optimal time for insemination can high conception rates be expected.

This and a subsequent paper outline and elaborate the results obtained by using a simple and economically constructed artificial vagina, the insemination technique used and the vaginal cytological criteria on which the time of insemination was based.

METHODS AND MATERIAL

Twenty-two ejaculates were collected from fourteen dogs of various breeds, for the purpose of obtaining semen to inseminate bitches where natural service proved unsuccessful, or was contra-indicated.

Semen was collected by means of artificial vaginas. Two sizes of rubber hosing were used as the vaginal casing (fig. 1). For medium and larger breeds of dog the diameter of the casing was 6.5 cm and the length 13 cm. For smaller breeds the casing was 4 cm in diameter and 10 cm long. Each outer casing was fitted with a valve approximately 5 cm from the posterior end. To complete these vaginas, latex liners and cones were adapted from those used in bovine equipment for large and medium breeds and those in ovine equipment for small and toy breeds. For purposes of inflation and pulsation a combined double-bulb insufflator was fitted to the valve.



(1) Artificial vagina of dogs assembled and ready for use on dogs of medium and larger breeds; (2) outer casing of artificial vagina for use on small and toy breeds; (3) latex cone; (4) latex liner as used in artificial vaginas for rams, shortened to fit outer casing 2; (5) 15 ml. graduated glass tube; (6) double-bulb insufflator used as pulsator.

The temperature of the vagina was controlled between 42–44°C by introducing hot water between the outer rubber casing and inner latex liner. Prior to use the water was discarded, the posterior half of the inner liner was lubricated very slightly and a small amount of air introduced through the valve.

When a "teaser" bitch was not available the dog was sexually stimulated before collection by digital manipulation of the penis through the penile sheath. The glans penis and bulbus glandis[24] were exposed and pressure applied behind the bulbus glandis. On introduction of the glans penis into the vagina, pulsation, simulating vaginal contractions, was applied by means of the double-bulb insufflator. The inner pressure of the vagina and the rate of pulsation were adjusted and regulated according to the response of individual dogs.

Only the first and second fractions of the ejaculate were collected. Immediately after collection a drop of semen was examined for density, percentage alive and directional motility. Insemination was carried out within half an hour after collection. In each case the whole volume collected was inseminated.

Results and Conclusions

The results obtained using the artificial vagina to collect semen from the clinical material presented, are summarised in the table. The volume of the first two fractions of the ejaculate varied from case to case: the mean volume was 4.76 ml. Using density, percentage alive sperm and directional motility as criteria, all 22 ejaculates were subjectively evaluated and found to be of good quality.

Results of Semen Collection

Case No.	Breed	Age (mths.)	Volume of ejaculate (ml)**	Interval between collections (hrs.)	Time required to accept a.v.	Pre-collection stimulation with a female in oestrus
1	Boxer	24	4.0	once only	immediate	No
2	Boxer	18	5.0; 3.0	48	10 min.	Yes
3	Ridgeback	84	6.0	once only	immediate	No
4	Bulldog	20	3.0; 3.5	72	immediate	Yes
5	Afghan	72	4.0; 4.0	48	immediate	No
6*	Corgi	84	1.5	once only	15 min.	Yes
8	B.Mastiff	50	6.0	once only	immediate	Yes
9	Boxer	42	5.0; 3.5	48	immediate	Yes
10*	Corgi	84	2.0	once only	10 min.	Yes
11	Boer Hound (Boerboel)	59	5.0; 4.0	48	immediate	Yes
12	Bulldog	36	5.0; 3.0	48	immediate	No
13	Boxer	74	11.5	once only	immediate	Yes
14	Bulldog	30	7.5	once only	immediate	No
15	B.Mastiff	25	4.0	once only	immediate	No
16	Ridgeback	—	7.0; 8.0	48	immediate	Yes

*Same dog.
**Only first two fractions of ejaculate collected.

Although good results were obtained when collection was carried out without the presence of a bitch in oestrus, the use of a teaser facilitated collection, and yielded a better semen sample. Where a "teaser" bitch was not used, pre-collection sexual stimulation of the glans penis by digital manipulation was essential. This stimulation generally shortened the time required for acceptance of the artificial vagina. No breed difference in response to the use of the artificial vagina was noted.

The construction of the vaginas used in dogs is both simple and economical without entailing any elaborate procedures. This makes its use by the general practitioner a feasible proposition. Although little difference was found in semen samples obtained by masturbation or the use of an artificial vagina, the latter greatly facilitated the collection of a semen sample, especially in excitable or fractious dogs. It also contributed to a more accurate collection of a whole or fractionated semen sample.

Apart from the use of the artificial vagina for semen collection for A.I. or experimental purposes, semen samples can be readily collected from dogs requiring certification for fertility.

ACKNOWLEDGEMENT

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PRACTICAL ASPECTS OF A.I. IN DOGS II: INSEMINATION OF THE BITCH

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SUMMARY

The relevant data on 16 clinical cases in which A.I. had to be practised because natural service proved either unsuccessful or was contra-indicated, are recorded.

Conception occurred in 9 of the bitches. Two of the bitches subsequently aborted.

The EI and SCI were used as aids in pin-pointing the time of insemination.

Comparisons were drawn regarding the vaginal cell changes occurring in cases which conceived to A.I., those that did not conceive, as well as the cytological changes found in bitches where natural service took place.

INTRODUCTION

The use of artificial insemination in dogs has become increasingly necessary, not only to overcome geographical distances or to extend the use of a valuable stud dog, but also to breed from bitches showing abnormal oestrous cycles. Artificial insemination in bitches can also be of distinct value in certain pathological conditions of the female genital tract, as well as in psychological aberrations.

Harraop[1, 3] has pointed out that since it is now possible to extend, preserve and even transport dog semen over long distances, A.I. in dogs can be carried out with little difficulty. The conception rates documented to date, however, have been far from satisfactory[2]. One of the main factors which lowers the efficiency of A.I. in dogs is the absence of clear cut external symptoms to pinpoint the optimal time of insemination for maximal conception. It is well known too, that the normal breeding cycle of the bitch may vary quite considerably.[4, 5, 6]

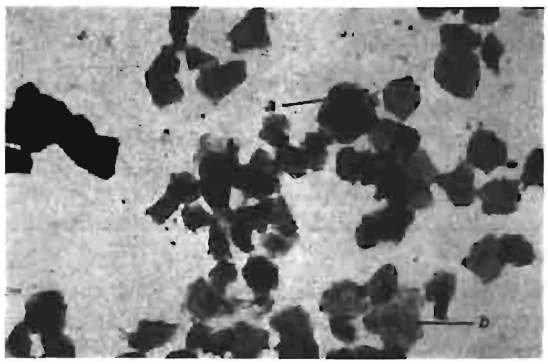

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Fig. 1. — The typical picture of a vaginal smear taken during oestrum, characterised by a predominence of large keratinised squamous cells. The darker staining cells (a) are eosinophilic while the lightly stained cells (b) are non-keratinised.

Several methods, all basically similar, have been used in demarcating the different phases of the oestrous cycle in the bitch, as well as pinpointing the time of ovulation.[4, 6, 7, 8, 9] Vaginal cell indices have been used extensively in humans to evaluate the effect of hormonal stimulation.[10–15] Similar indices have been shown to give reliable results in bitches as well.[6]

The present paper outlines the use of these indices in determining the optimal time for insemination in bitches, especially in those cases beset with breeding problems.

METHODS AND MATERIALS

Over the past two years 87 bitches with a variety of breeding problems were admitted to this clinic. Artificial insemination was carried out in twenty one of these cases, as natural service proved unseccessful or was contra-indicated. The relevant breeding history of sixteen of these cases is presented in table 1.

TABLE I.—SUMMARY OF BREEDING HISTORY

Case No.	Breed	Age (Months)	Current Oestrous Cycle	Partus Data	Reasons for A.I.
1	Boxer	24	2	Monoparous	Vaginal prolapse
2	Boxer	36	4	Monoparous	Refused to accept male
3	Ridgeback	62	5	Monoparous	Refused to accept male
4	Bulldog	11	1	Nulliparus	Inexperienced male.
5	Afghan	58	4	Nulliparous	Refused to accept male
6	Corgi	12	1	Nulliparous	Refused to accept male
7	Boxer	35	4	Nulliparous	Use of Imported Semen
8	B. Mastiff	48	5	Multiparous	Male unable to serve
9	Boxer	40	5	Nulliparous	Vaginal prolapse
10	Corgi	22	3	Monoparus	Refused to accept male
11	Boer Hound (Boerboel)	78	—	—	Refused to accept male
12	Bulldog	32	5	Nulliparous	Vaginal stenosis
13	Boxer	36	4	Nulliparous	Refused to accept male
14	Boxer	30	3	Monoparous	Size incompatability
15	B. Mastiff	36	4	Nulliparous	Size incompatability
16	Ridgeback	18	3	Nulliparous	Refused to accept male

With the exception of case No. 1, all the cases were admitted as soon as possible after pro-oestrum bleeding was noted. Vaginal smears, taken every 48 hours, were stained according to Shorr's method[16] and evaluated according to the method suggested by Schutte.[6]

Insemination was carried out during the eosinophilic index peak (fig. 1 and graph). If the eosinophilic index (EI) still showed a high value 48 hours later, insemination was repeated. In all cases, except case No. 7, the semen used was undiluted as discussed in a previous paper.[17] Liquid semen imported from the U.S.A. was used for case No. 7.

Plastic cattle insemination pipettes were used for the inseminations. These pipettes were modified for smaller breeds of dog by warming them above a flame to facilitate tapering, and then cutting them down to the required length. Either a metal speculum or perspex vaginascope was used to aid the passage of the pipette through the vaginal canal. By deep abdominal palpation the tip of the pipette could be directed through the cervix into the body of the uterus, where the semen was deposited. When the cervix could not be penetrated, the semen was deposited as close to the cervix as possible. In these cases the hindquarters of the bitch were elevated for a few minutes to allow the semen to flow into the uterus, thus preventing excess semen spillage and loss, should the bitch sit down. As a general routine the bitches were walked for 5–10 minutes after insemination.

RESULTS AND DISCUSSION

The site of semen deposition and ensuing insemination results are shown in table 2. Nine of the sixteen bitches conceived, giving a conception rate of 56.2 per cent. Cases No. 1 and 4 aborted on the 58th and 42nd day of gestation respectively. The reasons for these abortions have not been determined.

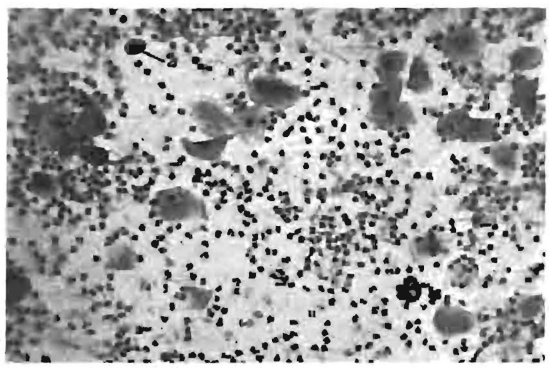

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Fig. 2. — An abnormal metoestrum smear depicting the presence of a high superficial cell index. A few parabasal cells (a) are also shown. Note the high concentration of leucocytes.

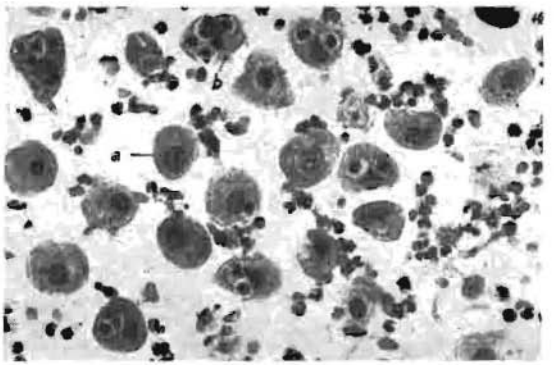

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Fig. 3. — The usual picture encountered in a metoestrum smear showing a predominence of small intermediate cells (a) and "metoestrum cells" (b). Note high concentration of leucocytes.

TABLE 2.—SUMMARY OF ARTIFICIAL INSEMINATION RESULTS

Case No.	Day of Cycle Inseminated*	Site of Semen Deposition	Volume of Undiluted Semen used (ml)	Litter size Resultant from Insemination
1	11	Ant. Vagina	4	3 pups aborted, 58th day
2	9, 11	Int. Cervical + Uterine body	5, 3	Not pregnant
3	11	Int. Cervical + Uterine body	6	Not pregnant
4	12, 14	Int. Cervical + Uterine body	3, 3.5	5 pups aborted, 42nd day
5	10, 12	Int. Cervical + Uterine body	4, 4	Not pregnant
6	12	Int. Cervical + Uterine body	1.5	4 pups (normal gestation)
7	11, 13	Ant. Vagina	10, 8 (Diluted)	Not pregnant
8	13	Ant. Vagina	6	5 pups (normal gestation)
9	11, 13	Int. Cervical + Uterine body	5, 3.5	Not pregnant
10	12	Int. Cervical + Uterine body	2	Not pregnant
11	11, 13	Int. Cervical + Uterine body	5, 4	6 pups (normal gestation)
12	10, 12	Int. Cervical + Uterine body	5, 3	Not pregnant
13	12	Int. Cervical + Uterine body	11.5	Pregnant
14	11	Int. Cervical + Uterine body	7.5	8 pups (normal gestation)
15	10	Int. Cervical + Uterine body	4	6 pups (normal gestation)
16	10, 12	Int. Cervical + Uterine body	7, 8	4 pups (normal gestation)

*Day on which pro-oestrum bleeding was first observed, is considered as first day of cycle.

Comparing the vaginal cytological data obtained from those bitches which did not conceive with those which did, a marked difference was noted in the cellular changes which occurred during the post insemination period (table 3). Both the superficial cell index (SCI) as well as the EI remained high in all cases where conception did not take place, although the leucocyte concentration tended to follow the usual pattern (fig. 2 and graph).

TABLE 3.—VAGINAL CYTOLOGY RECORDED DURING THE OESTROUS CYCLE OF ABERRANT BITCHES

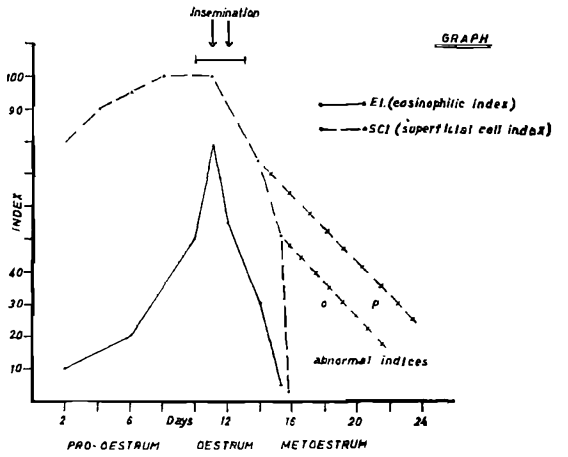
Case No.	Day of Cycle EI* Peak Recorded	Interval—EI Peak to < 10% (Days)	Interval—SCI** Peak <10% (Days)	Day of Cycle "Metoestrum Cells" First Observed
1	—	—	—	—
2	9	14	Remained high	None during first 22 day observation
3	10	4	10	None during first 20 day observation
4	12	5	5	17
5	12	4	8	18
6	9	6	7	17
7	13	4	Remained high	None during first 20 day observation
8	13	Case Discharged	Incomplete	Cytological Material
9	11	9	13	24
10	13	7	7	20
11	13	3	4	17
12	12	10	12	24
13	13	5	5	18
14	11	4	5	16
15	8	4	4	13
16	10	6	7	17

*Eosinophilic index.
**Superficial cell index.

TABLE 4.—VAGINAL CYTOLOGY RECORDED DURING THE OESTRUS CYCLE OF BITCHES IN WHICH NATURAL SERVICE WAS ALLOWED

Case No.	Day of Cycle Coitus Allowed	Day of Cycle EI Peak Recorded	Interval EI Peak to <10%	Interval SCI Peak to <10%	Day of Cycle "Metestrum Cells" First Observed	Resultant Litter size
17	11, 13	11	7	5	18	6
18	14, 16	14	3	6	17	5
19	13, 16	12	4	5	16	4
20	9	8	5	2	13	7
21	9, 11	12	4	4	16	5
22	14, 16	15	4	4	20	1
23	17, 19	19	5	5	24	7
24	10, 12	10	8	4	18	4

Where conception did occur, both in bitches inseminated artificially or those in which natural service was allowed, the cytological changes followed a similar pattern (cf. tables 3 and 4). In each instance the EI and SCI dropped below the 10% level within a 4–5 day period. Vaginal smears taken at this stage showed a predominance of small intermediate cells and "metoestrum cells",[6] as well as a high concentration of leucocytes (fig. 3). This was the typical cellular pattern encountered during normal metoestrum. Where conception did not take place, the drop of the cellular indices, especially the SCI, was markedly prolonged. In these cases too, the appearance of "metoestrum cells" was also delayed (table 3).



A schematic representation of the general pattern of the EI and SCI during the oestrus cycle of the bitch. Deviation from the usual SCI curve are shown by lines O and P.

Although the cellular picture remained one depicting maintained oestrogen stimulation in those cases which did not conceive, it is not possible at this stage to present a full explanation for this phenomenon. It seems feasible to suggest, however, that ovarian dysfunction may be implicated, as the cellular picture indicates oestrogen stimulation.

The term "conceived" has been used in broad concept in this paper. It is not possible, however, to rule out those conceptions which did occur with ensuing early foetal resorption. Further work on this aspect as well as on anovulatory oestrum should be carried out on an experimental basis, as clinical material cannot be used for this purpose.

Since it has been shown that the time of insemination can be established by vaginal smears in bitches with aberrant oestrus cycles with a resultant 56 per cent conception rate, there seems little reason why this method should not be more widely used to control insemination time in bitches with normal cycles, thereby further raising the conception rate.

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STAPHYLOCOCCAL MASTITIS: INTERRELATIONSHIP BETWEEN WHITE CELL COUNT AND NUMBER OF STAPHYLOCOCCI IN THE MILK

A. JANOVICS
Saphar Laboratories Limited, P.O. Box 256, Johannesburg

In the course of an investigation in which more than 2,000 milk samples were examined, staphylococci could be detected, in many cases, only after concentrating the microorganisms by centrifugation, incubation of the milk or by using heavy inocula. It was noted that these artifices were necessary with milks of low white cell count and suggested an interrelationship between white cell count and number of staphylococci in the milk.

The following considerations explain the significance of such an interrelationship and supplied the stimulus to systematic investigation. Elevated white cell count in the milk indicates an inflammatory process in the mammary tissues, i.e. mastitis. Staphylococcal mastitis spreads readily and often rapidly in the herd. In my experience (about 15 herds) staphylococci were found in at least one udder quarter of 50%–100% of all cows. The main source and reservoir of infection is the diseased udder that discharges staphylococci. If effective treatment or prevention of mastitis reduces the number of staphylococci shed in the milk, it will also curb the spreading of the disease.

It is known that in streptococcal mastitis a high white cell count is accompanied by large number of streptococci in the milk, but in the available literature on staphylococcal mastitis only one reference to white cell and bacterial count could be found. Udall, Johnson and Ferguson mentioned that, in milk samples taken from the four quarters of one cow, increased white cell count was associated with high staphylococcal count.

MATERIAL AND METHODS

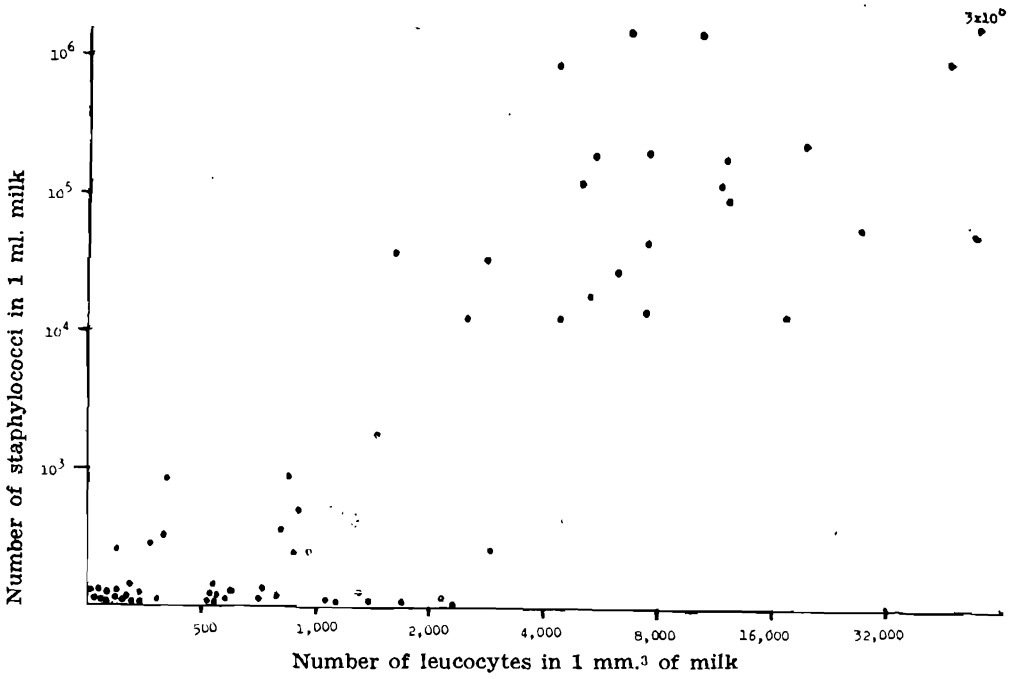
In this study 66 milk samples taken from single quarters have been examined. Samples must be taken in as aseptic a manner as possible into sterile containers and refrigerated without delay.

The white cell count was determined by Breed's method; to make counting easier, 0.01 ml of milk was spread over an area of 2 cm^2 instead of the recommended 1 cm^2.

Bacteriological examination was routinely carried out by culturing the milk on a solid and in a liquid medium and, when necessary, on selective media.

The solid medium was nutrient agar containing 7% sodium chloride and 0.1% potassium tellurite. This medium suppresses the growth of nearly all bacteria except staphylococci nearly all of which reduce tellurite and grow in the form of black colonies. These were transferred to nutrient agar and tested for coagulase activity. Coagulase-negative staphylococci were found occasionally, in one herd repeatedly, but it is doubtful if they were pathogenic. Coagulase-negative staphylococci were sometimes found in large numbers in milk with a normal white cell count and occurred often in milk samples that also yielded heavy growth of *Bacillus subtillis* and *Escherichia coli*. These observations indicate that coagulase-negative staphylococci were accidental contaminants.

The liquid medium used in this study contained 2% peptone, 0.5% sodium chloride, 0.1% lactose and 10% of a freshly prepared liver extract (calculated as fresh liver tissue). This medium also supports the growth of more fastidious bacteria, such as streptococci and pneumococci. Samples which yielded mixed populations of staphylococci and haemolytic streptococci or, in a few cases, *Pseudomonas pyocyanea*, were not included in the study nor were those with coagulase-negative staphylococci.



Correlation between numbers of staphylococci and leucocytes in milk.

To find the number of staphylococci, 0.1 ml of milk was plated salt-tellurite on medium. Within the limits of experimental error, it was immaterial whether the milk was added to the molten agar and plates poured or the sample was streaked out evenly on the surface of the plate. Whenever the number of colonies was too large to be counted, the test was repeated with dilutions of 1:10, 1:100, 1:1,000 and 1:10,000. In order to reduce the inaccuracy inherent in the method, caused mainly by the presence of clumps of staphylococci in the milk, the tests were performed in duplicate and for each dilution a fresh milk sample of 1 ml was used. The plates were read after incubation for 48 hours.

Results

The results are shown graphically and in two tables. In the graphical representation each dot represents a milk sample. It shows at a glance that the number of staphylococci was small in milks with low white cell counts. With increase of the leucocyte count the staphylococcal counts also increased. The number of staphylococci began to rise steeply when the leucocytes exceeded 2,000 in one mm^3; at about 4,000 white cells per mm^3 the staphylococcal count reached its maximum and remained at the same level in milks with still higher leucocyte counts.

Table 1 shows the frequency distribution of the number of staphylococci and white cells. The figures indicate the number of samples in each group. Most samples with low white cell count contained few staphylococci; with increasing white cell count a larger number of samples was found with a higher staphylococcal count and all samples with white cell counts of 4,000 and more per mm^3 harboured large numbers of the organism.

TABLE 1.—NUMBER OF STAPHYLOCOCCI AND LEUCOCYTES IN MILK. FIGURES ON THE RIGHT HAND SIDE OF TABLE INDICATE NUMBER OF SAMPLES IN EACH GROUP

No. of staphylococci in 1 ml of milk	No. of leucocytes in 1 mm³ of milk				
	0–500	500–1000	1000–2000	2000–4000	>4000
1–10	15	10	4	2	
10–100			1		
100–1,000	4	5		1	
1,000–10,000			1		
10,000–100,000			1	2	9
>100,000					11
Total	19	15	7	5	20

Table 2 gives information on the average number of staphylococci in 1 ml of milk compared with the leucocyte count per 1 mm³.

TABLE 2.—AVERAGE NUMBER OF STAPHYLOCOCCI IN MILK IN RELATION TO WHITE CELL COUNT

No. of leucocytes in 1 mm³ of milk	Number of staphylococci in 1 ml of milk
0–500	150
500–1,000	500
1,000–2,000	1,000
2,000–4,000	12,000
>4,000	800,000

DISCUSSION AND CONCLUSIONS

Allowing for the errors inherent in the methods of counting white cells and staphylococci in milk and for the comparatively small number of observations (66), the trend is clear: the number of staphylococci discharged in the milk of cows with mastitis was significantly larger than that in the milk of silent carriers. Table 2 shows that about 1,000 times more staphylococci were excreted in the same volume of milk of cows with mastitis than in the milk of cows in the carrier state.

The eradication of staphylococci presents great, almost insurmountable, difficulties. It was observed that the intensive treatment of animals with antibiotics to which the staphylococcus was sensitive in vitro failed to eradicate the microbe from the udders of the cows examined. In several herds which, from the clinical standpoint, were successfully immunized with vaccines, staphylococci could still be demonstrated in most milk samples if sufficiently large inocula were used.

This is a preliminary communication and the investigation is being continued with the intention of accumulating sufficient data for statistical evaluation.

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BOOK NEWS

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J. S. Afr. vet. med. Ass. 36 (3) 1965

STUDIES ON PARAMPHISTOMIASIS. VII. THE IMMUNISATION OF SHEEP, GOATS AND CATTLE. PRELIMINARY REPORT

I. G. HORAK

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SUMMARY

1. The successful experimental immunisation of adult sheep, goats and cattle against paramphistomiasis is described.

2. Cattle became immune six weeks after vaccination and the immunity was more complete than that in sheep or goats.

INTRODUCTION

During a study on the clinical pathology of *Paramphistomum microbothrium* infestation in sheep, Horak and Clark[1] reported resistance to reinfestation in some animals. In the light of this finding the present experiments were undertaken in an attempt to develop a method of immunising ruminants against paramphistomiasis.

METHODS

Sheep

Two unweaned lambs and three adult sheep were each infested with 40,000 metacercariae either in a single or two equal doses and then challenged with 186,000 ± 17,000 metacercariae. The control sheep each received a single infestation of 201,000 ± 1,000 metacercariae.

Goats

Two unweaned kids and two adult goats were each infested with 40,000 metacercariae either in a single or two equal doses and challenged with 184,000 ± 15,000 metacercariae. Three control goats each received a single infestation of 184,000 ± 16,000 metacercariae.

Cattle.

Metacercariae were exposed to 2 Kr of x-irradiation: Five adult cattle were each infested with 40,000 irradiated metacercariae and challenged at varying intervals with 250,000 non-irradiated metacercariae. Three controls were infested with 253,000 ± 3,000 metacercariae each.

At death or slaughter paramphistomes were recovered and counted (Horak[2]).

RESULTS

Sheep and Goats

The number of metacercariae dosed and the number of paramphistomes recovered from immunised and control sheep and goats are summarized in Table 1.

TABLE 1.—THE IMMUNISATION OF SHEEP AND GOATS AGAINST PARAMPHISTOMIASIS

No.	Immunisation Procedure		Days between end of immunisation and challenge	Challenge			
	No. of metacercariae dosed initially	No. of worms recovered		No. of metacercariae dosed	Days between challenge and slaughter	No. of worms recovered	% Recovered
	SHEEP						
1	Control			201,000	19*	96,163	47.8
2	Control			201,000	22*	107,641	53.6
3	Control			202,000	29	78,225	38.7
	LAMBS						
4	20,000 + 20,000, 32 days later	1,191	43	170,000	31*	70,697	41.6
5	20,000 + 20,000, 32 days later	1,926	43	169,000	33*	40,160	23.8
	ADULT SHEEP						
6	20,000 + 20,000, 32 days later	2,353	37	200,000	35	126	0.06
7	40,000	815	69	200,000	35	1,073	0.5
8	40,000	2,942	1,075	203,000	48	85	0.04
	GOATS						
1	Control			168,000	27*	47,307	28.2
2	Control			200,000	23*	86,135	43.1
3	Control			200,000	29*	71,579	35.6
	KIDS						
4	20,000 + 20,000, 32 days later	3,956	37	173,000	24*	45,046	26.0
5	20,000 + 20,000, 32 days later	654	37	169,000	46	3,372	2.0
	ADULT GOATS						
6	20,000 + 20,000, 34 days later	1,598	48	199,000	42	84	0.04
7	40,000	1,966	82	198,000	42	2,392	1.2

* = Died, paramphistomiasis.

TABLE 2.—THE IMMUNISATION OF CATTLE AGAINST PARAMPHISTOMIASIS

No.	Immunisation Procedure		Days between end of immunisation and challenge	Challenge			
	No. of metacercariae dosed initially	No. of worms recovered		No. of metacercariae dosed	Days between challenge and slaughter	No. of worms recovered	% Recovered
	CONTROLS						
1	Control			250,000	23	108,223	43.3
2	Control			256,000	33*	172,696	67.5
3	Control			250,000	28	102,103	40.8
	X- IRRADIATED METACERCARIAE						
4	40,000 (2,Kr)	1,122	42	250,000	29	107	0.04
5	40,000 (2,Kr)	1,490	56	250,000	22	33	0.01
6	40,000 (2,Kr)	1,204	71	250,000	28	3	0.001
7	40,000 (2,Kr)	1,007	71	250,000	28	10	0.004
8	40,000 (2,Kr)	1,002	76	250,000	28	7	0.003

* = Died, paramphistomiasis.

In the immunised group two lambs and one kid died from paramphistomiasis; the other kid and the adult sheep and goats were resistant to reinfestation. Two control sheep and all the control goats died as a result of the infestation.

Cattle

The numbers of metacercariae dosed and worms recovered are shown in Table 2.

Immunised cattle were solidly immune six weeks after initial infestation, whereas one of the control animals died of paramphistomiasis.

Discussion

Previously Horak and Clark[2] had shown that 170,000 ± 5,000 metacercariae were a lethal infestation for susceptible sheep. In the present experiments 185,000 ± 17,000 metacercariae proved lethal to sheep and goats and 256,000 metacercariae to cattle.

The animals that were successfully immunised not only survived but very few worms of the challenge dose remained. Adult sheep and goats were highly resistant to reinfestation, although a relatively small number of worms was still recovered. One sheep was still immune nearly three years after initial infestation. Adult cattle were solidly immune six weeks after immunisation.

The effect of x-irradiation on the viability and fertility of *P. microbothrium* and the immunisation of unweaned calves will be reported on later.

ACKNOWLEDGEMENTS

The Chief, Veterinary Research Institute, is thanked for permission to publish the results and Mr. J. H. D. Maré for his technical assistance.

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J. S. Afr. vet. med. Ass. 36 (3) 1965

SOME PRACTICAL CONSIDERATIONS ON THE PROTECTION OF SHEEP AGAINST PULPY KIDNEY DISEASE

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Systematic research on the protection of sheep against pulpy kidney disease has shown that a serum epsilon antitoxin level of 0.15 I.U. per ml and higher renders them immune[1]. This antibody level can be achieved by the use of epsilon toxoid in various forms. But a search of the available literature does not reveal a schedule of vaccination whereby sheep can be protected continuously. As a result of the lack of such information veterinarians and farmers have thus far applied rather haphazard vaccination regimens and erred on the side of too few injections and, more frequently, on the side of too many.

Pulpy kidney disease is difficult to diagnose and consequently frequently confused with other diseases presenting similar symptoms and post mortem changes. It is a natural tendency for veterinarians to advise farmers to use pulpy kidney disease vaccine when such a confusing disease complex occurs among his sheep. It is equally natural to recommend revaccination with the same vaccine if the mortality does not stop within a reasonable period, since definite information on the reaction following an injection of toxoid, whether it be a booster injection or not, has thus far not been available. There is no doubt that this confusion has resulted in an unnecessary use of vaccine at times.

Since pulpy kidney disease can be responsible for substantial losses among sheep, many farmers have adopted a policy of excessive vaccination in preference to taking any risk.

Furthermore, salesmen are inclined to persuade farmers to buy and use their vaccines in an effort to increase their sales. Unfortunately many farmers succumb to this practice. But with the narrowing of profit margins experienced by our South African farmers at present, the economy of protecting sheep against this scourge becomes of vital importance. Injecting sheep with vaccine implies the employment of labour to muster and handle them. It is, therefore, important to know the minimum number of injections required, and how they should be spaced, to protect sheep continuously. It is equally important to know how long lambs, passively protected through maternal colostrum, can be left before they have to be injected.

In order to provide a solution to many of the questions posed by veterinarians and farmers a series of vaccination experiments were conducted. Sheep were used as experimental subjects so that the results obtained would have direct application. The antigen used was a trypsin-activated, alum-precipitated epsilon toxoid similar to the vaccine issued by Onderstepoort for routine use in the field.

It was found that enterotoxaemia vaccine deteriorates on storage and should not be used later than one year after its preparation. Vaccine distributors, both wholesale and retail, should be encouraged to store their supplies for as short a period as is practically possible.

When sheep are vaccinated against enterotoxaemia for the first time in their life they have to receive two injections. The first and second injection a sheep receives are called the primary and secondary stimuli. A single dose of vaccine produces transitory protection and does not provide the injected animals with a sound basic immunity.

The vaccine as issued from Onderstepoort contains a quantity of antigen per dose far in excess of that required to produce a maximal immunity response. After storage for one year under reasonable conditions the vaccine still contains sufficient antigen to elicit a maximal response.

The interval between the primary and secondary stimuli may vary from two to six weeks without affecting the results. This allows a stock-owner some latitude in arranging the injection of his flock to suit his farming practice.

A striking feature brought out by these experiments is the difference in response by sheep receiving identical treatment. Two sheep receiving 90 Lf of toxoid per dose may show antibody levels of 150 and 10 I.U. per ml of serum.

A disturbing phenomenon demonstrated by these experiments is the fact that, as soon as nine weeks after a secondary stimulus, some sheep have antibody titres below the protective level. When a third injection is given twelve weeks after the second, some sheep are protected for a further period of only twelve weeks. If a fourth injection is given ten weeks after the third, all sheep are protected up to 21 weeks after the last injection. It appears that the period of protection becomes prolonged as the number of injections increase, but at its best the protection does not last for longer than about 21 weeks. These results, no doubt, account for the fact that farmers sometimes complain about losses from enterotoxaemia in spite of having vaccinated their sheep three months previously.

Where the incidence of the disease is seasonal it may be possible that a primary and secondary injection of enterotoxaemia vaccine will tide the animals over the danger period. But under conditions of intensive feeding sheep will have to receive single booster injections about every three months until a year has lapsed from the time of the primary injection.

When sheep have a sound basic immunity (such as is provided by primary and secondary injections of the Onderstepoort vaccine), the immunity following on a booster dose given twelve months after the primary stimulus will remain at a protective level for 12 months.

When, however, sheep are given primary and secondary stimuli, and at one and two years respectively afterwards booster injections, they will remain fully immune for at least three years subsequent to the last injection. This finding is significant to both farmers and veterinarians investigating outbreaks of disease among sheep. Farmers have the assurance that sheep vaccinated for three consecutive years are immune for virtually the rest of their life and no further money and effort should be expended on vaccinating them. Veterinarians can be certain that sheep vaccinated as above will not die of enterotoxaemia and, if mortality occurs, it must be ascribed to some other cause.

Enterotoxaemia does occur in lambs at a very young age and farmers are faced with the problem of having to protect them. In this series of experiments it was shown that the lambs born from ewes receiving a booster injection during pregnancy, are protected up to about 13 weeks. Furthermore it was shown that such lambs need not be vaccinated before they are 10 weeks old which eliminates the objection of having to inject them when too young. The ewes need not be vaccinated later than 4 weeks before parturition since farmers prefer to leave heavily pregnant ewes undisturbed.

The principles emerging from the studies reported in this paper should assist in reducing the uncertainties about the maintenance of an effective immunity against pulpy kidney disease. It is now also possible to work out a schedule of vaccination to suit a particular set of circumstances without applying excessive injections.

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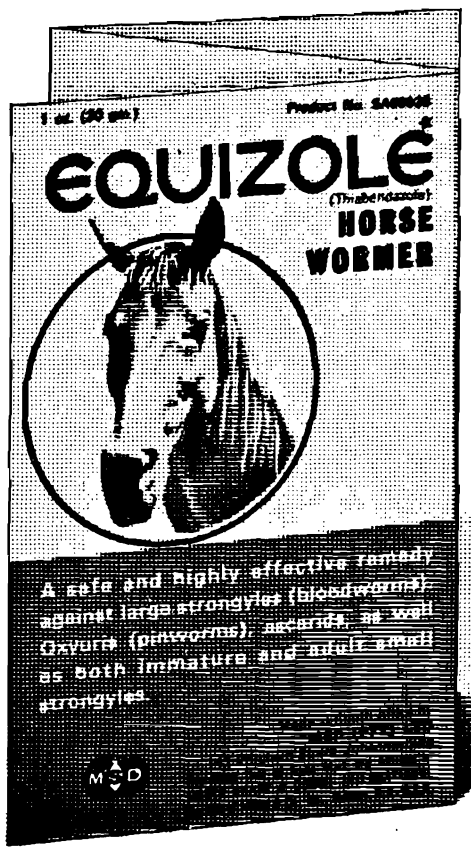

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THE EFFECT OF DIFFERENT LEVELS OF CYANURIC ACID IN SHEEP RATIONS IN WINTER

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SUMMARY

Cyanuric acid was fed at 4 levels (0, 8, 16 and 24 g per head per day) with maize meal as a daily supplement to 4 groups of sheep on a diet of low quality hay (4.31% C.P.) and a lick of equal parts of salt and dicalcium phosphate.

The test ran for 77 days and the results indicated that the daily amount of cyanuric acid required to maintain liveweight was between 8 and 16 g per head. The sheep receiving 24 g increased in weight but at this level of feeding the efficiency of cyanuric acid utilization tended to decrease.

INTRODUCTION

Efficiency in the utilization of urea by ruminants is dependent on the rate at which ammonia is released in the rumen from this source of non-protein nitrogen. When urea is fed in small amounts throughout the day, a large proportion of the ammonia evolved is taken up by the micro-organisms. However when a large single dose of this compound is ingested in a short period, the rate of ammonia release exceeds the rate at which it can be absorbed by the microflora. The excess passes through the rumen wall and is largely no longer available to the animal as a source of nitrogen.[1, 2, 3]

Cyanuric acid (2,4,6-trihydroxy-1,3,5-s-triazine) has been shown to be neither acutely nor cumulatively toxic to ruminants and it has also been demonstrated that this compound can be used by sheep as a source of non-protein nitrogen.[4, 5] It is suggested that the lack of toxicity of cyanuric acid is due to a slow rate of hydrolysis by bacteria. From this it was considered that, by using cyanuric acid as a source of supplementary nitrogen in protein deficient diets, it might be possible to achieve nitrogen retention levels which exceed the demands of maintenance.

A feeding trial was conducted to determine the effects of four levels of cyanuric acid on the liveweight and feed consumption of sheep receiving a ration consisting mainly of low quality hay.

METHODS

Forty maiden ewes aged two years were conditioned to pen feeding for a period of 64 days on a ration of teff hay (4.31% C.P.) and a lick of dicalcium phosphate and salt (equal parts by weight) *ad lib* plus a daily supplement of 114 g yellow maize meal and 20 g biuret per sheep.

At the commencement of the trial the ewes were divided at random into 4 groups. The basal ration of hay and lick was unchanged. Cyanuric acid at 4 levels replaced the biuret in the daily supplement of 114 g maize meal per sheep. (See Table 1).

Throughout the trial the sheep were weighed fortnightly after 16 hours without food and water. The hay and supplements were fed daily and hay residues were weighed daily. Lick consumption was measured weekly.

During the final 3 days of the comparison, all groups were fed the control supplement to eliminate differences in weight which might have resulted from the variation in lick and water intakes between treatments.

The composition of the cyanuric acid used was as follows, (D.M. basis) cyanuric acid 96.56%, urea 1.05%, biuret 2.39%, total N 32.0%.

Results

Table 1.—Liveweights and liveweight changes of sheep and lick and hay consumption by sheep over a period of 77 days

	Group 1	Group 2	Group 3	Group 4
Cyanuric acid (g per head per day)	0	8	16	24
Average initial weight (Kg)	40.45	40.63	41.09	40.00
Average final weight (Kg)	38.59	39.95	41.86	41.31
Average gain or loss (Kg)	−1.86	−0.68	+0.77	+1.31
Average hay intake (g per head per day)	1,035	1,040	1,140	1,099
Average lick intake (g per head per day)	23.53	28.35	42.24	39.41
100 × initial weight/final weight	95.40	98.33	101.87	103.27
*No. of animals	9	10	10	10

**Note:* One sheep in Group 1 was injured and withdrawn.

In the analysis of final weights expressed as a percentage of initial weights, Groups 3 and 4 were significantly ($P = 0.01$) greater than Group 1. Group 4 was significantly ($P = 0.05$) greater than Group 2. All other differences were not significant.

Discussion

The data indicate that the response in hay consumption and liveweight to the additional nitrogen in the form of cyanuric acid increased from 0 to 16 g per sheep per day but the response beyond this level was not of the same magnitude. The reduction in response could be attributed to two factors:—

(*a*) The level of available carbohydrate (maize meal) could have limited the utilization at the highest level of cyanuric acid feeding.

(*b*) The rate of release of ammonia from cyanuric acid was possibly so slow that at the highest level of feeding a portion of the material passed out of the rumen before it was attacked by the micro-organisms.

It is of interest to note that the voluntary lick intakes tended to be correlated with hay consumption. This tendency has been noted previously but it is not known whether the requirements for salt and/or dicalcium phosphate were increased by cyanuric acid *per se* or whether the greater intake was merely a response to increased metabolic activity.

Within the limitations of the trial it can be concluded that cyanuric acid fed at the rate of between 8 and 16 g per sheep per day supplemented the protein deficiency of low quality hay (4.31% C.P.) to produce a maintenance ration for sheep. At a higher level of feeding (24 g per sheep per day) greater nitrogen retention was achieved but with less efficiency.

Acknowledgement

Mr. C. F. Clinning is thanked for his invaluable assistance in conducting this trial.

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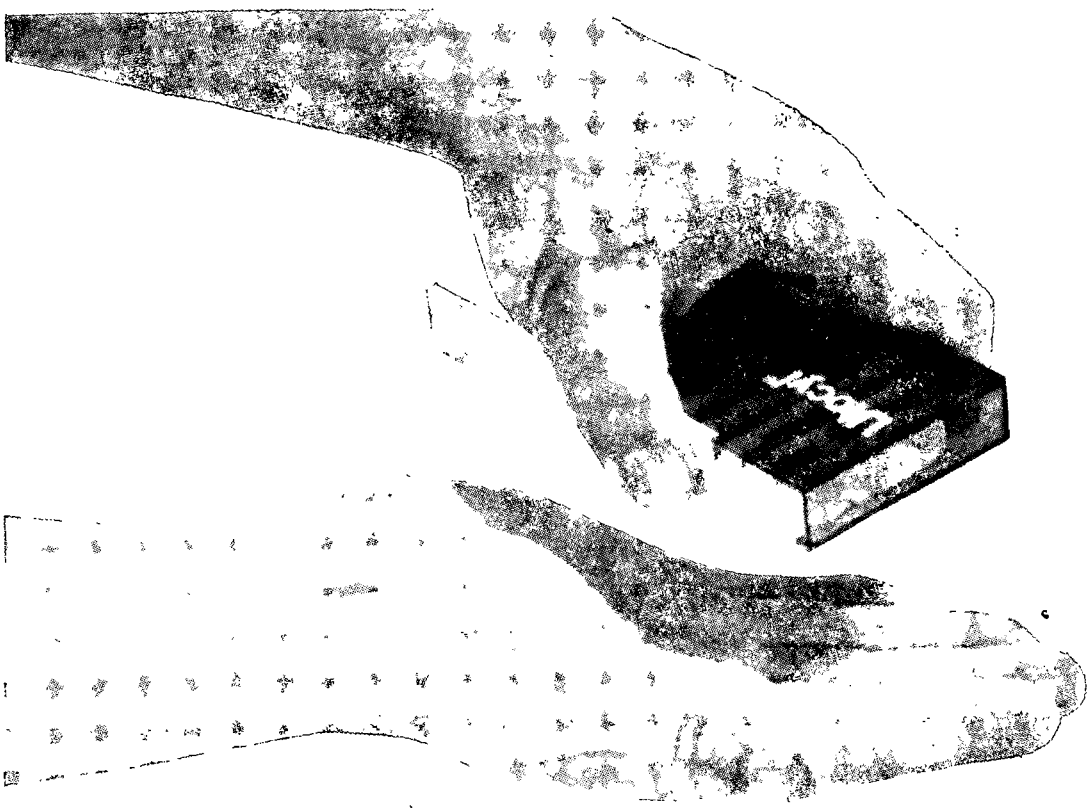

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J. S. Afr. vet. med. Ass. 36 (3) 1965

POLOXALENE AS A PROPHYLACTIC AND THERAPEUTIC AGENT FOR BLOAT IN CATTLE FED ON HIGH CONCENTRATE DIETS

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SUMMARY

Poloxalene (polyoxypropylene polyoxyethylene block polymer), a non-ionic surface active agent, has been found to be an effective agent for acute frothy bloat in cattle on high concentrate diets. The poloxalene was administered daily, either as a premix added to the feed, or as a solution by drenching. The solution, when administered as a drench, has also been found to be an effective therapeutic agent for the same condition.

It is generally accepted that frothing of the ruminal ingesta is by far the most important cause of pasture and feed lot (concentrate) bloat and that acute frothy bloat is a condition of major economic importance in South Africa.[1]

Bloat in South Africa is generally a problem in cattle grazing on lucerne and/or clover pastures, but in recent years there has been an increasing interest in feeding cattle on high concentrate diets when bloat also emerges as a problem from time to time.

Bartley[2] using identical twin cows with rumen fistulas, showed that poloxalene (polyoxypropylene polyoxyethylene block polymer),* a non-ionic surface active agent, was effective in preventing legume bloat for up to 12 hours. The poloxalene had no adverse effects upon feed intakes, health, reproduction or milk production. Meyer, Helmer and Bartley[3] found that when large quantities of C^{14} labelled poloxalene was administered to cows, no C^{14} was excreted in the milk or was present in the blood and tissues.

The use of poloxalene for the prevention and treatment of bloat in cattle being fed on high concentrate diets is reported.

* Smith, Kline & French compound No. 18667.

MATERIALS AND METHODS

Poloxalene is a bitter tasting liquid with a high molecular weight.

Experiment 1

(*a*) Poloxalene—30 ml. of a solution of 1 part poloxalene to 2 parts of water w/w was used as a drench for prophylaxis and therapy.

(*b*) Experimental Design—Twenty two cattle, 13 to 14 months of age, were assigned to 2 groups of 11 head each. The experiment was divided into 3 periods as follows:

	Group A	*Group B*
Period 1–13 days	treated daily	no treatment
Preiod 2–1 day	no treatment	no treatment
Period 3–13 days	no treatment	treated daily
Mean weight at start of trial	660 lbs.	690 lbs.

(*c*) Feed—The only feed presented consisted of a mixture of equal parts of chaffed lucerne hay and a concentrate meal, containing:

yellow maize meal	60 per cent
ground nut oil cake	10 per cent
sunflower oil cake	10 per cent
fish meal	10 per cent
molasses	4 per cent
wheaten bran	10 per cent
salt	2 per cent
dicalcium phosphate	2 per cent

(*d*) Stable routine:

7 a.m. poloxalene administered
7 a.m. to 11 a.m.—free access to feed only
11 a.m. to 1 p.m.—free access to water only
1 p.m. to 4 p.m.—free access to feed only
4 p.m. to 7 a.m.—free access to water only

Experiment 2

(*a*) Poloxalene—the solution was identical to that used in Experiment 1 and was also administered as a drench, but the dose was increased to 60 ml.
The premix was prepared to the following formulae:

poloxalene —36 per cent by weight
vermiculite —47 per cent by weight
lecithin —17 per cent by weight

One ounce of the premix containing 10 g. of poloxalene was poured over the pelleted feed.

(*b*) Experimental Design.—Ten head of cattle were assigned to 2 groups of 5 head each. Group C received the poloxalene solution and Group D received the poloxalene premix. The experiment consisted of two periods, as follows:

	Group C	*Group D*
Period 1–14 days	no treatment	no treatment
Period 2–14 days	poloxalene solution	poloxalene premix

(*c*) Feed.—The only feed presented consisted of pellets composed of:

yellow maize meal	29 per cent
wheaten bran	15 per cent
ground nut meal	5 per cent
sunflower meal	5 per cent
fish meal	1 per cent
dried brewers grain	5 per cent
lucerne meal	35 per cent
salt	1 per cent
molasses	3 per cent
dicalcium phosphate	1 per cent

(*d*) The stable routine was the same as in Experiment 1.

TABLE 1.—THE EFFECT OF THE DAILY ADMINISTRATION OF A SOLUTION OF POLOXALENE ON THE INCIDENCE OF BLOAT IN CATTLE ON A HIGH CONCENTRATE DIET.

Group	Period	Treatment	Number of cases of bloat		Number of cases Requiring Treatment
			Severe	Moderate	
A	1 (13 days)	none	6	9	6
A	2 (1 day)	none	0	1	0
A	3 (13 days)	poloxalene solution	2	0	2
B	1 (13 days)	poloxalene solution	0	0	0
B	2 (1 day)	none	1	0	1
B	3 (13 days)	none	3	1	3

RESULTS

Experiment 1

The results of Experiment 1 are presented in Table 1. It will be noted that there were 10 cases of severe bloat and 11 cases of moderate bloat during the periods of no treatment. Nine of the 10 cases of severe bloat were successfully treated with 30 ml. of the poloxalene solution administered as a drench. The one case of severe bloat which occurred in Group A during period 2, did not respond to treatment with either poloxalene solution or another anti-foaming agent. A trocar and canula had to be used to release the gas.

Two cases of severe bloat occurred in Group B during the period of treatment. One case was successfully treated with poloxalene solution, the other case was similarly treated but the bloating had to be relieved with a stomach tube.

TABLE 2.—THE EFFECT OF THE DAILY ADMINISTRATION OF A POLOXALENE PREMIX AND SOLUTION ON THE INCIDENCE OF BLOAT IN CATTLE ON A HIGH CONCENTRATE DIET

Group	Period	Treatment	Number of cases of Bloat	Number of cases requiring treatment
C	1 (14 days)	none	62	54
C	2 (14 days)	poloxalene premix	17	1
D	1 (14 days)	none	56	52
D	2 (14 days)	poloxalene solution	31	5

Experiment 2

The results of Experiment 2 are presented in Table 2. All the animals which required therapeutic treatment were given 4 fluid ounces of raw linseed oil.

DISCUSSION

Poloxalene has been shown to be an effective compound for the prophylaxis and therapy of acute frothy bloat in cattle and offers several distinct advantages over compounds in current use. The dose is small, the action persists for up to 12 hours, no side effects have been observed and no residues have been detected in milk, blood or tissues of treated animals. The bitterness of the compound, which some animals appear to find unplatable, is a disadvantage. These animals which appear to be poor feeders, will eat the treated feed after a short period of adaptation.

The poloxalene solution in the first experiment and the poloxalene premix in the second experiment were most effective in reducing the incidence of bloat in cattle on high concentrate diets. The poloxalene solution in the second experiment, despite double dosage, was not as effective. A possible explanation for the poorer results obtained with this solution is that aqueous solutions of poloxalene have been found to be unstable. The incorporation of an antioxidant with the poloxalene has overcome this problem.

The majority of cases of bloat in cattle are due to frothing of the ruminal ingesta and the administration of a surface active agent will break down this froth and enable the animal to eructate the gas. In some cases of bloat, although the gas is free (the froth may have been broken down by a surface active agent) the animal is unable to eructate the gasses due to overfill or paralysis of the rumen or some other cause. Two such cases occurred in the first experiment. In one case the gasses could be released by stomach tube but in the other case recourse had to be made to the use of a trocar and canula.

Two cases of severe bloat occurred in animals dosed with poloxalene in Experiment 1. One of the cases has been referred to above, the second case responded to the administration of a further 30 ml. of poloxalene solution. These apparent failures are probably due to inadequate dosage. It was occasionally observed that an animal did not always swallow the whole dose but would allow some of the solution to run out of its mouth.

An observation made before the commencement of these experiments was that many of these cases of bloat would respond to either the administration of an anti-foaming agent or the use of a stomach tube. This suggests that, in these conditions, the bloating was due to a combination of factors; the frothing of the ruminal ingesta acting in the same manner as over-filling of the rumen and thus preventing the eructation of the free gas trapped above the ingesta.

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J. S. Afr. vet. med. Ass. 36 (3) 1965

AN INVESTIGATION INTO A PROBLEM OF URINARY CALCULI IN MERINO WETHERS

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SUMMARY

Mortality as result of urinary calculi occurred in a high level diet group of sheep during an investigation on the influence of nutritional level on the wool of Merino wethers. Feed analyses revealed that the high level group had received more protein, phosphorus and magnesium, but less calcium, than the low level group. The differential mineral levels were also reflected in the blood analyses. It is concluded that the excess blood phosphate and magnesium was excreted in the urine, resulting in the formation of magnesium phosphate calculi.

INTRODUCTION

Five months after commencement of an experimental investigation into the influence of a high and a low level diet on the wool of Merino wethers[1], two sheep on the high level diet died from obstruction by urinary calculi and one sheep was found to have a calculus lodged at the root of the *processus urethralis*.

It was decided that chemical analyses of the feed, blood and calculi could offer an explanation for the formation of the calculi in animals of the high level diet group.

MATERIAL AND METHODS

Twenty Merino wethers had been divided into two equal groups. Group A had received a highly nutritious diet and group B had received a low level diet. They had been kept indoors in pens on slatted floors. The feed consumption per sheep was established over the period 17.3.1964 to 10.8.1964, by which time group A had been reduced to six sheep as result of mortality.

Each component of the feed was analysed separately for protein (on an air-dry basis), calcium, phosphorus and magnesium.

The blood was collected in heparin and analysed by the following methods:

(i) Calcium: Both the titrimetric method of Clark and Collip[2] and the colorimetric method of Ferro and Ham[3] were employed.

(ii) Inorganic phosphate: Analysed by the colorimetric method of Fiske and Subbarow.[2]

(iii) Magnesium: Analysed by the colorimetric method of Neill and Neely.[4]

(iv) Blood glucose: Analysed by the colorimetric method of Folin and Wu.[2]

(v) Blood urea nitrogen: Analysed by the titrimetric method of Brown.[5]

(vi) Erythrocyte and leucocyte counts were done by means of a Spencer haemocytometer.

(vii) Haematocrit determinations were done according to the Wintrobe method.

(viii) Haemoglobin was determined according to Drabkin's cyanmethaemoglobin method.[2]

The calculi were analysed qualitatively.

RESULTS

The composition and mineral analysis of the feeds are shown in table 1, the total amount of feed, protein, calcium, phosphorus and magnesium consumed per sheep is given in table 2 and the results of the blood analyses in table 3. In

the latter respect there were no significant differences as regards blood glucose, urea nitrogen, erythrocyte count, leucocyte count, haematocrit and haemoglobin values.

TABLE 1.—COMPOSITION AND MINERAL ANALYSIS OF THE FEEDS

Components	High level	Low level	Protein	Calcium	Phosphorus	Magnesium
	lb	lb	%	%	%	%
Oat Hay	3	100	3.0	0.17	0.17	0.06
Mealie Meal	110	30	9.6	0.03	0.35	0.14
Lucern Meal	80	60	11.9	0.90	0.21	0.21
Oat Meal	4	4	9.8	0.12	0.31	0.10
Lupin Seed Meal	3	6	24.3	0.24	0.33	0.18
Bonemeal*	—	—	—	22.96	10.25	0.35

*Formed 55% of a mineral mix of which 1 ounce was added to every 3 lb of feed.

TABLE 2.—TOTAL AMOUNTS OF FEED, PROTEIN, CALCIUM, PHOSPHORUS AND MAGNESIUM CONSUMED PER SHEEP

Component	High level feed	Low level feed
	lb	lb
Total food	418.29	442.53
Protein	44.53	32.91
Calcium	2.71	2.80
Phosphorus	1.71	1.36
Magnesium	0.73	0.55

TABLE 3.—THE ANALYSES OF THE BLOOD OF THE TWO GROUPS

	High level group		Low level group	
	Average	Range	Average	Range
Calcium mgm%	8.37	6.12–11.56	11.63	11.00–12.80
Inorg. Phosphate mgm%	11.84	9.45–12.92	6.72	5.23–8.27
Magnesium mgm%	3.54	2.75– 4.50	2.20	1.60– 2.70
Glucose mgm%*	40.88	37.25–45.20	36.92	32.52–39.00
Urea Nitrogen mgm%*	21.60	14.40–32.40	18.54	14.40–25.20
Erythrocyte Count X10⁶/cub. m.m.*	6.63	6.27– 7.22	6.13	5.57– 6.67
Leucocyte Count/cub m.m.*	9208	8800–9650	8685	7250–10050
Haematocrit %*	37.80	36.00–41.00	38.20	31.00–44.00
Haemoglobin Gm%*	10.30	8.70–11.60	10.10	8.90–11.60

*No significant differences between the groups.

The qualitative analysis of the calculi showed them to contain phosphorus, magnesium and a slight trace of calcium and were thought to be either magnesium phosphate or magnesium ammonium phosphate.

From tables 2 and 3 it was calculated that on an average each animal in Group A consumed:

32.11% more protein
3.21% less calcium
25.73% more phosphorus
32.73% more magnesium

and its blood contained:

28.03% less calcium
76.19% more inorganic phosphate
60.91% more magnesium

than the average animal in group B.

Discussion and Conclusions

It is concluded that in this experiment there was a direct influence of the diet on the blood picture, and that this was so marked that the normal calcium: phosphorus ratio of the blood of ± 2:1 was reversed in Group A to 0.73:1 as opposed to the Group B ratio of 1.17:1.

It may be deduced that in order to compensate for this imbalance, the animals in the high level group excreted the excess phosphate and magnesium in the urine, resulting in the formation of the magnesium phosphate calculi.

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OBSERVATIONS ON THE POST NATAL DEVELOPMENT OF THE PENIS IN MERINO RAM LAMBS AND WETHERS: THE POSSIBLE RELATIONSHIP TO THE PASSAGE OF URINARY CALCULI

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SUMMARY

Birth weight and weight gain have a significant positive effect on the resolution of the infantile adhesion of penis to prepuce in Merino ram lambs, irrespective of the age of the animal. Castration arrests the resolution of the adhesions if done at three to six weeks of age. The bearing of these observations on the obstruction caused by urinary calculi is considered.

INTRODUCTION

During the course of the investigation of the occurrence of urinary calculi in a group of merino wethers, clinical examination of these animals revealed that on everting the prepuce, the *processus urethralis* was found to be embedded in, and adherent to, the preputial mucous membrane, while the penis itself could not be differentiated due to fusion with the prepuce. These adhesions could be broken down only with difficulty and resulted in profuse haemorrhages.

As the infantile state of the penis, with the concomitant adhesions, could possibly play an important role in the retardation of the passage of urinary calculi, resolution of the adhesion was the subject of the investigation described below.

MATERIAL AND METHODS

A total of 444 Merino and German Merino sheep selected at random and comprising 150 wethers, 18 cryptorchids, 20 rams and 256 ram lambs were examined by everting the prepuce and recording the presence of adhesions. These animals were examined in flocks in the Stellenbosch, Malmesbury, Caledon, Middelburg and Bedford districts.

Concurrently the birthweight, average daily weight gain over the first 100 days of life, and age at the time of examination, in 68 German Merino ram lambs, maintained at the Stellenbosch-Elsenburg College of Agriculture, were recorded for the purpose of statistical analysis. The animals were divided into two groups, namely group X consisting of 14 head, in which any stage of fusion was still present, and group Y consisting of 54 head, in which there was complete separation of penis and prepuce.

RESULTS

1. WETHERS: One hundred and fifty wethers of all ages were examined. Of these 148 had been castrated at the age of three to six weeks and in all the animals the *processus urethralis* and penis were found to be completely fused. The other two sheep, aged two years, had been castrated with a Burdizzo when four months old; in these two animals there was complete separation of the penis and *processus urethralis* and these organs were comparable in size to that of a ram of similar age.

The adhesions were broken down in 50 of the wethers and treated with proprietary barrier creams. No recurrence of adhesions were noted after a period of four months had elapsed.

2. CRYPTORCHIDS: Eighteen cryptorchids of all ages were examined. In 15 there was complete separation of the penis, while fusion was present in the remaining three. These three were very young and underdeveloped.

3. RAMS: Twenty rams, two of which had been vasectomised, were examined, and in these animals a normal free-lying penis was found.

4. RAM LAMBS: The ram lambs, 256 in number, varied in age from 14 to 240 days old. In 166 of these there was complete separation, while in 90 animals varying stages of separation were noted, i.e. from complete fusion to where the shaft of the penis and prepuce only were still fused.

A salient feature of the results recorded in the examination of the animals described above, was that there appeared to be definite correlation between early separation of the genital organs and bodyweight gains.

To confirm these findings observations on the Stellenbosch-Elsenburg flock of 68 German Merino ram lambs were subjected to statistical analysis, as explained above. The mean age in days of group X (fourteen lambs in which resolution was incomplete) was 150 with a range of 141–166, whilst that of group Y (54 lambs in which resolution was complete) was 152 with a range of 139–165. This difference did not prove to be of any significance.

The mean birthweight of group X was 8.0 lbs with a range of 5.0–11.0, and that of group Y 10.5 lbs with a range of 6.0–15.0, a difference significant at the 1% level.

The average weight gain in pounds per day had a mean value of 0.40 lbs; with a range of 0.24–0.59 in the case of group X. in the case of group Y this amounted to 0.63 lbs, with a range of 0.47–0.83. The difference between the two groups was found to be highly significant at the 1% level.

DISCUSSION

Both Johnstone[1] and Arey[2] referred to the work of Hunter[3] who concluded that during the development of the male generative organ, the penis is at first naked and the prepuce gradually extends over it, becoming adherent to it. Johnstone further referred to Burrows[4] who stated that at an early stage the balano-preputial fold consists of embryonic epidermis only a few cells thick and that, during development, which varies for different species, this tissue becomes changed into a stratified squamous epithelium. Later, the middle layer of these epithelial cells undergo keratinization, and these keratinized cells no longer adhere to each other, so that the layer of epithelial cells covering the glans gradually separates from the layer of cells lining the prepuce.

Johnstone concluded that in well-grown Merino ram lambs this resolution was complete at about five months of age, but that under poor nutritional conditions it generally took longer. He further stated that castration arrested both the mucosal resolution and development of the penis, with the result that in the adult wether the penis resembled that of the uncastrated lamb 15 weeks of age or younger.

Wiggens and Terrill[5] determined the influence of various factors upon the post-natal development of the penis in the Columbia, Targhee and Rambouillet breeds of ram lambs and agreed with the statements of Johnstone that the mucosal resolution was influenced by the testicular hormones and that the live bodyweight played a dominant role in this process.

Watson, Sapsford and McCance[6] found a correlation between the weight of the testes, their general histological characteristics and bodyweight in Merino ram lambs. These authors found that the critical weights were from 18 to 27 kg and that little separation of the prepuce from the penis had occurred in animals weighing less than 18 kg, while separation was complete in ram lambs over 27 kg, irrespective of the age at which this weight was attained.

The results of this investigation indicate that the age of the animal is not of critical importance, whereas birthweight and weight gain must by inference be correlated to testicular function which results in the mucosal resolution.

Although only a few cryptorchids were examined, the indications were that in the cryptorchid the development of the penis followed the same course as that in the ram.

Excluding the two animals previously mentioned, all the wethers examined were castrated at the age of 3 to 6 weeks, at which stage resolution had not yet commenced, with resultant non-

development of the genitals. All had infantile organs which were enveloped by the adherent prepuce. It is reasonable to assume that this underdevelopment and attachment of the organs predisposes to occlusion of the urethra by urinary calculi to a greater extent than in the free-lying, well developed penis of the ram. Further work is necessary to evaluate the practical significance of castrating rams after the penis is fully developed and free-lying, especially in those areas where the incidence of urinary calculi causes such havoc amongst wethers.

Conclusions

1. At birth the penis of a lamb is in an infantile state and is enveloped by and adherent to the prepuce.

2. This adhesion resolves under the influence of the testicular hormones.

3. Birth weight and weight gain have a significant bearing on the resolution of the adhesions, irrespective of the age of the animal.

4. Castration arrests the resolution of the adhesions and the subsequent development of the penis, with the result that the penis of the adult wether, if castrated at three to six weeks of age, resembles that of a newborn lamb.

5. It is surmised that this arrested development of the penis is of considerable importance in arresting the passage of urinary calculi.

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'N VERSLAG OOR DIE VERSAMELING VAN SAAD VAN 'N SWART RENOSTERBUL (*DICEROS BICORNIS*)

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Nasionale Dieretuin van Suid-Afrika, Pretoria*

OPSOMMING

'n Eenvoudige masturbasiemetode word beskryf waarvolgens semen van 'n swartrenosterbul, van die Nasionale Dieretuin in Pretoria, gekollekteer was.

SUMMARY

A simple masturbation technique is described whereby semen was collected from a Black Rhinoceros bull in the National Zoological Garden, Pretoria.

INLEIDING

Enige verwysing na 'n effektiewe metode om saad van 'n swartrenoster te kollekteer, kon nog nie opgespoor word nie.

Alhoewel die swartrenosterkoei van die Pretoriase Dieretuin gereeld bronstig geword het, kon die swartrenosterbul nog nie daarin slaag om haar te dek nie. Hierdie bul is, soos die meeste ander swartrenosters, agressief, maar hy laat 'n mens dikwels toe om aan hom te vat.

Die gedagte het ontstaan om die koei kunsmatig te insemineer. Aanvanklik was daar gepoog om saad met behulp van kunsvagina's (perd- en beestipes) te kollekteer, maar om praktiese redes moes hierdie metode laat vaar word. Die natuurlike kromming van die renoster se penis (fig. 1) het die gebruik van die bogenoemde kunsvagina's onmoontlik gemaak. Die renosterbul kon op geen manier beheer word nie en dit sou 'n gewaagde onderneming wees om die bul elektries te stimuleer. Met die metode, wat hieronder beskryf word, was drie semenmonsters op drie verskillende geleenthede versamel. (Sien tabel).

Fig. 1

Fig. 2.

METODE

Die renosterbul is na die heining gelok waar hy 'n bietjie meel ontvang het. Die bul is dan in die lies, om die skede en oor die perineum

*Huidige adres: Nasionale Kruger-Wildtuin, Pk. Skukuza.

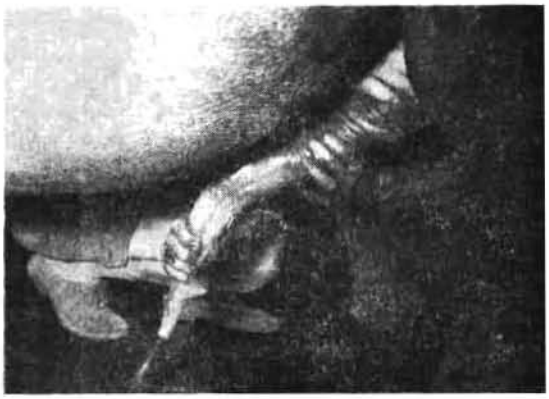

Fig. 3.

gekrap totdat die penis deur die skedering verskyn het. Die een hand word dan versigtig in die skede opgedruk en die penis word vasgevat en verder uitgetrek. Terwyl die penis daarna met die een hand aan die vlerkvormige verdikkings vasgevat word, word die punt van die penis met die ander hand in 'n afwaartse rigting masseer (fig. 2). Terwyl die penis verleng, word die proksimale gedeelte uit die skede getrek. Hierdie gedeelte van die penis bevat 'n klomp kronkelende, vlak bloedvate. Sodra druk oor hierdie bloedvaatryke gedeelte toegepas word swel die penis baie vinnig en neem die penis 'n meer reguit vorm aan (fig. 3).

Wanneer hierdie stadium van ereksie bereik word, word 'n latex tregter met 'n gegradueerde proefbuis aan die punt oor die penis gevou en die penis word aanhoudend van agter na voor gemasseer. Die renoster trap baie rond en jaag 'n mens dikwels uit die kamp. Dit was dus soms nodig om die werk vir 'n kort rukkie te staak totdat die bul weer kalmeer het. Die bul moes gewoonlik vir meer as 15 minute gemasseer word voordat enige saad opgevang kon word.

Resultate

Die volgende monsters was op drie verskillende geleenthede gekollekteer:

Datum	Tyds-duur van Massering	Volume van Monster	pH	Kleur	Digtheid
Nov. '64	min. ±20	15 ml.	8.0	Geel	Dun melkerig
Mrt. '65	15	3 ml.	—	Geel	Dun melkerig
Apr. '65	45	3 ml.	7.3	Geel-bruin	Dik melkerig

By mikroskopiese ondersoek van die monsters was daar baie dooie spermatozoa, plaveiepiteelselle en neutrofiele gevind. Die spermatozoa het oor relatiewe lang sterte beskik en die ovaalvormige spermkoppe was relatief skerp.

Bespreking

Hierdie voorlopige resultate dui slegs daarop dat dit wel moontlik is om deur middel van massering van die penis semen van 'n swartrenoster te kollekteer. Die saadmonsters was van 'n swak gehalte, maar dit sluit nie die moontlikheid uit dat beter monsters deur die toepassing van hierdie metode verkry kan word nie.

PRELIMINARY REPORT ON THE TRANSMISSION OF FILAROIDES OSLERI (Cobbold, 1879) IN DOGS

J. E. DORRINGTON

Private Practitioner, 4 Kort Street, Bellville, C.P. Received for publication, April, 1965

The life-cycle of the nematode worm, *Filaroides osleri* is unknown. Various workers,[1, 2] have unsuccessfully attempted to transmit the disease and it has been assumed that as with those of closely related species, some invertebrate vector probably plays a rôle. The purpose of this report is to describe two successful transmission experiments thereby indicating the probability of a direct life cycle.

In the first experiment a non-infected bitch was kept under fly and insect proof conditions. She produced a litter of eight pups (four of each sex). The bitch pups were given an average *daily* dose of 50 eggs *per os* for 14 consecutive days commencing from the date of birth. The eggs were obtained from nodules removed on alternate days from infected bitches and kept in N-saline at room temperature. All eight pups were reared together under the insect proof conditions and examined at four months of age using the direct illuminated bronchoscopic technique. Three of the four bitch pups had numerous well developed lesions in their tracheae yielding numerous eggs on microscopic examination, the fourth bitch pup remained as yet unaffected whereas all four male pups were free of any signs of infection.

In the second experiment all the conditions were identical excepting that the dosing was reduced to 100 eggs approximately at weekly intervals for three weeks commencing at the time of birth. Examination at nearly four months of age showed two pups to have already developed a *few small* nodules, the others were regarded as suspicious whereas all the controls were free of any signs of infection.

From earlier observations,[3] it was apparent that pups picked up the infection at a very early age whereas older pups or adult dogs remained much more resistant to infection. The possibility of some anatomical peculiarity in newly born pups enabling the larvae of *F.osleri* to establish themselves more easily, may account for the success of the abovementioned experiments. This possibility is now being investigated.

Further research is also being undertaken at present but from these two successful transmission experiments, it would appear that infection can take place without any invertebrate intermediate vector.

ACKNOWLEDGEMENTS

This work is supported by a grant from the University of Pretoria and will form part of the thesis for the D.V.Sc. degree.

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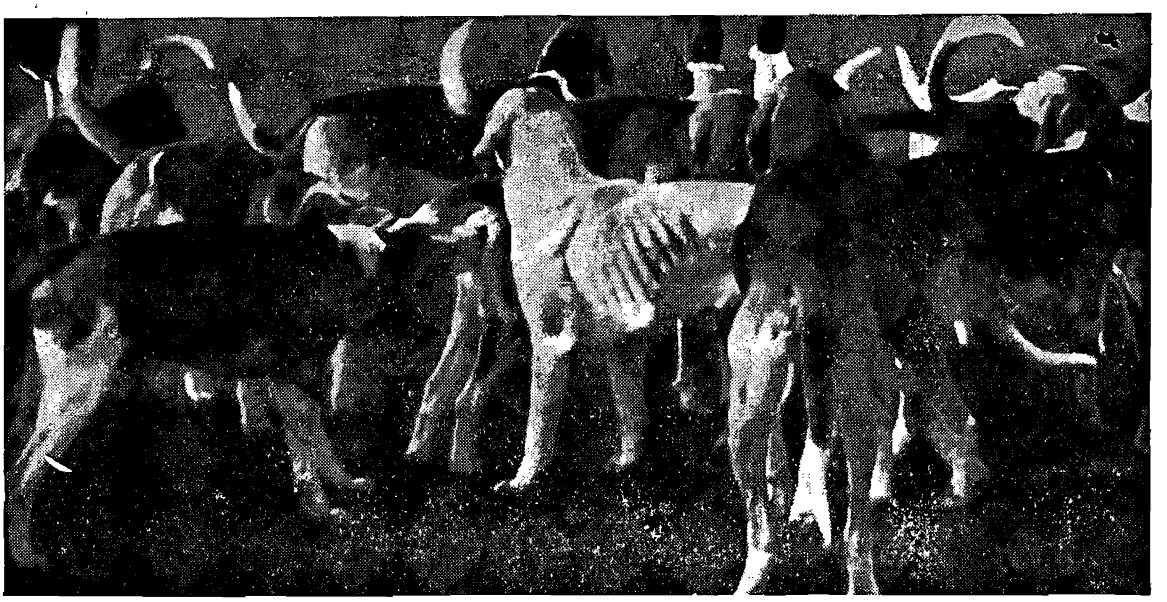

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A NOTE ON THE OCCURRENCE OF TRANSMISSIBLE ENTERITIS (MONOCYTOSIS) OF POULTRY IN THE WESTERN CAPE

P. M. S. MASTERS

Veterinary Investigation Centre, Stellenbosch

This disease is characterised by symptoms of sudden anorexia, watery diarrhoea, cyanosis of the head and general depression. Mortality is usually low (5%) with 20% morbidity accompanied by a severe drop in egg production and is often complicated by a partial moult. The course of the disease is usually 1 to 2 weeks while egg production usually takes 4 to 6 weeks to return to normal.

The term "transmissible enteritis" for this disease syndrome is preferred by many investigators since it implies that the disease is caused by an infectious agent whereas Monocytosis is not pathognomonic for this condition since an increase in monocytes also occurs in Newcastle disease and acute tuberculosis. Unfortunately the name has stuck and is unlikely to be changed.

Although the disease was originally believed to be a metabolic condition, Sieburth[1] and Watanabe[2] have proved the existence of a filterable virus-like organism as the causative agent with intestinal bacteria as complicating factors. The filterable agent, when inoculated, has caused the disease, and transmission of immunity from parents to offspring has been demonstrated. Repeated attempts to isolate the causative agent from the blood or organs of infected birds have failed, whereas embryonated egg preparations containing contaminating intestinal bacteria have been found highly infective. It is considered possible that an endotoxin is liberated due to lysis of the enteric group of streptococci.

Two severe outbreaks of the disease occurred in the Western Cape, in February, 1964. Both outbreaks were preceded by a severe heat wave in the area with temperatures of 102°F being recorded.

Farm A

The adult birds on this farm number 26,000, most of which are kept in Californian houses. Replacements are reared from day-old to 4½ months on a seperate holding 7 miles away.

On 16th January, 1964, 5,000 four-month-old Rhode Island Red replacement pullets were brought over to the parent farm.

Two weeks later it was noticed that these pullets were not developing as expected and that the mortality rate was abnormal. Birds of the same age group which had been brought to the parent farm at the same time and placed on open range under arcs showed no symptoms.

Six live pullets were brought to the Veterinary Investigation Centre for examination. Two birds were slaughtered immediately and examined *post mortem*. On gross macroscopic examination, pancreatitis and soiling of the vent feathers was detected. No other lesions were found. Two birds were slaughtered one week later. On necropsy, pancreatitis, catarrhal enteritis with mucoid casts in the ileum and petechial haemorrhages of the abdominal fat were observed. Five days later, one of the remaining two birds died and on autopsy the classical symptoms of uric nephritis (viz. pin head sized crystals of uric acid on the kidneys, liver, heart and peritoneum) were present. The remaining bird was slaughtered two days later and similar lesions were found. During the course of the disease 150 pullets died and 400 were culled, and total loss was equivalent to 11%.

Farm B

This farm has a total poultry population of 5,000 birds. The disease affected pullets aged

5 months kept in houses 250 yards from each other. Older birds in houses adjoining those containing the affected birds did not contract the disease.

The pullets in different houses were affected by the disease as follows:

1. *Californian House No.* 8.—Contained 500 White Rock pullets aged 5 months of which 9 died and 50 were clinically affected (11.8%).
2. *Californian House No.* 12.—Contained 130 White Leghorn pullets aged 5 months of which 8 died and 10 were sick (13.8%).
3. *Battery Laying House.*—Contained 1,272 first Cross pullets of which only 6 died (0.47%).

Comment

On Farm A it was recommended that a medicated mash of 200 gms oxytetracycline/ton of feed be fed for a period of 5 days following which the antibiotic was reduced to 100 gms/ton of feed for the next 10 days.

Mortality ceased after the 6th day of medication and by the 12th day no further culling was necessary.

On Farm B no treatment was given to the birds in the battery laying cages because of the sporadic and isolated incidence of the disease. The birds in the two Californian houses were fed furazolidone at a rate of 200 gms/ton for 10 days. Mortality ceased 12 days after medication was commenced.

It is doubtful that the infective agent is waterborne, otherwise a much higher incidence of the disease could have been expected in the battery house on Farm B. Transmission by the droppings is most probable, especially so in intensive houses where birds are on litter in close contact with one another.

Although mortality is usually low (5%), the disease is of economic importance because of the serious drop in egg production and concomitant moult, so that in some outbreaks it may take months before production returns to normal. Early diagnosis is essential if a severe set-back in egg production is to be avoided. By the time that the classical symptoms of uric nephritis are noticed on necropsy, the disease is usually too far advanced to be satisfactorily controlled by mash medication.

For early diagnosis particular attention should be paid to the following:

1. Soiling of the vent feathers — usually yellow green in colour.
2. Petechial haemorrhages in the abdominal fat.
3. Pancreatitis—instead of the normal pinkish grey colour, this organ has a chalky appearance due to necrosis.
4. Catarrhal enteritis of the ileum—the contents of which consist of casts of thick mucus resembling curdled milk.
5. Oophoritis.
6. Uric nephritis (visceral gout).

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ACKNOWLEDGEMENTS

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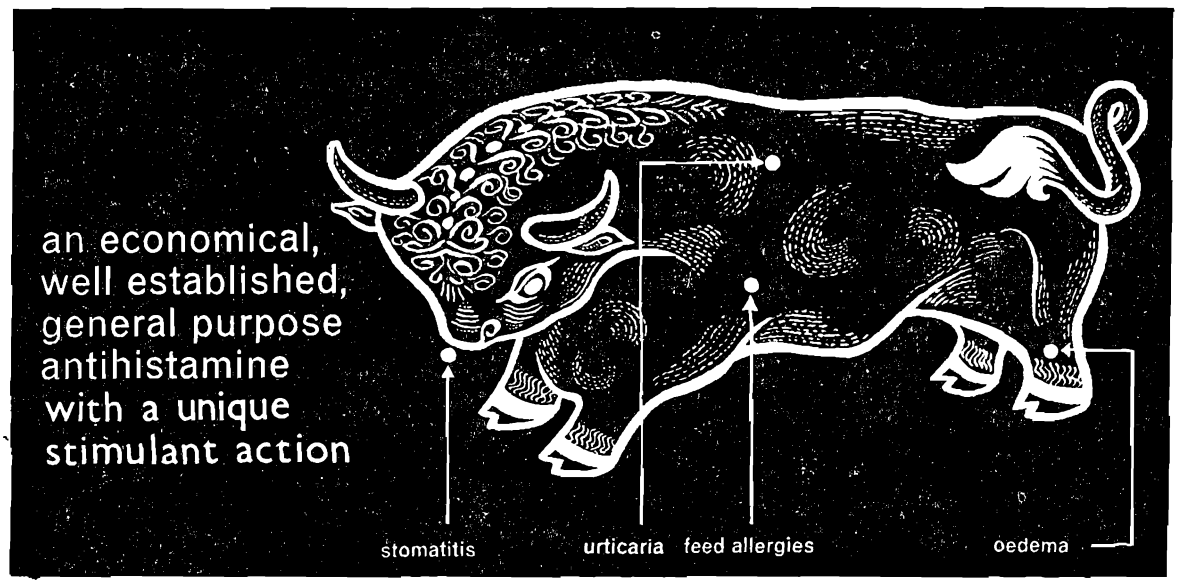

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INVESTIGATIONS ON THE SENSITIVITY OF TUBERCULOUS AND NON-TUBERCULOUS CATTLE TO VARIOUS MYCOBACTERIAL SENSITINS

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SUMMARY

The results of an investigation on herds in which non specific reactions interfered with the interpretation of tuberculin tests is given. Cattle from these herds were tested with P.P.D. sensitins prepared from *M.bovis*, *M.avium*, *M.kansasii*, *M.smegmatis*, *M.fortuitum*, a scotochromogenic strain, and an unclassified strain isolated from a bovine "skin lesion". The multiple comparative test easily recognises sensitization caused by bovine tuberculosis. The sensitivity pattern in 16 herds indicates that the cattle in these herds were sensitized by *M.avium* or closely related avian-like strains. The comparative avian-bovine tuberculin test could be used to great advantage in South African herds where non-specific reactions cause problems of interpretation of tuberculin tests.

INTRODUCTION

Non-specific reactions to mammalian tuberculin in cattle are usually caused by infection with *Mycobacteria* which are related to M. bovis. The most common are *M.avium*, *M.tuberculosis*, acid fast "skin lesions" organisms and *M.paratuberculosis* in countries where Johnes disease commonly occurs.[1, 2, 3] Infections with these organisms may cause sensitization without any apparent lesions, small regressive lesions,[4, 5] in rare cases progressive disease[6, 7, 8, 9] or lesions typical of the specific disease as in Johnes disease and "skin lesions". The so-called atypical Mycobacteria can also sensitize animals to bovine tuberculin[10] and natural infection with similar organisms may cause granulomatous lesions and sensitivity to tuberculin in cattle.[11]

In some countries the occurrence of non-specific reactions reaches such proportions[12, 13, 14] as to seriously interfere with the efficacy of the single intradermal test when mammalian tuberculin only is used. The comparative test using avian and bovine tuberculin simultaneously is therefore frequently used for distinguishing non-specific reactors from tuberculous cattle.[12-19]

In South Africa our knowledge of the occurrence of non-specific reactors is limited. Extensive tuberculin testing, in an organized tuberculosis eradication campaign has not yet been started, and detailed analysis of the available data has not been undertaken. Problem herds, in which the occurrence of non-specific reactors makes the interpretation of tuberculin tests difficult are, however, encountered. Skin lesions occur quite frequently in some areas and both *M.avium*[20] and *M.tuberculosis*[21] sensitization have been demonstrated. *M.kansasii*[22] non-chromogens,[23] scotochromogens, *M.fortuitum* and *M.phlei*[20] have been isolated from cattle tissues in this laboratory. Lambrechts and Kleeberg have isolated a number of strains of Mycobacteria which probably constitute a new species from "skin lesions". These strains are fairly closely related to *M.phlei* but there is some doubt about the role played by these organisms, they are probably not the primary etiological agent of "skin lesions", as typical skin lesions cannot be produced by injecting the strains into cattle. The cause of non-specific reactions in South African Cattle is still mainly a matter for speculation. A commonly held opinion has been that avian tuberculin is of little use for comparative testing in South Africa as avian tuberculosis in poultry is rare and Johnes disease virtually non-existent. Although avian tuberculin produced from the strain D_4 has been made available by Onderstepoort for many years the demand has been low, only 2,000 doses being

issued to every 140,000 doses of bovine tuberculin issued.

The need for a more thorough investigation of the causes of non specific reactors is therefore apparent.

Materials and Methods

Although there is considerable overlapping in the sensitivity caused by various Mycobacteria, reactions to sensitins prepared from Mycobacteria are highly specific. Reactions to a sensitin prepared from a strain homologous to that responsible for sensitization are larger than reactions to a similar dose of a heterologous sensitin.[24, 25, 26, 27] Purified protein derivative (P.P.D.) sensitins prepared from the strains shown in table 1 by a method similar to that described by Green[28] were used in this investigation. The strains were proved to be culturally, biochemically and allergenically typical of the species they represented.

Table 1.—Strains of mycobacteria used for P.P.D. production

Strain	Species	Source
IXOPO	M. sp.	Bovine skin lesion isolated H. H. Kleeberg S.A.
Lacticola	M. smegmatis	Received from the Trudeau Laboratory, Saranac Lake, New York, U.S.A.
W33	M. kansasii	Isolated from bovine lymph gland S.A.
20485	M. avium	Isolated from lymph gland lesion of a pig, S.A.
AN_5	M. bovis	Received from Centraal Diergeneeskundig Instituut, Rotterdam.
Fortuitum	M. fortuitum	Received from the Trudeau Laboratory, U.S.A.
Cole	Scotochromogen	Received from the Trudeau Laboratory, U.S.A.

The field investigation was undertaken in conjunction with the Division of Veterinary Services (Field). A circular letter was sent to all state veterinarians explaining the purpose of the investigation and the method of study. Suitable herds were selected on the basis of their testing history by the state veterinarians concerned. Following a routine tuberculin test on a herd, between 6 and 24 suspected non specific reactors were selected for further investigation. These animals were then retested with six P.P.D. sensitins. The sensitins contained 0.5 mgm of dried P.P.D. powder per ml (25,000 sensitin units per ml) and were given code numbers. They were injected in 0.1 ml amounts (2,500 SU) in three sites on each side of the neck, the sensitins being rotated in the different sites according to charts supplied. The skinfold measurements before and 72 hours after injection were recorded and the results sent to this laboratory where they were decoded and analysed.

The test method was apparently followed accurately by all participating veterinarians except in 3 herds where the injection sites were not varied. In some cases cattle had lost their sensitivity when tested with the six sensitins. This may have been due to the long time lapse of up to six months between the routine test and the multiple comparative test in some herds. For this reason only animals which showed skinfold increases of 2 mm or more to at least one of the sensitins were included in the final analysis. In the first 18 herds the following sensitins were used Ixopo, Lacticola, W33, 20485, AN_5 and Fortuitum. It then became apparent that sensitivity to the sensitins Ixopo and Lacticola was generally of a very low level. The sensitin Cole was therefore substituted for Ixopo but unfortunately only 3 herds were offered for investigation with this sensitin.

Results

The 21 herds selected for the investigation were widely distributed over the eastern and northern parts of the country. Unfortunately no herds were tested in the Western Province where "skin lesions" and other non specific reactor problems are frequently reported.

In two herds the general sensitization pattern was of such a low level as to be of no importance, a further two herds were infected with bovine

tuberculosis, another showed a type of sensitization of undefined etiology, and in sixteen herds sensitization was apparently caused by *M.avium* or closely related organisms.

Herds infected with bovine tuberculosis

In the herd We. there was a history of reactions of an atypical nature at previous tuberculin tests. Post mortem examination of one such case had failed to reveal any lesions, and cultures from pooled lymph glands sent to this laboratory failed to yield any Mycobacteria. The results of the multiple comparative test are given in table 2. Subsequently cow 141 and bull 303 were sent to Onderstepoort where typical lesions of bovine tuberculosis were found at autopsy. The diagnosis was further confirmed by isolation and identification of *M.bovis*.

In herd G there was a history of tuberculosis but interpretation of reactions at previous tests was lenient. Tuberculous animals with low sensitivity were not removed and the disease had, therefore, continued to smoulder in the herd. The results of the multiple comparative test are given in table 3. Cow No. 78 was subsequently slaughtered and typical lesions found at autopsy. The diagnosis was further confirmed by isolation and identification of *M.bovis*.

TABLE 2.—SKINFOLD INCREASES IN HERD WE. AT MULTIPLE COMPARATIVE TEST

No. of Animal	Sensitin					
	Ixopo	Lacticola	W33	20485	AN_5	Fortuitum
C321	0	0.5	0.7	4.1	3.8	1.7
C303 ~	0.5	0	3.9	5.4	5.7	1.0
C141 ~	1.3	0.5	9.5	4.3	16.6	3.3
C272	0	0.5	3.5	5.1	6.0	1.7
C283	0	0.3	2.3	1.8	5.2	0
C210	0.3	0	2.6	0 6	5.5	0.4
C303	2.8	2.7	8.0	2 3	16.4	2.5
C317	0.3	0.6	4.2	0 4	6.2	6.7
C274	0.4	1.3	2.0	0 6	3.3	0.6
B10	0	0	4.0	6 6	7.7	0.3
BRU	0.6	0.2	10.3	4.9	13.0	1.6
C115	0.2	0	4.1	2.7	3.9	0.3
Total	6.4	6.6	55.1	38.8	93.3	20.1
Mean	0.5	0.6	4.6	3.2	7.8	1.7

TABLE 3.—SKINFOLD INCREASES IN HERD G AT MULTIPLE COMPARATIVE TEST

No. of Animal	Sensitin					
	Ixopo	Lacticola	W33	20485	AN_5	Fortuitum
7	0.8	0.1	0.4	1.1	3.8	0
56	0	0.6	0.1	0.3	3.6	0.6
68	0.7	1.1	4.0	2.4	4.3	0.2
78~	0.5	0.3	2.7	10.6	14.0	2.1
120	0.4	0.9	2.0	2.0	5.8	0.7
149	0	0	0.6	3.7	1.5	0.4
184	0.5	0	0.2	0.1	2.0	0
222	2.3	0.5	4.5	7.7	8.6	1.2
Total	5.2	3.5	14.6	27.9	43.6	5.2
Mean	0.7	0.4	1.8	3.5	5.5	0.7

Herd showing non-specific sensitivity of unknown etiology

This herd (VI) was previously infected with tuberculosis but the disease was eliminated by isoniazid treatment and gradual removal of reactors. The cattle selected for these tests were from that part of the herd in which no case of tuberculosis had been detected in 3 years of constant control and testing. Reactions occurred most frequently in calves, young heifers and oxen and were generally hard and circumscribed. The results of the multiple comparative test are given in table 4.

TABLE 4.—SKINFOLD INCREASES IN HERD VI. AT MULTIPLE COMPARATIVE TEST

No. of Animal	Sensitin					
	Ixopo	Lacticola	W33	20485	AN_5	Fortuitum
505	3.0	2.6	2.5	2.5	6.7	2.0
63–0	0.5	0.2	1.3	2.0	0.1	0.6
415–0	1.1	0.2	4.5	1.2	3.8	1.3
236	1.5	0.3	2.0	3.2	1.5	0.7
52	1.4	2.4	1.0	5.4	2.8	1.0
501–3	1.8	2.5	1.9	1.8	5.8	2.1
99–1	0.9	1.0	0	2.0	0	1.6
504	1.5	1.0	1.4	6.0	3.5	0.8
490	1.9	2.2	5.2	2.7	2.2	2.2
272–3	0.7	0	2.8	2.7	8.0	1.0
62–2	0	0	1.3	3.5	1.3	0.2
209–3	0.6	2.3	1.3	1.3	4.4	1.0
22	1.0	0	3.1	3.8	3.0	0.7
294	2.7	1.0	4.0	5.5	2.1	0.7
Total	18.6	15.7	32.3	43.6	45.2	15.9
Mean	1.3	1.1	2.3	3.1	3.2	1.1

Herds showing sensitivity to avian tuberculin

In the remaining 16 herds the sensitivity pattern was similar. The highest reactions occurring to avian P.P.D. followed by the reactions to the scotochromogen (in the few herds tested) and *M.kansasii* sensitins. The means of the reactions to each sensitin in the 16 herds are given in table 5 and the frequency distributions of the reactions to *M.bovis*, *M.kansasii* and *M.avium* sensitins are shown in histogram form in Figs. 2, 3 and 4.

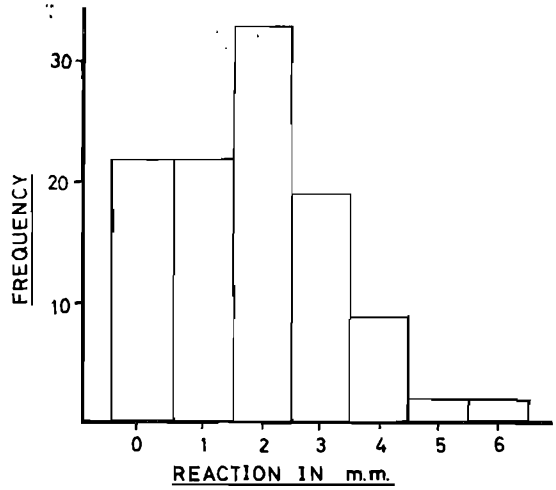

Fig. I. — Frequency distribution of reactions to 2,500 S.U. of AN5 (M. bovis) PPD in non specifically senstized cattle.

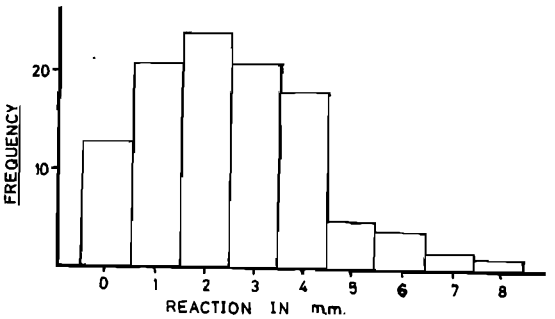

Fig. II. — Frequency distribution of reactions to 2,500 S.U. of W33 (M.kansasii) sensitin in non specifically sensitized cattle.

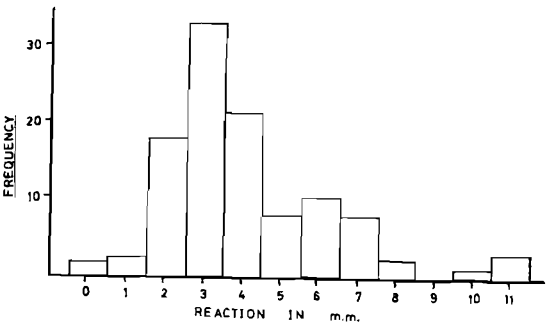

Fig. III. — Frequency of reactions to 2,500 S.U. of 20485 (M. aviam) P.P.D. in non specifically sensitized cattle.

TABLE 5.—MEAN SKINFOLD INCREASES AT MULTIPLE COMPARATIVE TESTS IN 16 HERDS

Area of Origin	No. Tested	No. Reactors	Sensitin						
			Ixopo	Lacticola	W33	20485	AN_5	Fortuitum	Cole
Eastern Cape	9	7	2.1	2.3	3.4	3.2	2.5	2.6	—
Natal	6	6	3.6	1.0	3.7	7.6	2.2	1.3	—
Eastern Cape	4	4	1.5	1.5	3.0	4.6	2.5	2.2	—
Natal	7	7	1.0	0.9	2.0	4.4	1.3	1.0	—
Transvaal	9	8	1.3	1.0	3.4	4.3	2.2	0.8	—
Natal	25	14	0.8	0.4	2.0	3.0	1.4	1.0	—
N.W. Cape	7	5	0.2	0.2	0.8	3.9	1.0	0.4	—
N.W. Cape	12	6	0.6	0.6	1.1	3.7	0.8	1.1	—
N.W. Cape	14	8	0.5	0.8	2.4	4.1	2.8	1.9	—
Transvaal	8	7	2.0	1.7	4.3	5.7	2.9	2.8	—
Eastern Cape	9	6	1.5	1.6	2.7	3.0	1.4	2.1	—
Natal	11	6	0.7	0.9	2.1	2.1	1.4	0.8	—
Natal	8	8	0.3	0.4	1.3	3.7	1.6	0.9	—
Natal	6	5	—	1.3	2.3	3.1	1.4	1.8	2.6
Eastern Cape	16	7	—	0.7	2.3	3.6	1.7	1.3	2.6
Eastern Cape	9	5	—	1.2	2.8	3.6	1.7	3.4	3.1
Mean			1.17	0.97	2.45	3.92	1.81	1.52	2.76
Standard Deviation			1.27	0.96	1.74	2.14	1.40	1.32	—

Discussion

The inclusion of two herds infected with *M.bovis* in this study was fortunate because it was shown that bovine tuberculosis could be readily recognised by the method and with the sensitins employed in this study. In both herds there were cases where the sensitization was possibly not due to bovine tuberculosis e.g. Herd G No. 149 and Herd We. No. C321. The inclusion of doubtfull cases did not influence the overall picture of an infection caused by *M.bovis*. Of considerable interest is the fact that the degree of sensitivity to the *M.kansasii* sensitin (W33) in herd We. was second only to the sensitivity to bovine tuberculin. This was expected as a similar picture is seen in guinea pigs sensitized by *M.bovis*.[20] In the case of herd G the reactions to *M.kansasii* sensitin (W33) were lower than the reactions to the *M.avium* sensitin (20485). This unexpected result may have been due to some procedural error such as a leaking syringe which delivered the incorrect dose.

The sensitivity pattern in the other herds was in marked contrast to that seen in the tuberculous herds. In one herd (VI.) equal sensitivity to avian and bovine tuberculin was seen, which may indicate a sensitization by another organism which is related by both *M.avium* and *M.bovis*. No definite conclusions can be made on the results from a single herd.

In the other 16 herds a very uniform pattern was seen. The greatest degree of sensitivity was demonstrated to the *M.avium* sensitin followed in descending order by the sensitivity to the scotochromogen, *M.kansasii*, *M.bovis* and *M.fortuitum* sensitins. Sensitivity to the *M.smegmatis* and the *M.sp.* sensitins was of a very low order. This sensitivity pattern is exactly analogous to that seen in guinea pigs sensitized with *M. avium*.[20] The difference between the means of the reactions to the *M.avium* and *M.bovis* sensitins was significant at the 1% level. The difference between the means of the reactions to the *M.avium* and *M.kansasii* sensitins was not significant. The number of cattle tested with scotochromogen sensitin were too few to be considered statistically. Bacteriological confirmation of the type of sensitization was also provided in one of these herds. Lymph glands from a no visible lesion reactor cow were sent to this laboratory where *M.avium* (virulent for chickens) was isolated. In five herds in which comparative avian-bovine tests have subsequently been performed on the whole herd it was seen that the comparative test greatly facilitated the interpretation of reactions. It seems reasonable to assume that the main cause of sensitization in these herds was *M.avium* or a closely related acid fast organism. The avian-bovine comparative test should therefore greatly facilitate the interpretation of reactions in many South African herds in which non specific reactions occur. It is of interest to note that about 70% of tuberculous-like lesions in the lymph nodes of swine in South Africa are caused by avian or avian-like mycobacteria.

ACKNOWLEDGEMENTS

The Chief, Veterinary Research Institute, Onderstepoort, is thanked for permission to publish this article, and the assistance of Dr. Mansveldt and all the members of the Field Services who cooperated in this experiment is greatly appreciated.

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LETTERS TO THE EDITOR

Crib-biting in Cattle

The interesting account by G. D. Sutton (J.S. Afr.vet.med. Ass., **36**(1) 1965, p. 123) of a case of crib-biting in an ox has prompted me to briefly report two other cases of a similar behaviour pattern in cattle.

Case 1.—A 5 year old Friesian dairy cow was frequently seen to grasp, and to manoeuvre as far back into her mouth as possible, a smooth horizontal bar which formed part of the front of her tie-up stall. She would then champ on this bar and produce quantities of saliva. Few swallowing movements were made and the saliva generally overflowed her lips and drooled on to the floor. There were no obvious lesions or abnormalities in her mouth, and in all other respects she was considered quite normal. The cowman thought that this behaviour usually occurred just after the cow had been milked.

Case 2.—A 6 year old Friesian cow (on a different farm from Case 1) behaved very much as the previous animal, except that she did not produce as much saliva, and she sometimes licked the horizontal bar before grasping it. In all other respects this animal was also considered quite normal. The owner of the cow believed that this behaviour mainly occurred after the feeding of concentrate foods.

Whether my two cases can be compared with Dr. Sutton's case, or whether any of the three can be directly compared with crib-biting in the horse, is difficult to decide. Most accounts of equine crib-biting emphasise the grasping of a fixed object with the incisor teeth, the arching of the neck and the swallowing, or attempted swallowing, of air. Neither Dr. Sutton's case nor my two cases really conform to this description. In some ways the behaviour patterns shown by these three cattle are reminiscent of the sucking of the edge of the empty feeding bucket which is sometimes seen when a calf has just finished drinking its milk. One possibility is that this bovine crib-biting is a carry-over of a calfhood habit into adult life. It may be relevant to notice that Hafez, Williams and Wierzbowski (1962) have suggested that wind-sucking in the horse first appears in the foal at weaning and may originate as a substitute for suckling.

Comparatively little work has been done on abnormal behaviour in domesticated animals. References to crib-biting in cattle do occur in the literature, but they tend to be somewhat dated: e.g. Friedberger and Fröhner (1908); and it is to be hoped that the publication of Dr. Sutton's case will encourage other workers to report further on this and other abnormal behaviour patterns of the domesticated animals.

ROGER EWBANK

University of Liverpool

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Diagnosis of Pregnancy in Mares

Sir,

Dr. C. H. van Niekerk is to be congratulated on his excellent article dealing with his observations on early clinical diagnosis of pregnancy in mares which appeared in your journal of March, 1965. In his introductory remarks he refers to thoroughbred breeding and states that according to information received from the Jockey Club lowered fertility in thoroughbred mares is a more serious breeding problem in thoroughbreds than in any other class of farm livestock and mentions in addition that surprisingly little intensive research has been done in the Republic in this connection. As the Jockey Club operates

on certain restrictive rules and regulations which materially affect the breeding efficiency of thoroughbred mares in S.A. it is essential that the student of equine breeding acquaints himself thoroughly with all factors which influence it. I shall therefore be grateful if you will permit me to publish certain findings of an exhaustive study conducted during an investigation of this problem.[1]

Under Jockey Club Rule 353 Section (B) 8, application for registration of foals in "The Stud Book" has to be made annually between the 15th January and 31st May of the racing year (i.e. from 1st August to 31st July) in which the foal is born. The result of this rule is that although the foaling season starts from the 1st August to comply with the ageing Rules on Racing, registration of foals in The Stud Book is not permitted before the 15th of January of the same racing year. During the interval of birth of the foal and application for registration there is a certain percentage of foals which die or which are destroyed because of illness or accident. Such foals are therefore naturally never registered and the mare is regarded as not having reproduced.

During the same study[1] a summary of monthly conception percentages were compiled as supplied by Pfaff[6] for the different provinces, which for the entire Republic were as follows:—August 22.2%, September 44%, October 62.2%, November 73.6%, December 94.5%, January 91.3%, February 100.0%. These figures were correlated with the average rainfall over a 30 year period as well as with the curve for the average hours of day-light. It was regarded as significant that the ascending rate of conception is closely connected with increasing day-light hours and rainfall with its consequent improved plane of nutrition and warmth. These observations confirm the findings of other investigators of the existence of a definite breeding season during which reproduction is successful and which according to Küpfer[5] varies under natural veld conditions from the end of October to the end of March. The importance of these environmental factors have been fully stressed by Quinlan[7, 8] and Belonje[1, 2]. For racing purposes the ageing of thoroughbreds is taken from the 1st of August each year which compels the breeder to drop his foals as soon as possible after the 1st of August to be able to supply youngsters to compete in the 2- and 3-year old races. This is the reason why in thoroughbreds an artificial breeding season is created stretching from approximately the 15th September to 15th December each year and which does not correspond with the natural breeding season of horses.

Many of the thoroughbreds studs are situated in the sheep farming areas of the Cape Province. It is therefore of interest to record that a similar situation occurs in the sheep breeding industry in those areas described by Van Rensburg[9]. Here too, insistence on a restricted autumn lambing season has proved to be the major cause of poor fertility when rams are put to ewes during late spring and early summer when drought conditions frequently occur. Conception figures of 40% are not unusual for that time of the year indicating that it is not only the thoroughbred in which a low fertility is observed. The investigations of Hugo[3] have proved beyond doubt that here too management is the major cause of lowered reproduction. Jooste and others[4] have further shown that far too few rams are used, and sheep farmers are now urged to have an autumn as well as spring lambing season each year.

This lowered breeding efficiency is not encountered in other breeds of horses in S.A., i.e. Arabs, Palomino's, fivegaiters, etc., because of their unrestricted breeding season which usually stretches from November to March each year with conception figures ranging between 90–100%.

The Jockey Club and Thoroughbred Breeders Association are fully aware of the fact that the ageing rule for racing purposes is the basic cause of the breeding difficulties in thoroughbreds. The Jockey Club is however unable to change their ageing rule to suit breeding requirements.

C. W. A. BELONJE.

East London.

April, 1965,

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PUBLIKASIE VAN SEKERE BESLUITE VAN DIE VEEARTSRAAD

Dit is ooreengekom dat die volgende besluite van die Raad wat op die vergadering van 3/3/65 geneem is vir publikasie vrygestel word:—

1. Die professie in kennis gestel word van die voorneme om die gedragskode te hersien en versoek dat voorstelle betreffende wysigings of toevoegings sonder versuim aan die Registrateur van Veeartse, Pk. Onderstepoort gestuur moet word.

2. Die Registrateur stappe sal neem teen die optrede van sekere aptekers wie middels en tablette sonder voorskrif verskaf en handelinge verrig wat spesiaal tot die beroep van veeartse behoort.

3. Klagtes van adverteerdery met die oog op professionele gewin of die aanmoediging of toelating van sodanige advertensies en die uitreiking van adreskaartjies aan die publiek, is deur die Veeartsraad oorweeg en die nodige stappe is teen die betrokke veeartse geneem.

4. Die Veeartsraad het aandag geskenk aan 'n saak wat op 3/9/64 in die Landdroshof te Malmesbury gedien het en waarin 'n persoon wat nie as Veearts geregistreer is aangekla was in verband met die onthoring van beeste.

5. REGISTRASIE AS VEEARTSE

   Die volgende persone is onlangs as Veeartse in die Republiek geregistreer:

   Christoffel Jacobus Visser.

   Pieter Johannes van der Lingen.

   Audrey Violet May.

   Gert August Kronsbein.

   Hendrik Jan Bertschinger.

6. REGISTRASIE VAN KANADESE GRADUANDI

   Korrespondensie tussen die Saskatchewan Veterinêr-Mediese Vereniging, die Hoof van die Navorsingsinstituut vir Veeartsenykunde en die Registrateur van Veeartse is deur die Veeartsraad oorweeg.

   Die Raad is van mening dat die Kanadese curriculum baie gunstig met die Suid-Afrikaanse leerplan vergelyk en indien hulle bereid is om aan die vereistes van Seksie 8 van die Veeartswet te voldoen, daar geen beswaar teen registrasie sal wees nie. Die M.R.C.V.S. moet egter nie as 'n voorbehoudsbepaling wees nie.

7. Toestemming is aan die firma Buckland Publishers gegee om die lys van geregistreerde veeartse in die Farmer's Encyclopedia te publiseer.

8. Klagtes teen lesings en na-doodse ondersoeke gedoen deur handelsreisigers is deur die Veeartsraad sorgvuldig oorweeg. Die

Raad is van sienswyse dat die handelsreisiger 'n gebied betree wat uitsluitlik tot die beroep van 'n Veearts behoort maar voel dat hierdie saak moet aangepak word d.m.v. samesprekings tussen die Raad van die SAVMV en die betrokke firmas.

9. 'n Klagte teen 'n veearts dat hy hondsdolheid entstof teen R2.10 per dosis aan 'n kliënt verkoop het, is deur die Veeartsraad oorweeg. Die bevinding van die Raad was dat hondsdolheid entstof nie deur privaat praktisyns aan die publiek verskaf mag word nie, aangesien die entstof nie onder die wet geregistreer is nie. Wat die prys betref is daar geen regverdiging om die entstof teen so 'n buitensporige prys te verkoop nie. Veeartse se profyt op die verkoop van entstowwe bestaan uit die 12½% afslag wat die Staat aan geregistreerde Veeartse toegestaan het.

10. 'n Klagte teen 'n sekere Mnr. Calder wat hom as 'n Veearts voordoen terwyl hy nie as sulks geregistreer is nie, is onder die aandag van die Veeartsraad gebring. Stappe wat tot dusver deur die Registrateur geneem is om hom op te spoor het misluk en die saak is nou aan die polisie gerapporteer.

Die uwe,

W. S. VAN JAARSVELDT.

Registrateur van Veeartse.

28 April 1965.

OBITUARIES

CECIL JACKSON

Amongst men occasionally there is one, who, by intellect and temperament, is destined to tread paths beyond the commen ken. Such a man was Cecil Jackson.

He was born in London in 1905. He entered what was then known as the Johannesburg University College and graduated in Veterinary Anatomy (first class) and in Physiology, thereby obtaining the B.Sc. degree, in 1924. During

1925 he was Registrar to the Hon. Mr. Justice R. A. Tindall. He subsequently studied veterinary science and obtained the B.V.Sc. (S.A.) degree in 1929, whereupon he was appointed Research Officer at the Veterinary Research Laboratory, Onderstepoort, in the section of Pathology, as from 1930. A year later he received the additional appointment of Lecturer on Veterinary Anatomy in the Faculty of Veterinary Science of the Transvaal University College, as it was then known. By 1936 he had completed his monumental thesis on "The Incidence and Pathology of Tumours of Domesticated Animals in South Africa" for which the degree of D.Sc. (S.A.) was conferred upon him. This work gained him world-wide renown. In the same year he succeeded Professor H. H. Curson to the Chair of Anatomy, a post he was to hold with great distinction until his resignation in September, 1955, when he was appointed part-time Lecturer on Comparative Haematology in the Department of Physiology of the Medical School of the University of the Witwatersrand, and simultaneously acted as consultant to clinical laboratories in Johannesburg. The following year he was appointed Senior Research Fellow of the National Cancer Association of South Africa.

In 1957 he left South Africa to take up the position of Consulting Pathologist to the Animal Health Trust in London and a year later became Head of the Preclinical Veterinary School and Professor of Veterinary Anatomy at Makere College, Kampala, Uganda. The following year he was also appointed Head of the British Empire Cancer Campaign's Comparative Cancer Research Unit at Kampala. In 1961 he accepted the post of Principal Research Officer of the Biological Research Institute at Achimota, Ghana and was appointed the ensuing year as Acting Director of the Institute, at present renamed the Ghana Academy of Sciences Laboratory (Entomology and Parasitology). Since 1957 he produced a further ten papers, besides the previous ones well known to his colleagues in this country. They covered various aspects of pathology and infectious diseases: eight of them have been published.

With regret we received the news of his death in hospital in Ghana on January the fifth, 1965.

During his life he had held many honorary positions. He had been Editor of this Journal from 1937 until 1946. From 1952 until 1955 he had been member of the Tumour Nomenclature Committee of the International Cancer Research Commission. In 1960 he had been member of the Committee on Research Planning in Comparative Medicine of W.H.O. and in 1961 at the International Symposium on Cancer of the Bladder held under auspices of the Union Internationale contre le Cancer. In 1963 he had been President of the Ghana Veterinary Medical Association and representative for the African Continent on the Editorial Advisory Committee of Pathologia Veterinaria.

Shortly before his death he was informed that he had been elected Fellow of the Royal College of Veterinary Surgeons: the actual certificate of election was dated January 8th.

A great scientist has passed from our midst: a man every inch a scholar, with a magnificent command of language. From himself he exacted the utmost in his dedication to his work. Much of what he has accomplished will probably only be appreciated at a later date. Those who worked close to him will ever remember the intellectual experience that a session with him at his beloved instrument, the microscope, always proved to be.

Our sincerest sympathy goes to his son, presently at Balliol College, Oxford, and married daughter, living in Pietermaritzburg.

LESLIE LOMBARD DALY

We regret to announce the death of Major Leslie Lombard Daly, who passed away at Pietermaritzburg on the 22nd June, 1965, after a short illness.

He was born in Kroonstad on the 15th October, 1900. He qualified at the old Transvaal University College (now the University of Pretoria) in 1925 and soon afterwards joined the Department of Agriculture. He spent several years at Louis Trichardt, where, incidentally, he did most of his travelling by mule waggon and was later transferred to Barberton where he married Miss Dorothy Massam, then the Matron of the Barberton Hospital. He joined up in World War II in 1940 and had a distinguished military career, attaining the rank of Major in the S.A. Veterinary Corps. On demobilisation he was recalled to the Eastern Transvaal to take charge of a serious outbreak of foot and mouth disease. Soon afterwards (1945) he was appointed Sub-Director in charge of Natal and Zululand, with headquarters at Pietermaritzburg, and remained here, except for a year in the Eastern Cape, until his retirement in 1960. During the years in Natal he was largely responsible for the total eradication of East Coast fever from the province.

He was a forceful and forthright character; he was respected by his colleagues and by all who had the privilege of working under him. His work in the Eastern Transvaal but particularly in Natal will forever remain a monument to his memory. He was a keen race follower and owner, and an old member of the Victoria Club, Pietermaritzburg, as also a Freemason.

He leaves a widow, a son, who is now a Pietermaritzburg City Councillor, and a married daughter, Mrs. Orr of Kimberley.

RAYMOND ALBERT ALEXANDER

It is with great regret that we record the death of Dr. R. A. Alexander which took place in Pretoria on the 8th of July, 1965.

Dr. Alexander was born in Benoni, Transvaal, in 1899. He obtained the degree of B.Sc. (Agric.) in 1922 and that of B.V.Sc. in 1925. During 1931 he worked and travelled extensively overseas as a Fellow of the Empire Marketing Board. In 1935 he was awarded the degree of D.V.Sc. for his work on the neurotropic virus of horsesickness. During his career he attended numerous international and world conferences and gave expert advice to foreign governments, notably to Egypt in 1944 over horsesickness and to the United States of America in 1953 over bluetongue.

He was appointed Director of Veterinary Services in 1950 and Professor of Infectious Diseases in the Faculty of Veterinary Science in 1958.

He was awarded the degree of D.Sc. (honoris causa) by the University of Cape Town in 1955 and was also a Fellow of the Royal Society (South Africa), Honorary Professor of the University of Madrid, Honorary Member of the Medical Association of South Africa and Honorary Member of the Section of Comparative Medicine of the Royal Society of Medicine.

He retired in 1961 but remained active as an advisor to the Council for Scientific and Industrial Research.

We extend our sincere condolences to his widow and to his son and daughter.

ALEXANDER MACDONALD DIESEL

It was with feelings of great shock and deep sorrow that members of the veterinary profession and his many other friends received the news of "Don" Diesel's death on the 23rd July. Very few knew that for months past his health had not been good and that he had to undergo a serious operation. This was performed three days before he died and only ten days after he had suffered a grievous loss through the death of his wife. While first reports of the result of the operation were promising and held out good hoped for complete recovery, he suffered a severe attack of coronary thrombosis on the second day, and this proved fatal.

Born in Bloemfontein on the 3rd September 1897, Don spent his early childhood on the farm Floradale in that district, where his father was a well-known breeder of Shorthorn cattle. No doubt this early contact with all classes of animals on the farm instilled into him that love of animals which he showed throughout his life.

He matriculated at Grey College in Bloemfontein, and in 1917 was awarded a government scholarship to study veterinary science at the Royal Veterinary College in London. After qualifying in 1921 he returned to the Union and joined the Veterinary Field Division of the Department of Agriculture in May 1922.

For the next eleven years Don served as Government Veterinary Officer in various parts of the country, but mainly in the Transvaal and Natal, where the campaign against East Coast Fever was then at its height. In July 1933 he was promoted to Senior Veterinary Officer of Natal, a post which he held until he was transferred to

Pretoria on promotion to the post of Assistant Director of Veterinary Services in November 1944. Two and a half years later in May, 1947 he was promoted to Deputy Director of Veterinary Services (Field). He vacated this post on reaching the retiring age in September 1958, but immediately resumed service in the Department by accepting a temporary position at Onderstepoort, which he held until July, 1961.

Don also served the Veterinary Faculty for 13 years, from 1945 to 1958 as Senior Lecturer in State Veterinary Medicine, a subject on which he was a recognised authority.

While serving at Onderstepoort he was appointed by the Minister of Agriculture: Technical Services as chairman of a Committee of Enquiry into the control of Foot and Mouth Disease. The report which he presented was so comprehensive and important that he received a special letter of appreciation from the Minister for what the latter described as a "formidable task culminating in an admirable and well-thought out report".

During his 39 years service with the Department of Agriculture Don rendered valuable service to the animal industry of this country and played no mean part in the final eradication of some of the most obstinate and dangerous stock diseases of Southern Africa such as East Coast Fever and Sheep Scab. His remarkable organizing ability, his profound knowledge of the Stock Diseases Act and its regulations, and his strict impartiality in applying these in conjunction with his inborn sense of justice and fairness in his dealings with both the stock owners and the officials serving under him, earned for him the admiration and respect of all with whom he had to deal.

As meritorious as his achievements on behalf of the livestock industry are the services rendered by him to the veterinary profession and particularly to this Association. First elected to the Council of the Association in 1945 he served it continuously in some or other capacity up to the time of his death. He was President from 1952 to 1956 and was elected Honorary Life Vice-President in 1957. On the termination of his service with the Department of Agriculture in 1961 he became the first full-time Secretary of this Association and served it as such till a few days before his death. The loss of such a capable official at this stage is certainly irreparable. Further, Don has served as a representative of the Association on the Veterinary Board continuously since December 1947, and here too his sound knowledge of veterinary jurisprudence and his unbiassed views will be sadly missed.

The funeral service was held in St. Andrews Presbyterian Church where he served for many years as an elder of the Church. A fitting testimony to his wide popularity was the exceptionally large attendance which included numerous colleagues some of whom had specially come from distant parts of the Republic, many bowlers from the Pretoria City Bowling Club where he was a popular player, and a large number of other professional and business people. The Association was represented by the President, Dr. H. P. Steyn, who acted as a pall-bearer.

Don is survived by a married daughter, Mrs. Molly Reed, who with her husband and little daughter lives in Port Elizabeth. Our deepest sympathy goes out to them in the double bereavement that they suffered in less than two weeks.

S.W.J.v.R.

PROFESSIONAL PROVIDENT SOCIETY OF SOUTH AFRICA

After seeing the recently published report and accounts of the Professional Provident Society for 1964, I feel compelled to comment on the remarkable growth of this Society which was founded by the professions some twenty years ago exclusively for the benefit of professional people.

The assets of the Society increased by the record figure of R1,124,307 and amounted to R4,770,009 by the end of 1964. The income from investments rose to R226,752 while the subscriptions paid by members, including contributions to the various supplementary schemes offered by the Society were just short of R1.5 million. Over R250,000 was paid to members and their dependants in sick pay, hospital, life assurance and provident fund benefits. R557,120 representing over 86% of the subscription income was credited to members' provident fund accounts by way of the annual dividend allocation while the interest credited to these accounts at the rate of 6.25%, amounted to R174,207.

An interesting fact pointed out in the report is that, because of the dividend allocation, the average actual cost of R325 per month sickness insurance was only R30 per annum. Younger members paying the minimum subscription rate only paid R6.50 per annum for the same cover.

It is also mentioned that the development and experience of the Society have been such that its Board has proposed a substantial increase in benefits without any increase in contribution rates.

In the light of this it is surprising that there are still many professional people eligible for membership who have not yet availed themselves of the benefits and financial security which the Society offers. If you are interested in membership, full details can be obtained from:

The Manager,

Professional Provident Society of S.A.,

P.O. Box 6268,

JOHANNESBURG.

ERRATUM.

J.S. Afr. vet. med. Ass. 36, (2), *June* 1965.
"Blowfly (*Lucilia cuprina*) strike in sheep: A comparison of prophylactic efficiencies of chlorfenvinphos (AC 4072) and VC 13." by G. E. Thompson.
Page 245, second line of summary; for 0.5 per cent read (0.05 per cent).

THE PROFESSIONAL PROVIDENT SOCIETY OF SOUTH AFRICA ANNOUNCES INCREASED BENEFITS

The Board of the Professional Provident Society has recently announced very substantial increases in its sickness and permanent incapacity benefits which will greatly enhance the value of membership. The sickness benefit has been increased from R1.25 per share per week to R1.40, giving a total benefit of R364 per month on the maximum shareholding of 60, and the limitations in regard to the period of payment have been removed, thus providing completely continuous protection. The permanent incapacity benefit has been almost doubled, so that a member with the maximum shareholding receives an income of R182 per month. This benefit becomes payable automatically after a member has drawn sickness benefit continuously for 52 weeks for the same or a related illness. Whilst drawing the permanent incapacity benefit a member pays no share subscriptions, but his provident fund account continues to receive the normal credits, so that the lump sum payment he will receive on retirement is unaffected.

The Society which was established by the professions in 1941, specifically to meet the professional man's need for insurance against loss of income during his working life, has maintained a record of steady progress. In addition to the sickness insurance, it offers provident fund benefits, hospital benefits, group life assurance cover and retirement annuity (pension) schemes, and the protection afforded by membership is today an essential part of the professional man's insurance portfolio.

The following combination of membership and optional schemes, showing costs and benefits for a man aged 35 next birthday, will be of interest.

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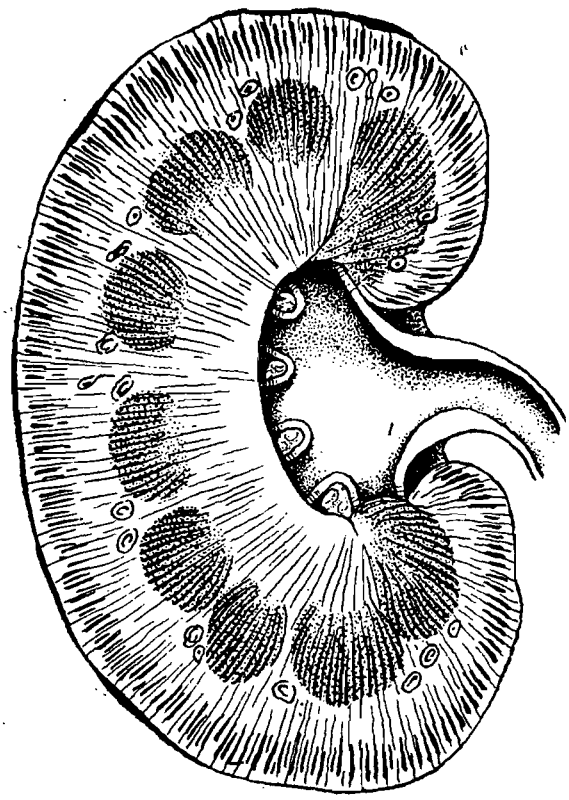
	Permanent incapacity benefit per month	R180
	Estimated provident fund at age 65	R9,560
R7.56	**Group Life Assurance R12,000 (optional)**	
	Sum Assured payable on death	R12,000
R1.50	**Hospital Scheme (optional)**	
	Benefit per day R5—yearly maximum	R1,000
R25.00	**Retirement Annuity (optional)**	
	Initial Sum Assured (life cover)	R8,960
	Sum Assured plus bonuses immediately before retirement at age 65	R21,735
	Cash payment on retirement at age 65	R7,245
	plus monthly pension, payable for life but in no circumstances for less than 10 years	R104.35

If you would like further information, you should write to the Manager, P.O. Box 6268, Johannesburg.



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BRANCH MEETING

The Eastern Transvaal Branch of the S.A.V.M.A. held its annual general meeting at Lydenburg on 5 June, 1965. The meeting was well attended despite the various veterinary difficulties the Eastern Transvaal was experiencing at the time of the meeting.

During the business meeting of the branch it was decided that:—

(*a*) The annual subscription be reduced from R3.00 to R1.00 per annum.

(*b*) That the meeting in 1966 be held at Pietersburg on Saturday, 4 June.

The following committee was elected for 1965–1966:—

Dr. M. J. N. Meeser, Chairman

Dr. C. J. Coetzee, Secretary.

Dr. P. V. A. Davies, Member.

The Chairman expressed the Branch's appreciation for the services rendered by the previous secretary, Dr. Sue Solomon, who after her marriage had settled in Kenya. Three new members, Drs. Veenstra, Pullinger and Young, were elected to membership. The papers read during the course of the meeting were of a high standard:

Dr. A. J. Snyders of M.S.D. in his talk on parasites in cattle told of some of the experimental work being done in Eastern Transvaal. Amongst others he warned against the injudicious use of drugs and stressed the fact that as parasitism affected young cattle severely up to the age of 18 months it was advisable to select a good drug with a wide range of paricidal activity for treatment of affected young animals.

Professor Steve van Heerden read a much needed and most instructive paper on the examination of the bull for fertility. He illustrated his paper with slides showing different testicular and other related conditions.

Mr. P. J. le Roux, Senior Biologist to the Provincial Fisheries Institute at Lydenburg read a paper on "Diseases of Fresh Water Fish". This paper revealed the existence of a wide open field for veterinary research. Mr. Le Roux's description of the various skin conditions seen in fresh water fish caused by fungi, protozoa and bacteria, of some diseases of the internal organs caused by bacteria, and of the various intestinal parasites of fish not only made most interesting listening but also pertinently brought home to the veterinarian the lack of his knowledge of diseases of fish.

Mr. Le Roux then took the meeting to the Institute where a visit to the aquarium elicited many questions. A brisk question-and-answer session followed Mr. Le Roux's demonstration of artificial insemination of trout and a visit to the trout hatchery.

Professor van Drimmelen read a paper on Brucellosis. He emphasised the increasing danger of *Br. melitensis* in man and gave an extensive description illustrated by slides of his investigations into the *Br. melitensis* problem of the Bantu of Sekhukhuniland.

A most enjoyable and instructive day was topped by an excellent congress dinner.

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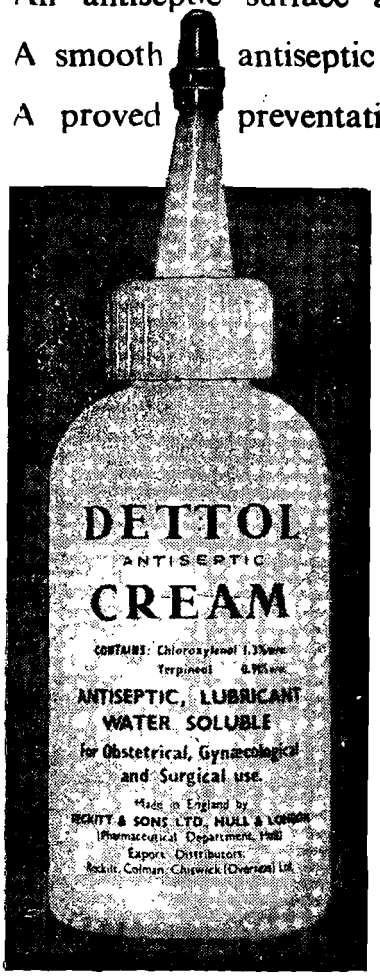

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BOOK REVIEWS

"BIOCHEMISTRY"

BY S. P. DATTA AND J. H. OTTAWAY

(Concise Medical Textbooks; Baillière, Tindall & Cassell, London)

360 pages; Overseas Price 21/–

It was with a keen sense of anticipation that I opened my copy of this new book; a worthy successor to the well-known "Aids to Biochemistry" by the same authors, which has been prescribed for student use in the Faculty of Veterinary Science, University of Pretoria for some years now. One of the greatest difficulties facing the presentday lecturer in biochemistry is the choice of a suitable textbook for use by students taking this subject as part of their curriculum for Bachelors' degrees in Medicine, Veterinary Medicine, Agriculture and numerous other allied disciplines. We are frequently asked to recommend suitable textbooks in this field for use by the officers of regional laboratories, research workers in numerous fields and even practitioners who wish merely to "brush-up" their knowledge of biochemistry or who wish to gain a general background to the subject without being subjected to profound and often irrelevant or painful detail. The array of general textbooks in biochemistry to choose from is formidable; the difference in subject matter between most of them is often of little consequence. General textbooks written by specialists in any particular field of a rapidly expanding subject are only too often biased in favour of the author's speciality or contain outdated information or perpetuated erroneous statements pertaining to fields with which he is not familiar. Biochemistry textbooks are no exception. The outstanding general textbooks on this subject are bulky and unfortunately often too expensive for casual reading or recommendation for student use unless they form part of a readily accessible library.

This new book by Datta and Ottaway strikes a happy medium. It takes the reader through a brief discussion of the chemistry of the major constituents of tissue and foodstuffs and their digestion, on to a more detailed but very lucid exposition of the major metabolic pathways. The concluding chapters which include a concise discussion of acid-base balance, the general chemistry of blood, excretion and detoxication deal appropriately with hormonal control mechanisms of metabolism and give a useful introduction to such topics as hereditary metabolic defects, metabolic blocks and other biochemical variants of interest.

Since this book is intended for use by students in medical disciplines, it presupposes a knowledge of the elements of inorganic, organic and physical chemistry. It can do no less since this knowledge is taken for granted in any course of biochemistry. It is not a book for those who want detailed accounts of the chemistry of carbohydrates, proteins, lipids, minerals or vitamins. These are today specialized topics, much of which falls without the scope of general courses in this subject. To my mind the greatest value of this book lies in its excellent succinct presentation of metabolism which after all is the most important part of a medical or veterinary student's biochemical curriculum.

This new book by Datta and Ottoway has been considerably expanded; not only by the inclusion of many new and excellent figures but by the virtual rewriting of most of its chapters, with the inclusion of much new and useful material. Its final chapter on techniques is a most welcome one and includes useful descriptions of what is involved in electron microscopy, differential centrifugation, measurements of gas exchange, spectrophotometry, electrophoresis, chromatography and isotope work. These are in the words of the authors, techniques which the student has to know but seldom sees.

The volume is slightly larger than the original pocketsized edition. It is very well written,

reads easily and is singularly free from irrelevant detail or historical references which can be found elsewhere. The subject matter is freely illustrated with clearly drawn figures, while tables have been kept to a minimum. From the point of view of veterinarians the sections on trace minerals and sex hormones are disappointingly brief, while ruminant biochemistry and nutrition has for obvious reasons been largely ommitted.

I have little hesitation in recommending this little volume to those who want a general background in biochemistry. It will continue to be prescribed for use by students in this faculty.

J.M.M.B.

MICROANALYSIS IN MEDICAL BIOCHEMISTRY

by I. D. P. WOOTTON

(J. & A. Churchill Ltd., London.) 246 pages;

Overseas price 30/-

This book succeeds one of the same title written by the late E. J. King and I. D. P. Wootton. In its present form it has been considerably expanded with the inclusion of much new material. This book is one of methods for use in the clinical biochemistry laboratory. It covers the commonly performed analyses for clinical and routine biochemical purposes on blood, urine, cerebrospinal fluid and tissues. It is a standard source of reference in many medical institutions and most of the methods are currently in use in the various clinical or biochemical laboratories at the Onderstepoort Veterinary Research Institute. This book is recommended for use by veterinarians in regional diagnostic laboratories and private practitioners who are interested in clinical chemistry. Most of the methods for determining blood constituents and some of the urinary constituents require a good photoelectric colorimeter or photometer, which rather limits the use of this book for general practitioners. The volume forms a very useful companion to "Biochemistry" by Datta and Ottaway.

J.M.M.B.

MYIASIS IN MAN AND ANIMALS IN THE OLD WORLD

by F. ZUMPT

Butterworth and Co. (South Africa) Ltd., Durban, p.p. 267. Price R13.00

This book should serve a pressing need and have a wide appeal to both practising veterinarians and research workers.

The work commences with a description of parasitism by the larvae of dipterous flies and is followed by a statement of the objects aimed at, namely, to make possible the identification of the species of larvae so frequently encountered in living animals and supply the information required from such findings.

Evolutionary trends in myiasis producing flies are discussed, numerous examples to support the theories advanced being given.

For the specialist a brief description of morphology and terminology, illustrated with excellent line drawings follows, which in turn is followed by keys to the larvae of the myiasis producing flies.

The subject is treated in an entirely practical manner which serves to simplify the recognition of the larvae involved, in that these are dealt with in accordance with the situations in which they are encountered.

Hereafter follows the main body of the work; the numerous species of flies whose larvae are associated with myiasis in the Old World are dealt with systematically in regard to their morphology, biology and pathogenesis in brief but comprehensive fashion.

A host-parasite list arranged to deal with myiasis in wild and domestic animals is included and a section on technique describing methods of collection and rearing of larvae and adults, their preservation and dispatch when accurate identification by specialists is necessary adds considerably to the value of the work. Short notes on control measures with references to detailed descriptions of methods employed, where required, are included in the comprehensive literature references covering the subject matter of the volume.

Throughout, extensive use is made of illustrations in the form of line drawings of a very high standard, photographs and a single plate which make the recognition of the parasite in question a simple matter for the less experienced investigator.

This well bound volume is printed on high grade moderately glazed paper.

R.M.DUT.

COMPARATIVE PHYSIOLOGY AND PATHOLOGY OF THE SKIN

Edited by ARTHUR J. ROOK and G. S. WALTON, Blackwell Scientific Publication, Oxford

Date of Publication: 1965 p.p. 794: Price: £7. 15s.

The book contains 55 papers delivered at a symposium at Cambridge by 46 authorities drawn from the medical and veterinary field.

The papers can be classified under the following headings:

Comparative dermatology

Under this heading the present state of our knowledge of human and veterinary dermatology is compared and tasks, possibilities and aims of comparative dermatology are discussed.

Hair

The genetics of hair growth, replacement of hair, normal and abnormal hair in man and animals are some of the more important aspects included under this heading. This is a very valuable contribution to the veterinary literature as very little is known in this field of veterinary dermatology.

Nutritional Influences on the skin

Nutritional influences on the skin in man and in animals constitute the next group of papers. Emphasis is placed on the pathological lesions produced by deficiencies.

Porphyria and light sensitisation

This interesting subject is very well covered by authorities in the human and veterinary fields. The various types of porphyrias are discussed in great detail.

The Mast cell

This forms a very valuable section of the symposium. The history, pharmacology and pathology of the mast cell in man and animals receive attention.

Immunology

In a very comprehensive series of lectures all the various aspects of skin sensitivity are discussed. This includes papers on allergy, anaphylaxis, contact dermatitis, urticaria, atopy and reactions to arthropods and drugs.

Tumours of the Skin

Various aspects of skin tumours receive attention. Normal skin, the distribution and incidence of skin tumours are some of the important subjects under discussion. The aetiology, with special reference to virus and chemical agents as skin carcinogens, forms another major part of the lectures.

The symposium certainly covers a very wide field. It is a valuable contribution to our knowledge of skin pathology. The application to the veterinary field of the knowledge available in human dermatology and the adoption of a uniform scientific terminology offer immense prospects for immediate advances in diagnosis and therapy and for planned research.

This book forms a most welcome addition to the library of the pathologist, clinician and general practitioner.

J.D.S.

NEMATODE PARASITE POPULATION IN SHEEP AND ON PASTURE

by H. D. CROFTON

Technical Communication No. 35 of the Commonwelath Bureau of Helminthology, St. Albans. Price 10/-

This booklet, containing 271 references, can most conveniently be considered to have four different sections.

The first deals with the parasite population in the sheep and enters into discussions on such phenomena as the "spring rise" in egg counts, the interrelationship between different parasites in the one host, the significance or otherwise of egg counts per se, and the "self cure" phenomenon.

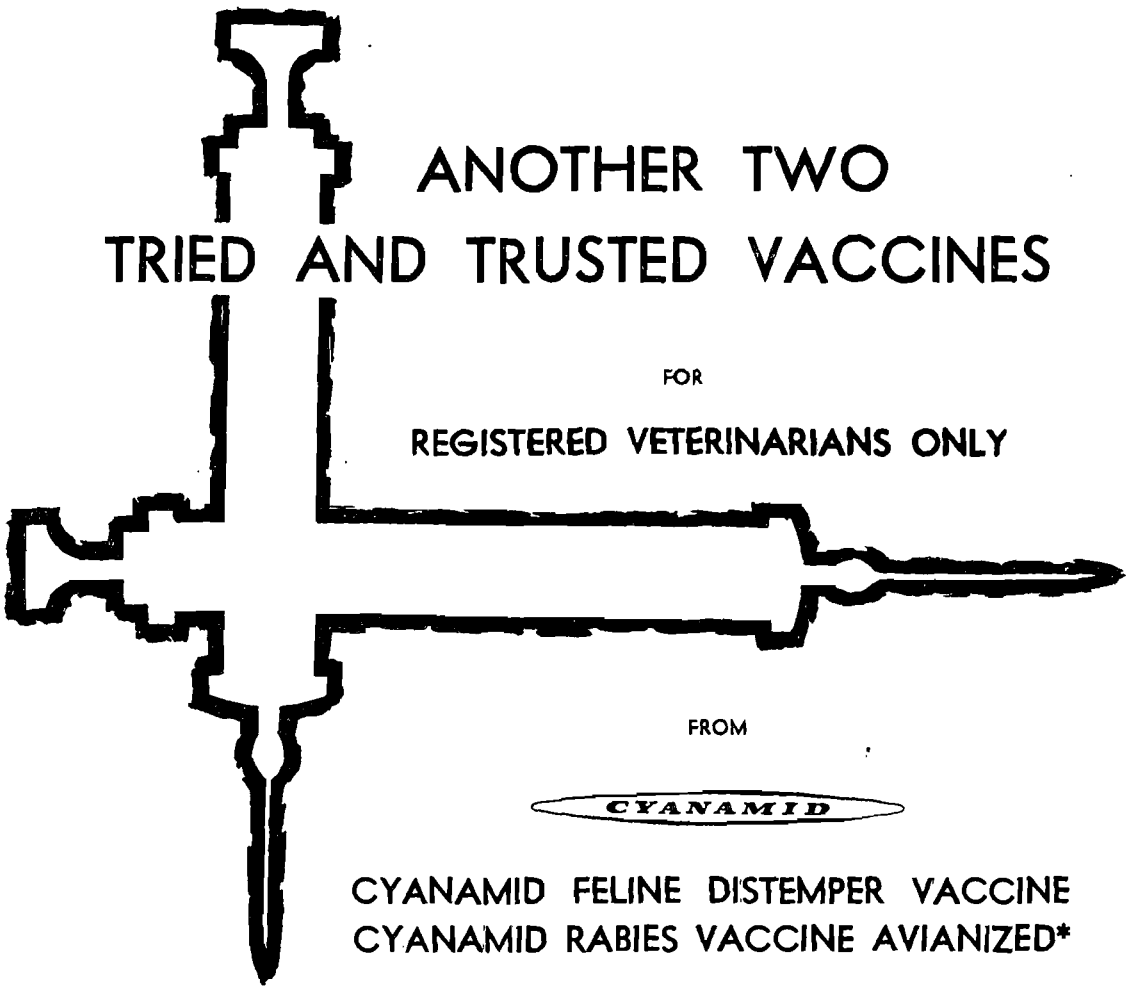
The second section deals with the parasite population on the pastures. Factors affecting this population such as the grazing and defeacation habits of sheep, the microclimates in the veld, and the varying degrees of resistance to temperature and desiccation evinced by the different pre-parasitic larvae are considered.

Section three deals with the life histories of the most commonly encountered parasitic nematodes, and the fourth section with theoretical considerations of control measures and the author's conclusions. When read together, they should be of great interest to veterinarians who frequently have to advise farmers on the complex problem of internal parasite control, and wish to go the desired one step further than merely recommending the anthelmintic of choice.

A little thought is required to convert northern hemisphere July summers, and such comments as "even after three week's drought, the relative humidity can be over 90% on a normal well-drained pasture", to conditions such as we know them in South Africa.

This booklet was first published in 1963 by the Commonwealth Agricultural Bureaux, Farnham Royal, Bucks, England, and may be obtained directly from them.

P.J.S.A.



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BRIEF REPORT

of the 9th meeting of the Permanent Committee of the World Veterinary Association, held in Paris on 27th May, 1965

Present: Bureau of the Permanent Committee:

PROF. DR. W. I. B. BEVERIDGE, President

PROF. DR. H. C. K. WAGENER, Vice-President
PROF. DR. JAC. JANSEN, Secretary-Treasurer,

26 delegates representing 26 countries, 8 representatives of associate members, observers from the "Office International des Epizooties", World Health Organization, Food and Agriculture Organization, Council for International Organizations of Medical Sciences and the International Women's Auxiliary to the Veterinary Profession, and Prof. Dr. Dr. h.c. H. C. Bendixen and Prof. Dr. H. Merkt, guests of the Bureau.

1. The PRESIDENT opened the meeting at 2.15 p.m. He informed those present that 8 apologies had been received. A moment of silence was observed in remembrance of Prof. J. Verge and Prof. Dr. St. Angeloff, who had passed away in 1964.

2. *Minutes of the 8th Meeting of the Permanent Committee of the World Veterinary Association held in Paris on 21st May*, 1964.

3. (*a*) *Report of the Permanent Committee of the World Veterinary Association for the year ending 31st December*, 1964.

   (*b*) *Balance sheet of the Secretariat of the Permanent Committee for the year ending 31st December*, 1964.

   (*c*) *Draft Budget of the Secretariat of the Permanent Committee for the year ending 31st December* 1965.

4. (*a*) *Report on the Association Fund of the World Veterinary Association and Financial Statement for the year ending 31st December*, 1964.

   These documents (2, 3, 4 (*a*)) were approved.

   (*b*) *Payment of contributions*

   Four members were in arrears. If no payment should have been received on or before 1st August, 1966, they should automatically be suspended from membership.
   As from 1st August, 1965, the contribution would be 1½ shillings per veterinarian represented by each member. The members of the Association Fund Committee were re-elected.

   (*c*) *Appointment of auditor for the following year*

   Mr. R. Mulder was re-appointed.

5. *Affiliation of Specialist Associations to the World Veterinary Association; alterations, if any, of Rule* 4.

   The following recommendations of a subcommittee, which had studied a possible change of Rule 4 of the W.V.A., were accepted:

   (1) To accept the *scheme for the headings of Rule* 4 as proposed by the President of the Permanent Committee, amending however, the wording of headings from 9 up to 14 inclusive to: Clinical Studies of the different species of animals (instead of Medicine). (See enclosure, - a -).

   (2) To change in the *working scheme for sections of World Veterinary Congresses* No. 8: Surgery (incl. Anaesthesia and Radiology) which should read: Experimental and Developmental Surgery. (See enclosure, - b -).

6. *Applications for membership/associate membership*

(*a*) *World Association for Buiatrics (associate membership)*

It was agreed to accept the World Association for Buiatrics as an associate member under heading 10: Clinical Studies Ruminants, on the understanding that at World Veterinary Congresses they would include in their section papers, if any on Clinical Studies Small Ruminants.

(*b*) *Any others*

It was agreed to accept the application for associate membership received from the *World Association for Veterinary Surgery* under heading No. 8: Surgery (incl. Anaesthesia and Radiology), on the understanding that at World Veterinary Congresses they will deal only with experimental and developmental surgery.
At World Veterinary Congresses the specialist associations would be concerned with clinical studies (medicine and surgery) of their respective animal species.

7. *XVIIIth World Veterinary Congress, Paris, 1967*

Prof. VUILLAUME, President of the Organizing Committee, said that 60-70 working sessions of half a day had been planned. Perhaps this number could be enlarged if it would be possible to make the festivities of the bicentenary of the veterinary school at Alfort last half a day instead of a whole day.
Each session could be divided into two so that a total of 70-140 sessions could be organized. It was intended to arrange these sessions as *symposia*, each dealing with an important topic. For each topic one, two, three or four speakers would be *invited*. Both members and associate members had already been asked to submit propositions for subjects and from the answers received a list of proposals for subjects had been made by the Organizing Committee. This list would be circulated to members and associate members of the W.V.A. They would be asked to give their opinion and propose other subjects if so wished.
A division of speakers would be made roughly according to the number of veterinarians for which contribution is paid. There would be ample time for discussion. There would also be an opportunity to submit short communications to be published in summary in the Congress proceedings. The Congress fee would be 150-200 N.F. (approx. £11-£15 or US$29-US$40.). One or two months before the Congress, all congressists would receive a set of the Congress documents, printed in "Offset". Within six months after the Congress, a volume containing the discussions at the various symposia would be published. It was agreed to remit to the Organizing Committee Dfls. 60.000.—from the Association Fund.

8. *Film Work*

It was decided to continue the film work of the W.V.A. and to publish a second edition of the film catalogue in 1966.

9. *Any other business*

The attention was drawn to the 5th Pan-American Congress of Veterinary Medicine and Zootechnics, Caracas, Venezuela, 18-24 September, 1966, and to the 4th Symposium of the World Association of Veterinary Food Hygienists, Lincoln, Nebraska, U.S.A., 26-30 July, 1965.

10. The meeting was closed at 3.45 p.m.

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