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RECENT DEVELOPMENTS IN VETERINARY SCIENCE OF IMPORTANCE TO THE PRACTITIONER IN SOUTH AFRICA*

B. C. JANSEN**

In a discussion of the subject under the above title it is important to enlighten the veterinary profession at large on the recent reorganization effected in the State Veterinary Services of our country. Prior to 1962 the Field Veterinary Services functioned under the control of the Director of Veterinary Services who was simultaneously director of the Onderstepoort Veterinary Research Institute. From 1962 the two organizations were separated and the Veterinary Research Institute and the Division of Veterinary Services (a new designation) functioned as separate, autonomous bodies each under its own director. Within the Department of Agricultural Technical Services the Division of Veterinary Services was classified with the Extension Services and the Research Institute with the Directorate of Research.

It soon, however, became apparent that there was insufficient cohesion in the interest of veterinary science between these two organizations. A feeling that they were drifting apart was engendered. In due course it was also felt that veterinary affairs were sufficiently distinct not to be so closely linked with the administration of purely agricultural matters. The result was that from November, 1968, the entire state veterinary enterprise was again unified under one Director-general who is directly responsible to the Secretary for Agricultural Technical Services. Each of the two sections is now under the control of its own Director assisted by two Deputy-directors, but their functions are closely integrated and co-ordinated by the Director-general.

The new veterinary organization is geared to the solution of the livestock problems as they have been identified in the different regions of the country. At five widely separated localities Regional Diagnostic Laboratories have been established to provide State Veterinarians with laboratory aids. This will assist them in identifying the causes of problems and will relieve the scientists at the Veterinary Research Institute of routine duties distracting their attention from essential research activities. These centres are: Stellenbosch, Grootfontein, Allerton, Windhoek and Onderstepoort. A veterinarian working in the field will now be able to identify a problem with the assistance of the Diagnostic Laboratories and then transfer himself and the problem to the Veterinary Research Institute. At the Institute he will be able to find the final solution with the assistance of teams of scientists and sophisticated facilities. Furthermore the facilities and accomodation for State Veterinarians are being improved to such an extent that they can function efficiently.

At the Veterinary Research Institute a large, modern building complex has recently been taken into use. It houses the entire administrative section, the vaccine production unit, the section for the distribution of vaccines, the Poultry Pathology unit and the whole of the section for research in Bacteriology. As a result, the remaining sections at the Institute have been provided with ample additional space in the laboratories vacated by the sections which moved into the new building. It has also been possible to make an entire building available for a Diagnostic Laboratory.

A modern isolation building for research on internal parasitism has been completed.

The Institute has completed plans for the erection of a laboratory complex for research on exotic virus diseases, mainly foot-and-mouth disease. It will be provided with maximum security isolation facilities. Plans have been drafted for the construction of buildings to house poultry which will be kept free from pathogenic viruses such as the agent

* Congress Lecture presented at the 64th Annual Congress, Cape Town, September 1969.
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of Marek's disease. The objective is to produce eggs free from foreign agents for the preparation of human and poultry vaccines.

From the above brief review it will be evident that the State Veterinary Services are making great efforts at solving the problems of the country and I wish to appeal to the private practitioners as a body to assist them as far as possible and also avail themselves of the diagnostic facilities created. Few of us would doubt that fruitful areas of cooperation between the State Veterinarian and the private practitioner already exist and more have recently been created, e.g. the introduction of the Tuberculosis Eradication Programme. Many veterinarians making a living in various fields of practice seek opportunities for research — they can be assisted by the Research Institute and the Diagnostic Laboratories. These people do it for the sake of interest, pleasure, satisfaction and for the sake of advancing the animal industry. One does not expect the busy practitioner to unearth many new facts or to make striking advances in basic research. His searching is of a different intensity spread over the broad area of his practice and his projects should be concerned with some aspect of his regular work. The private practitioner is in an ideal position to study the epizootiology of diseases. Where the cause of a disease is obscure and consequently there are no means of producing it artificially, the epizootiologist is primarily depended upon to elucidate the unknown phenomena and pave the way for the laboratory worker to take over.

Knowledge in veterinary science is expanding exponentially. Already there is justification for the introduction of specialities in veterinary practice, since it is impossible for a person to master the whole field adequately to discharge his functions to the satisfaction of his clients. At this stage it would seem that specialization on a species basis rather than in subjects such as radiology, orthopaedics, parasitism and chemical pathology offers the best solution. As agricultural production units become specialized, all those who hope to offer them useful advice must similarly specialize. It seems likely that in predominantly farm animal production areas, the general practitioner will be replaced by the species specialist. Specialization can be through formal courses at the Veterinary Faculty. Although this presents difficulties for the busy rural practitioner conducting a one-man practice, the results obtained from continued education is worth the temporary sacrifice. A second way in which a veterinarian can become a specialist is by attending to the problems encountered with a particular species of animal to the exclusion of others. This informal method takes longer and is well suited only to the members of a group practice. As a profession we shall soon have to decide whether or not specialization should be formally recognized. At the same time it will have to be determined what the criteria for recognition should be. Should the proof of competence be based solely on postgraduate study or will extensive publications suffice?

During the past decade an increasing number of veterinarians have become involved in the care of animals in zoological gardens, studies on animals in game parks and research on primates in association with specialists in human physiology. They are expected to give guidance on the requirements of animals in health and disease and, equally important, to advise on the possibility of the transmission of disease from animals to man. Well-known diseases such as rabies and tularaemia readily come to mind, but recently a most unusual human disease hazard derived from laboratory monkeys has been described. It concerns the *Herpesvirus simiae* which causes a benign clinical disease in monkeys. The lesions are similar to the herpetic ulcers frequently seen in man. They appear on the tongue, the muco-epithelial border of the lips and in the buccal cavity. These ulcers heal fairly rapidly and leave no scars. The lesions do not cause any difficulty with eating. Sometimes an infected monkey suffers from conjunctivitis and a nasal discharge. When an infected monkey bites another, lesions on the body result. Histological examination of the tissue at the edge of the ulcer shows intranuclear inclusion bodies.

Man becomes infected through the bite of an infected animal or contamination of cuts with infective saliva. Instances are on record where laboratory workers have become infected by handling infected tissues. Although man rarely becomes infected, the disease is invariably fatal. Only a few recoveries have been reported. The essential lesions consist of an ascending myelitis and encephalitis.

Prophylactically, monkeys of unknown disease status should not be handled without protective clothing and the use of anaesthe-

tics. Clinically diseased animals should be destroyed.

A grave responsibility towards human and animal health has been placed on our shoulders by the discovery of episomal or transferable resistance to antibiotics in bacteria. This phenomenon concerns the process whereby resistance to antibiotics can be transferred from one member of the Enterobacteriaceae, e.g. *E. coli*, to another member of the same family, e.g. *Salmonella typhi*, by mere contact without the second organism ever being exposed to the drug. It is claimed that the inclusion of antibiotics as growth stimulants in animal feeds leads to the emergence of intestinal bacteria resistant to the drug. The resistance can be transferred to pathogenic bacteria from the non-pathogenic species persisting in the intestines even after the feeding of the antibiotic has been stopped. If at any time the pathogenic bacteria were to attack the animal body, any attempt at treating the resulting disease with the same or a closely related antibiotic would be futile. Furthermore, the resistant bacteria can be transmitted to human beings attending the animals in which they occur. When they are established in the intestines of man, their resistance may be transferred to human pathogens with the same serious consequences as in animals. Whether the feeding of antibiotics at a low level is more conducive to the emergence of episomal resistance than their chemotherapeutic application, is not yet conclusively established, but we as veterinarians must be cognisant of this phenomenon and apply antibiotics in our practice with the greatest possible discretion. The British Government regarded this matter of sufficient importance to appoint a special committee to investigate the practical significance of episomal resistance and to devise means of controlling it, if necessary. The committee has not yet issued a report, but we are anxiously looking forward to receiving it.

Scientists concerned with the study of basic neuro-physiology have found the dog a most suitable experimental animal. On account of its advanced state of domestication it is sociable and responsive to humans and its environment. Detailed studies of the normal development and function of the dog's brain have been conducted and have made it possible to relate functional changes to altered behaviour and reflex responses. The name of M. W. Fox should be mentioned among the pioneers in animal behavioural studies. These experiments have also brought to light information on the behaviour of dogs which has practical significance to practitioners who are often called upon to advise owners on puppies showing aberrant behaviour. For instance, it has been shown that pups hand-reared in isolation do not associate with other pups when brought into contact with them, and remain shy and withdrawn.

By about 4 to 5 weeks the most rapid stage of development of the pup's brain is over; after this time the growth of the brain is much slower in relation to the rest of the body. It seems that this change of rate of growth indicates the point at which the pup's power of perception and locomotion is sufficiently developed for it to start learning various things and associating with humans. This is also the stage at which it starts barking and playing with its litter mates. This period of adaptability ends at about 12 weeks and, if they have not had contact with humans up to this age, they remain unfriendly. The attachment to human beings increases from 5 weeks old and reaches a maximum at 12 weeks. Hence reward training should be commenced when the pup is about 12 weeks old on the basis that at this stage it takes a pride in pleasing its owner. It is claimed that learning is unstable or transient in young pups aged 5—6 weeks, but more stable at 8—9 weeks. Inhibitory training, e.g. house training, is best done at 8—9 weeks, when punishment makes a more permanent impression and before the emotional bond between the pup and its master is too firm.

Behavioral studies have shown that the best stage to transfer a pup from the litter to its owner's home is at the age of 7 weeks. By then it should have had sufficient time to socialize with its own species to prevent asocial behaviour towards other dogs in later life, as exemplified by states such as sexual impotence. It will also allow time for house training and establishing emotional ties with the new owner. It is true that extreme shyness and agression are prevalent in certain breeds, independent of the effects of environment and early experience, but we as veterinarians should influence breeders not to select for conformation only while neglecting personality.

The objective study of animal behaviour has become a well-defined scientific subject with marked practical significance. The welfare of animals kept under systems of intensive husbandry has prompted such great in-

terest that animal behaviour is being taught as a separate subject at some veterinary faculties. This is evidenced by an increase in publications on the behaviour of pigs, fowls and cows kept in groups.

A condition which may be of interest to veterinarians has been described under the unusual name of the Chediak-Higashi syndrome. This is an inherited disease occurring in man, mink and cattle in a particular geno type. In all affected humans and animals the granulocytes of the body contain abnormally large granules. Forty-seven cases have been reported in humans. The condition is found wherever mink are raised and its presence led to the development of the "blue" or so-called Aleutian colour phases in mink. Two affected herds of cattle have been reported in the U.S.A.

The Chediak-Higashi syndrome is inherited as a simple recessive trait. Affected humans usually die before they are five years old and cattle before they are six months old. Mink also die young except when outbred. The essential features of the syndrome are the following: lysosomal abnormality in various cell types, partial albinism, photophobia and susceptibility to infections. The detection of abnormal peripheral leukocytes is still the easiest and most convenient method of diagnosis.

In mink the colour phase in affected animals was called Aleutian, because the colour of the pelt resembled that of the Aleutian fox. It should not, however, be confused with Aleutian disease of mink which is caused by a virus producing severe bile duct proliferation with periportal fibrosis, fibrinoid degeneration of numerous arteries and arterioles and fibrinoid degeneration of the glomeruli. It appears as though Aleutian mink are more susceptible to the Chediak-Higashi syndrome —hence the confusion in the names.

At birth affected calves, e.g. of the Hereford breed, are either entirely white or have a fawn colour in areas which are normally red. They have decreased pigment in the iris.

Affected children and animals are more susceptible to a variety of infectious diseases of viral or bacterial origin. They usually suffer from boils, abscesses, enlarged lymphnodes, splenomegaly and hepatomegaly.

Scrapie, a disease of sheep which has been known for many years and has been the subject of research for a long time, has consistently evaded a universally acceptable explanation as to the exact nature of the agent causing it. Some researchers say that it is a 'slow virus' of a small size and very resistant to chemical and physical influences, but that it does not contain nucleic acid in spite of its ability to multiply. The mass of evidence is in favour of replicating viruses containing nucleic acid and, therefore, some scientists maintain that the scrapie agent must also contain nucleic acid. There is also a suggestion that it might be a replicating polysaccharide. We in South Africa are vitally interested in any findings that can elucidate the problem, since we have had some cases of scrapie among imported sheep during the past few years.

A disconcerting aspect of foot and-mouth disease is the recent finding that cattle can act as carriers of the live virus. By obtaining pharyngeal scrapings from recovered animals at regular intervals the live virus could be demonstrated on the mucous membranes of the throat for a period up to 24 months after clinical recovery. Even a fair percentage of vaccinated animals became carriers of the virus after challenge, which showed that the immune status of cattle exposed to the virus did not prevent the establishment of the carrier state. A serological survey conducted in Rhodesia showed that a number of species of game had suffered from infections of all three Southern African types of virus without the domestic stock in the same area having shown evidence of foot-and-mouth disease. Virus was also isolated from pharyngeal scrapings of buffalo in areas where the disease has not been present in cattle for some years. Information such as this is of importance to us, because in Southern Africa foot-and-mouth disease has been associated with the presence of game in all outbreaks.

Until fairly recently the mycoplasmas, except for the causal agents of contagious bovine pleuropneumonia and contagious agalactia of sheep and goats, did not draw much attention as pathogenic organisms. Since, however, they were intentionally looked for and investigated with improved techniques, some interesting information has come to light. Mycoplasmas found in humans and animals can be either saprophytic or parasitic. Where they are parasitic, concurrent infection of bacteria or viruses may aggravate the condition caused.

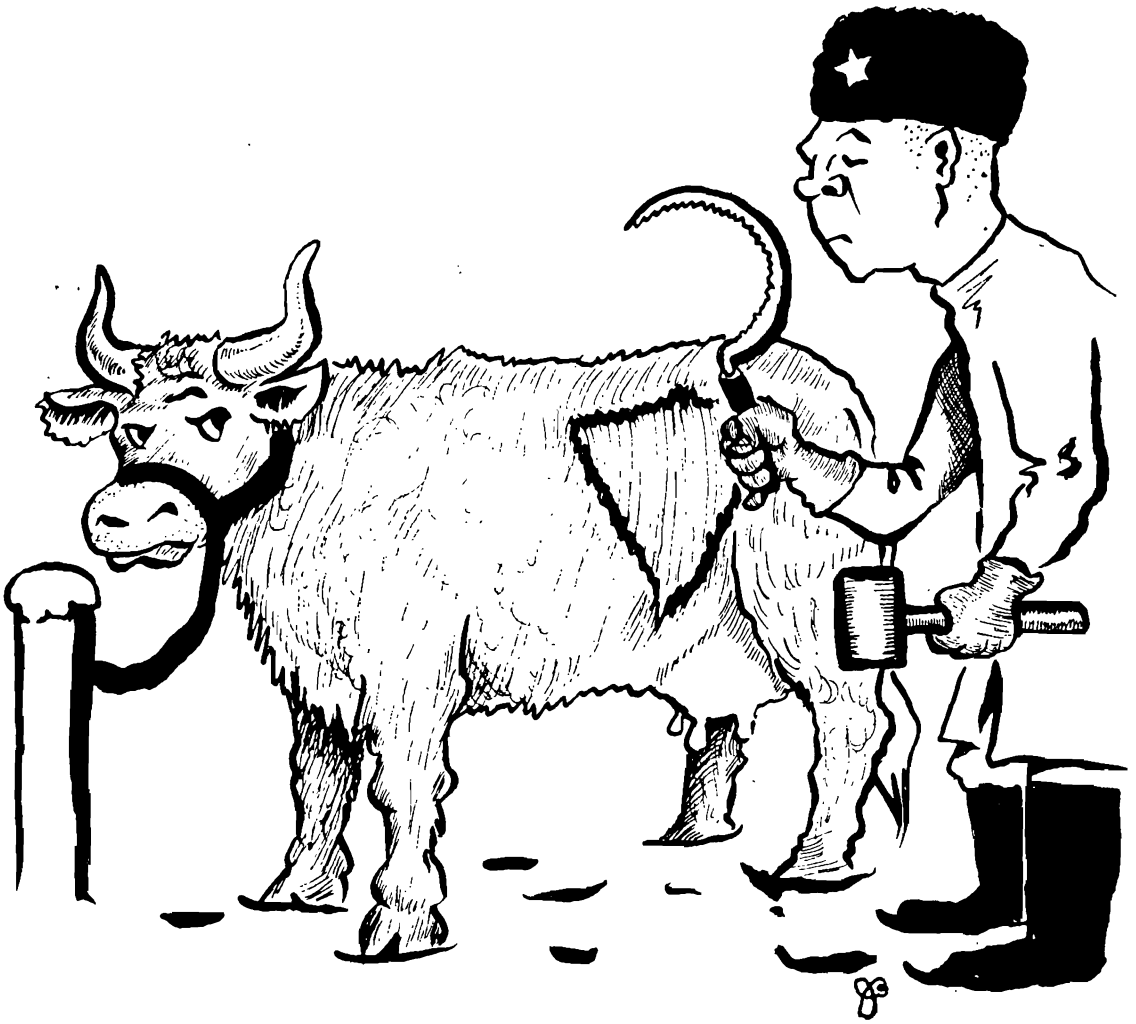
Mycoplasmas have been isolated from upper respiratory tract lesions in chickens and turkey sinusitis; the female bovine geni-

tal tract, where they caused inflammation predisposing to infertility; the male bovine genital tract; the diseased udder as a definite cause of mastitis in cows; cases of fibrinous pericarditis. pleuritis, peritonitis and arthritis in swine; cases of chronic or enzootic pneumonia in swine. They have also been isolated from the respiratory tract of dogs and from cases of the post-parturient fever syndrome in sows.

Very few mycoplasma species are known to infect more than one species of host. Some of them, e.g. *Mycoplasma mycoides*, stimulate the production of protective antibodies resulting in a solid immunity. In such infections vaccines have a useful prophylactic value. In contrast, other species do not provoke an immunity. Mycoplasmas in general are resistant to penicillin and sulphonamides, slightly susceptible to streptomycin and the nitrofurans and very susceptible to the tetracyclines, erythromycin and tylosin. Therapeutic measures cannot, however, be relied upon to control mycoplasmosis effectively. This should rather be based upon one or more of the following: vaccination, slaughter of infected animals and the establishment of closed flocks free of pathogenic mycoplasms.

From what has been said, it is obvious that there is no end to the stimulating challenges being offered by veterinary science. It is the duty of every member of the profession to contribute to the solution of our problems. We have come a long way since the days when veterinary science was practised by blacksmiths and later when it was no more than a pseudo-science. But we have no reason for complacency. What about the large scale application of preventive medicine schemes? How far are we from leadership in this sphere? How many of us have combined a business outlook on agriculture with our veterinary knowledge? Can we apply the results of the latest developments in science to the benefit of our country? We are in possession of wonder drugs, vaccines and remedies to control epizootic and erosive diseases; shall we be able, through their use, to exert marked influence on our animal industry in the future? I am sure we will, if we set our minds to it.

Veterinarians around the World 5



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THE RESPONSE OF THE BOVINE ADRENAL CORTEX TO HALOTHANE ANAESTHESIA*

D. G. STEYN**

SUMMARY

The response of the adrenal cortex to stress, induced by halothane anaesthesia, was studied in some Africander, Jersey and Friesland cattle and assessed by means of various haematological and biochemical determinations.

Following a preliminary starvation period and induction over 5 to 12 minutes deep surgical anaesthesia of one hour's duration was effected. Recovery was rapid and uneventful.

Marked breed differences were apparent in the nature of the adrenal cortical response evoked. In general, differential leukocyte counts showed that this response consisted partly of a transient neutrophilia, lymphocytopaenia and eosinopaenia. The neutrophilia was absolute in the case of the Friesland and Jersey cattle, but relative in the case of the Africanders. The latter animals also showed linear decreases in erythrocyte counts and haematocrit readings during the period of observation. Africander cattle apparently possess higher plasma cortisol levels than Jerseys and Frieslands and also maintain elevated plasma levels of this hormone for longer periods after anaesthetic stress. In the latter animals free plasma cortisol rises rapidly after the onset of anaesthesia decreasing rather abruptly to normal levels after recovery.

Africander cattle also respond to anaesthetic stress with a mild but prolonged hyperglycaemia. Some thoughts are advanced regarding the genesis of this phenomenon.

The eosinopaenic response followed a typical pattern in all breeds. After an initial sharp decrease in eosinophile counts immediately after anaesthesia, an eosinophilia became apparent during the subsequent forty eight hours.

No significant changes could be demonstrated in the plasma electrolyte levels of the representatives of the three breeds used.

INTRODUCTION

Since the discovery by Addison in 1849 that malfunction of the adrenal gland could be the cause of disease, many hormones with numerous functions have been isolated from the adrenal gland and more specifically from the adrenal cortex. Of the 27 steroids listed by Dorfman and Ungar[1] the C21 steroids cortisol, corticosterone and aldosterone are the most important, cortisol and corticosterone accounting for most of the corticosteroids secreted[2]. Various authors found low plasma levels in cattle compared to those in human beings[3,11].

Selye was the first to point out that stress elicits a non-specific response by the organism. The stimuli that are able to act as stressors activate the pituitary-adrenal system to promote metabolic responses that help the organism to face the altered circumstances[12]. In acute emergencies the organism calls primarily upon its carbohydrate reserves. This is characterized by an initial hyperglycaemia dependent upon the release of adrenalin and a secondary hyperglycaemia as a result of gluconeogenesis caused by the release of glucocorticoids.

Thorn was the first to make use of the decrease in eosinophils as a measure of adrenocorticoid activity. Various authors[13,14,15] demonstrated that adrenocorticoid activity was accompanied by an increase in the total white cell count, a neutrophilic leukocytosis, a lymphopaenia, and an eosinopaenia. The plasma sodium and chloride levels decrease during the shock phase of the alarm reaction. Hyperkalaemia and hyperkaluria may follow the liberation of much potassium from disin-

* Based on a dissertation submitted to the Faculty of Veterinary Science, University of Pretoria in partial fulfilment of the requirements for the degree M. Med. Vet. (Chir.)

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tegrating cell bodies at the time of the catabolic impulse [16].

It has been shown by various authors that surgical interferences also act as stressors and that the same non-specific changes can be anticipated after operation. Tension, pre-operative preparation, pain, shock, anaesthesia and the trauma of surgery may all contribute to the elevation of the plasma levels of 17-hydroxycorticosteroids (17-OHCS) [17-19]. The length of the operation and the extent of tissue damage could be correlated with the rise of steroids during and after the operation. Lewis [18] found a significant correlation between the rise in plasma hydrocortisone concentrations and the duration of halothane anaesthesia. Similar findings were recorded by the other authors in humans [20, 21] and in dogs [23].

Adrenocortical secretion in the bovine has been the object of study by various authors. Some of these studies have been based on indirect evidence of adrenocortical function [24, 25]. Plasma levels of 17 OHCS were however, recorded in normal cattle [5, 6, 8, 24, 26], in cattle in various physiological states [7, 27, 28], and cattle under stress caused by disease, surgery and other trauma [3, 11, 29, 30, 31]. In the bovine steroids are mainly excreted via the bile and steroid determination in the urine is, therefore, not a practical procedure [32].

The response of the bovine adrenal to stimuli appears to follow the same pattern as in other animals and man. It has been stated [18] that, in evaluating the adrenocortical response to operation, general anaesthesia, pain and shock are as important as actual tissue damage. The stress elicited by surgery cannot be fully assessed if the stress caused by anaesthesia is not known. Amongst the inhalation anaesthetic agents, halothane is becoming more popular. It is, therefore, the purpose of this investigation to determine the response of the bovine adrenal cortex to halothane anaesthesia.

METHODS AND MATERIALS

Experimental animals. Nineteen clinically healthy animals of various ages in good condition, were used. The group consisted of three Friesland cows, six Jersey cows, nine Africander heifers, and one Africander bull. One Jersey was lactating, five Jerseys were in various stages of pregnancy and the remainder of the animals were nonpregnant or nonlactating. The animals were kept in the same environment for six to eight weeks to adapt to prevailing conditions. They were taken through a crush once daily. Changes in local circumstances and unfamiliar procedures were therefore largely eliminated. They were fed a standard hospital ration and had free access to water.

Anaesthesia. The animals were starved for 24 hours and then anaesthetized without the use of premedication or basal narcosis for induction.

Anaesthesia was produced with halothane administered via a tight fitting face mask from a circle system anaesthetic apparatus. At the stage of light anaesthesia a cuffed endotracheal tube was introduced and the anaesthesia deepened to the level of surgical anaesthesia, as shown by a sluggish corneal reflex, protrusion of the third eyelid and disappearance of the anal reflex. This was maintained for an hour. The animals were then left to recover without being disturbed. The heart and respiratory rates were recorded at intervals of 15 minutes. The volume of halothane used during the entire procedure was recorded.

Collection of blood. Blood was obtained by jugular venipuncture. The first samples were taken before fasting and again 24 hours later immediately before anaesthesia. Subsequent samples were obtained at the termination of anaesthesia and 5, 24, 48 and 72 hours later.

Haematology. All counts were made on heparinized blood immediately after collection.

a. *Erythrocytes* were counted in a Spencer Bright-Line haemocytometer after being diluted 1 : 200 and mixed with Hayem's solution (mercuric chloride 0.5 g, sodium chloride 1./g, sodium sulphate 5.0 g, water 200 ml).

b. *Leukocytes* were counted after being diluted 1 : 20 in a solution containing glacial acetic acid 2.0 ml, 1.0 per cent solution gentian violet 1.0 ml, and water 97.0 ml.

c. *Eosinophils* were counted after diluting 1 : 20 in a solution of propylene glycol 50 ml, distilled water 40 ml, phloxine B (1 per cent aqueous solution) 10 ml, and sodium carbonate (10 per cent aqueous solution) 1 ml. Counts were done in duplicate on the same haemocytometer each time.

d. *Differential leukocyte counts.* Smears were prepared from blood flowing freely from the jugular vein without applying pressure to distend the vein and were stained with May-Grünwald-Giemsa and 200 cells were counted in each smear.

e. *Haematocrit* readings were determined on

samples from six animals using a micro-method technique.

Chemical determinations. Heparin was used throughout as anticoagulant. All determinations were performed on plasma and completed as soon as possible after sample collection. Plasma cortisol levels were determined by a method developed by van Rensburg (personal communication) embracing extraction and sol vent partition, thin layer chromatography, and spectrophotometry. This method is specific for free cortisol. Blood sugar was determined photometrically using the method of Lehman and Silk [33] and an Evans Electroselenium (EEL) portable model A photometer. Sodium and potassium were determined by flame photometry according to King and Wootten [34] and using the required accessories on a Zeiss PMQ — 2 spectrophotometer. Samples for determination of these two elements were often stored in the refrigerator until it was convenient to work through a batch. Plasma bicarbonate and chloride levels were both determined titrimetically according to the procedures of van Slyke, Stillman & Cullen [35] and Schales & Schales [36] respectively.

The values for the various determinations obtained from the samples collected 24 hours before anaesthesia served as control values for each animal concerned.

Statistical analysis. Since repeated measurements were performed on the same experimental unit, a method described by Cole & Grizzle [37] was used to analyse the effect of anaesthesia on the different values determined. In the multi-variate analysis of variance the test criterion of Lawley, viz. the sum-of-roots was used. Due to the limitations of the computer, the immediate and 5 hour post anaesthetic set of values and those obtained 24, 48, and 72 hours after anaesthesia had to be analysed separately. However, in all the analyses the breeds differed at $p = 0.01$. This suggested that the three breeds could be separated and an unvariate analysis of variance applied. This had two advantages, that the seven repeated determinations on the same animal could be used in the same analysis, and that transformations of the data could be applied. As a multiple comparison test, that of Scheffé was used since it is applicable when all contrasts are tested.

RESULTS

In the Jerseys and Friesland the mask was fitted in the standing position. The Africanders were fractious and had to be cast with ropes and the anaesthetic administered in the recumbent position. No clinical differences in the response of the animals to the anaesthetic could be found.

The time required to produce surgical anaesthesia varied from 5 to 12 minutes (mean 8.4). The average amount of halothane used for induction and maintenance of surgical anaesthesia for one hour was 43 ml (range 33 to 65 ml).

A slight tachycardia was recorded in some animals. The heart rate increased to 94 per minute after 45 minutes and then decreased to 80 per minute at the termination of anaesthesia. The respiratory rate showed a gradual increase from 26 to 32 per minute after 45 minutes at which rate it was maintained for the remainder of the anaesthetic period.

The animals regained a standing position 10 minutes after the halothane had been removed from the circuit.

Haematology.

a) *Erythrocytes.* There is a significant ($p = 0.1$) difference between the breeds. The following mean values were obtained: Frieslands 5.056×10^6, Jerseys 6.433×10^6 and Africanders 9.290×10^6/ cu. mm. The value for the Frieslands is lower than the values cited by various authors [24, 38, 39, 40, 41]. The value for the Jerseys is in agreement and the value for the Africanders considerably higher than those quoted.

The data obtained from the Frieslands and Jerseys showed no statistically significant differences or tendencies in the seven successive determinations. In the Africander cattle however, a significant ($p = 0.01$) linear tendency for the cell count to drop from 24 hours before to 72 hours after anaesthesia (Fig. 1A). During the one hour of anaesthesia the erythrocyte count decreased slightly but increased again during the five hours following anaesthesia. Similar findings were recorded in horses [42].

b) *Total leukocytes.* The mean for the Frieslands was 7586, for the Jerseys 7293, and for the Africanders 8132 leukocytes / cu. mm. These figures are in agreement with the figures quoted by Sholman [39], Osbaldiston [40], and Schalm [41] but lower than the figures of Canham [38] and Rusoff & Piercy [43].

In all three breeds there was a marked increase in the values obtained five hours after termination of anaesthesia i.e. at 30 hours. It is obvious from Fig. 1B that the changes in the Africanders were not as dra-

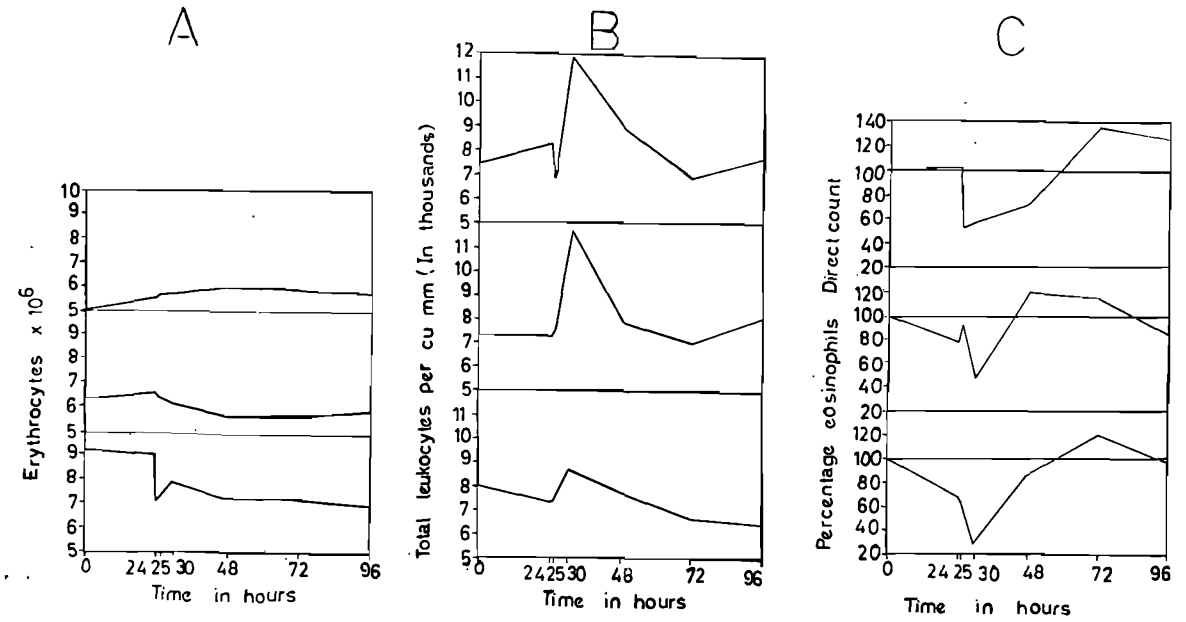

Fig. 1 The effect of halothane anaesthesia on A: the erythrocyte count; B: the total leukocyte count; C: the absolute eosinophil count. Top = Fries., Middle = Jersey., Bottom = Afr.

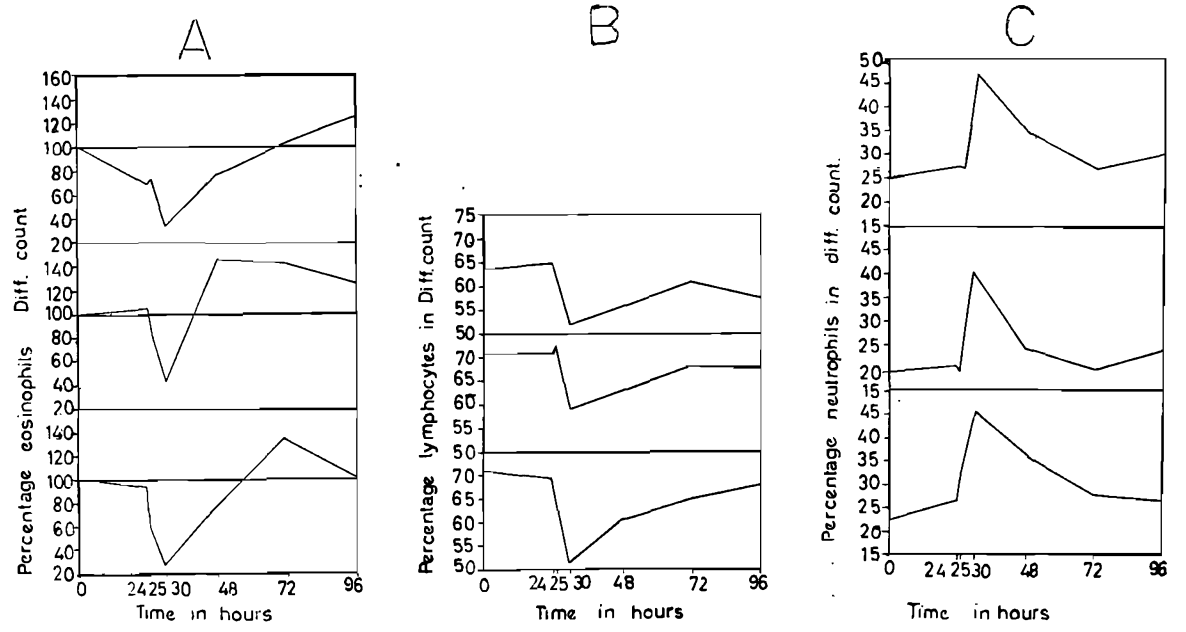

Fig. 2 The effect of halothane anaesthesia on A: the percentage eosinophils in the differential count; B: the percentage lymphocytes in the differential count; C: the percentage neutrophils in the differential count. Top = Fries. Middle = Jersey. Bottom = Afr.

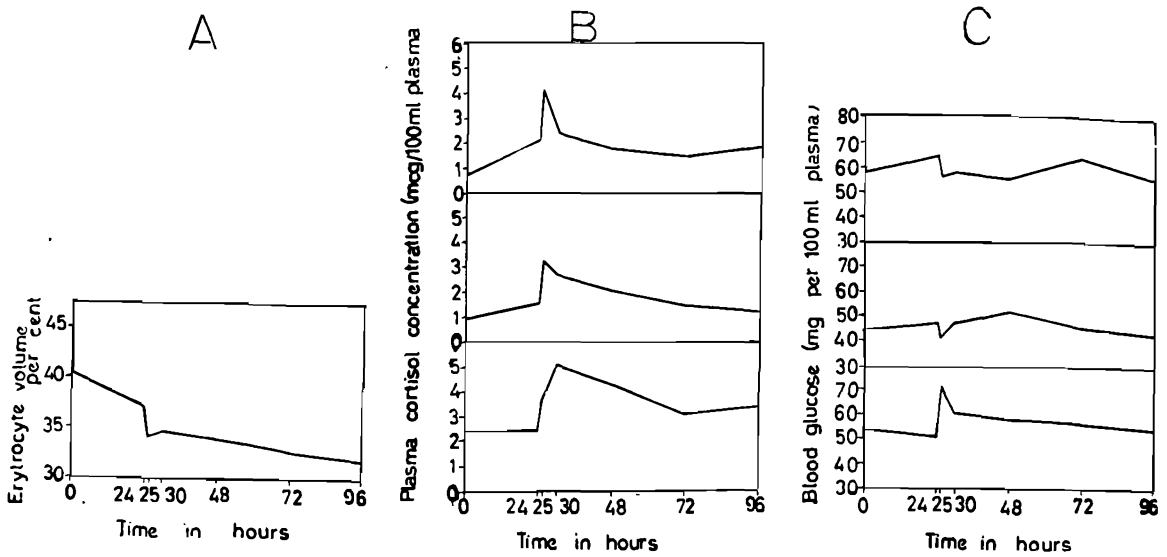

Fig. 3 The effect of halothane anaesthesia on A: the haematocrit readings; B: the plasma cortisol levels; C: the blood sugar levels. Top = Fries. Middle = Jersey. Bottom = Afr.

matic as in the other two breeds. In both Frieslands and Jerseys the values were still above normal at 24 hours after anaesthesia whereas the 48 hour values had decreased to below the baseline values. In the Africanders a significant ($p = 0.05$) linear drop occurred from 5 hours to 72 hours after anaesthesia although the latter mean was not significantly lower than the mean 24 hours before anaesthesia.

c) *Eosinophils.* The absolute eosinophil counts were as follows: Frieslands 502, Jerseys 473, and Africanders 293 eosinophils/cu. mm. These values are lower than the values quoted by Schalm [41] but correspond to those cited by Osbaldiston [40]. The values recorded for eosinophils in the differential leukocyte counts were 8.16 for Frieslands, 5.9 for Jerseys, and 4.65 per cent for the Afrikanders. These figures are higher than those given by Canham [38], Holman [39], and Schalm [41]; lower than the data of Osbaldiston [40] and Saroff [44], but compare well with the figures of Johnston, Rusoff & D'Armstrong [45].

If Fig. 1C and Fig. 2A are compared it is evident that they follow the same pattern. A marked decrease in the numbers and percentage of eosinophils in the differential count occurred between the immediate and 5 hour post-anaesthetic values. This was particularly evident in the data of the Africanders. After 24 hours the values had returned to within baseline limits. The 48 hour values were however all above these once more. At 72 hours the data were back to baseline in the Africanders, still elevated in the Frieslands, while the Jerseys showed a higher absolute count and a lower differential count than the normal. Similar findings were recorded by other authors following ACTH injections, electrical stress, adrenalin injections, and other stress factors [24, 30, 32, 46, 47, 48, 49]

d) *Differential leukocyte count.* The values obtained for the three breeds were Frieslands: lymphocytes 64 per cent neutrophils 25 per cent, and eosinophils 8.16 per cent; Jerseys: lymphocytes 70.8, neutrophils 19.7, and eosinophils 5.9 per cent; Africanders: lymphocytes 71.25, neutrophils 22.8, and eosinophils 4.65 per cent. The percentage lymphocytes found in the present work is therefore higher and the percentage neutrophils lower than the figures cited by Holman [39] and Osbaldiston [40].

e) *Lymphocytes.* In all three breeds the 5 hour figures were lower than at any other time during the experiment. The values were 81, 83 and 72 per cent for the Frieslands, Jerseys, and Africanders respectively (Fig. 2B). There was a gradual return to baseline values and although not statistically significant, the values were still slightly below normal at 72 hours, the last day of the experiment.

f) *Neutrophils.* From Fig. 2C it is clear that a marked rise in values occurred from the

post-anaesthetic sampling to the 5 hour sampling; the highest value being that of the Africanders with a 200.87 per cent increase. This corresponds with the decrease in lymphocytes during the same period. From this elevated level the values returned to near normal at 48 hours in the Frieslands and Jerseys but the Africanders were slower in returning to baseline levels.

g) *Haematocrit.* The haematocrit readings determined in six Africander cattle showed a significant ($p = 0.01$) linear drop from the 0 hour to the 72 hours post anaesthetic sampling. (Fig. 3A)

h) *Cortisol.* The mean values obtained for the three breeds were Frieslands 0.86, Jerseys 0.99, and Africanders 2.42 mcg/100 ml of plasma. These values are lower than the values recorded by various other authors [4, 5, 6, 8, 9, 10, 28, 29, 30, 44] but comparable with the values cited by Paterson [7] and van Rensburg (personal communication).

As illustrated in Fig 3B the initial value found in the Africanders was more than double the corresponding value from the Frieslands and Jerseys. This initial value was more than doubled after 24 hours fasting in the Frieslands and nearly doubled in the Jerseys whereas no changes occurred in the Africanders. The highest values were reached after one hour of anaesthesia in the non-Africander breeds but the value in the Africanders continued to rise to reach a peak at the 5 hour determinations. The stress of anaesthesia was therefore followed by an increase in the plasma levels of cortisol. The plasma cortisol level did not return to normal at the termination of anaethesia, but it remained elevated and only gradually returned to a level slightly higher than the initial values at the end of the experiment.

Elevated levels of plasma cortisol have been recorded in cattle following transport [50], electrical stress, shipping and injection of adrenalin [11, 30, 31, 44] and other stress factors [9, 29]. Various authors reported an increase in plasma cortisol levels in humans after anaesthesia and surgery [17, 18, 20, 21, 51, 52].

i) *Blood sugar.* The initial baseline values for blood sugar levels in the three breeds were Frieslands 58, Jerseys 44.8 and Africanders 54.5 mg per cent. This is in agreement with the figures cited by Cornelius & Kaneko [53] and Merrill & Smith [47].

In comparing the results in Fig. 3C one finds that in the non-Africanders a slight decrease in blood sugar occurred while under anaesthesia, whereas a definite increase was present in the Africanders. In the Africanders the blood sugar decreased in the five hours after anaesthesia compared to a slight rise in the other breeds. In the Africanders it remained above the normal baseline value for a longer time than the blood sugar value in the non-Africander.

j) *Sodium and potassium.* The mean of the baseline value for all the animals was 141 m Eq/l for sodium and 4.5 m Eq/l for potassium. These figures are in agreement with the figures quoted by Cornelius & Kaneko [53], Fisher [54], McSherry & Grinyer [55] and Coles [56].

No changes in either sodium or potassium values were evident in the post-anaesthetic samples as compared to the pre-anaesthetic values. Similar findings were reported by Muller [42] in horses after anaesthesia or anaesthesia and operations. Goetsch, McDonald & Odell [57] failed to demonstrate any changes in either sodium or potassium after the injection of four synthetic corticosteroids. In human patients however a rise in potassium, which was found to be relative to the duration and depth of anaesthesia, was found by other authors [49, 58-60].

k) *Bicarbonate and chloride.* The mean of the initial bicarbonate values as determined on nine Afrikanders was 24.5 m Eqil and that for chloride was 103.3 m Eq/l. The bicar bonate was lower than the value of 29.47 m Eq/l quoted by McSherry & Grinyer [55] and the value of 26.229 m Eq/l of Hauser-Hurlimann [61]. The chlorides compared well with those of other authors [55].

The values obtained during this experiment remained fairly constant and no significant changes could be demonstrated. This agrees with the findings of Müller [42] in horses.

DISCUSSION

Efficient anaesthesia demands maximum safety, ease of induction and maintenance, good relaxation and in the large animal, a rapid recovery [41]. Halothane seems to fulfil these criteria in animals and man.

The reason for the bradycardia [63, 64] and hypotension caused by halothane is not clear but a depression of the vasomotor centre and peripheral ganglionic block have been incri-

minated.[65]. In this experiment a mild tachycardia was seen in practically all the animals. The tachycardia cannot be explained by the liberation of adrenalin since the highest rate was seen some 45 minutes after induction in the Afrikanders. By that time the adrenalin liberated during the excitement stage of induction would have been metabolized. Although the Friesland and Jerseys did not resent the administration of halothane, tachycardia was also evident in these animals.

Halothane normally depresses spontaneous respiration and causes moderate hypercapnoea [42, 62, 64, 66]. During this study a slight increase in respiratory rate was found which is in agreement with the findings of Berge & Müller [63]. It was however noticed that the respiration was markedly shallower than in horses.

It has been shown in dogs [67] that bicarbonate or base excess falls when PCO_2 rises. The carbon dioxide combining capacity is reduced during respiratory acidosis. No deviations of the bicarbonate and chloride values were found in this study. Determination of PCO_2 and pH would have yielded more reliable information about this particular aspect.

The rise in erythrocyte count in the Friesland and Jerseys after withholding water and food for 24 hours was probably due to haemoconcentration. Dougherty & White [68] state that an initial rise in haemoglobin levels and red blood cell counts was observed within to six hours after a single injection of ACTH. Subsequently the haemoglobin concentration and erythrocyte count fell to subnormal levels which persisted for as long as 24 hours in mice. The tendency toward a decrease in erythrocyte counts and haemoglobin values as seen in man resembles the alterations in the blood picture seen in other species treated with adrenocortical extracts. Haemodilution could have accounted for the reduction in numbers of erythrocytes and in the haematocrit readings in this study. Dougherty & White [69] report however that erythrophagocytosis was observed within one hour following injection of ACTH or adrenocortical extracts. Köhler [24] found a slight increase in erythrocytes 24 hours after ACTH injections.

Various authors have studied the effect of ACTH and adrenocortical hormones on the leukocyte picture and lymphatic tissues of animals and man [13, 15, 47, 57, 68, 69, 70, 71, 72, 73].

The administration of one of these preparations was usually followed by an eosinopaenia, a lymphopaenia, and a neutrophilia.

The effect of stress factors on the leukocyte picture has been the object of investigation by a number of authors [7, 13, 14, 24, 48, 74, 75].

A large number of drugs, bacterial products, tissue metabolites. and disease states have been found to produce adrenocortical mediated lymphopaenic responses. This phenomenon is mediated by the discharge of ACTH from the adenohypophysis and is induced by those steroids with an oxygen on carbon 11 [48]. An increase in neutrophils of 85.78 per cent in the Jerseys, 88 per cent in the Frieslands, and 100.87 per cent in the Africanders recorded at the 5 hour sampling is in agreement with the findings of the authors mentioned. The total leukocyte count in the Afrikanders increased by only 7.7 per cent. The differential leukocyte count showed a decrease in lymphocytes to 72.42 per cent and the eosinophils to 26.88 per cent. From this it appears that although the neutrophils increased to a value of 200.87 per cent, this increase was largely neutralized by the great decrease in lymphocytes. The neutrophilia was therefore relative in nature.

Dougherty [13] stated that the duration of the lymphopaenia is related to the amount of adrenocortical steroids secreted, the period over which accelerated adreno-cortical secretion continues and the rate of restoration of lymphocytes to the blood. The cortisol levels and lymphocyte counts in the Africanders showed a definite correlation. Kumagai & Dougherty stated that it is possible that the lymphocytotic response or the lymphocyte reaction to stress stimuli could be developed clinically as a tolerance test to determine the capacity of the individual to react to such agents.

The eosinopaenic response, known as the Thorn test, has perhaps been most widely used of all indirect tests of adrenal function [53]. Following the administration of ACTH, the drop in the number of eosinophils expressed as a percentage of the pre-injection count, is approximately twice as great as the drop in the number of lymphocytes [48]. The mean drop in the three breeds was about 21 per cent in lymphocytes compared to 66.5 per cent in eosinophils.

A eosinopaenic response does not necessarily mean that adrenocortical activity is increased, since such a change ensues after adrenalin liberation [17, 32, 76, 77]. The eosinophil count should also be related to the possibility

of an allergic response or detoxification in decomposition of body protein. The liberation of adrenalin in the experimental animals used in the present study especially the Africanders could account for part of the eosinopaenic response.

The eosinopaenic response is generally typical in nature and comprises the following phenomena: after a dramatic fall in eosinophils, the level of these cells rises to above baseline values before returning to pre-stress levels. This was also clearly illustrated in the present study.

Stress was defined by Selye [78] as: "The state manifested by a specific syndrome which consists of all the non-specifically induced changes within a biological system".

This stress elicits nervous stimuli which are transmitted into the median eminence where a neurosecretory product, corticotrophin releasing factor is secreted into the primary capillary plexus of the hypophyseal portal system. The portal system carries this product to the venous sinuses of the adenohypophysis where the glandular cells are excited to secrete corticotrophin[79]. This in turn activates the adrenal cortex and glucocorticoids are secreted. The rate of steroid production can be very rapidly increased. In less than half an hour the plasma concentration of 17—OHCS may be increased to relative high levels under the influence of ACTH[80]. The concentration of cortisol hormones in plasma depends upon the secretion rate, rate of uptake by the tissues, rate of destruction by the liver, the amount bound to protein, and the rate of excretion. The disposal of steroids occurs mainly in the liver where they are degraded into many metabolites. Apart from these factors one should in the interpretation of analyses obtained from determinations of cortisol in plasma, consider the diurnal rhythm as observed in man [81] and horses [75]. Shaw, Dutta & Nichols [28] could find no such rhythm in cattle.

Only small increases of plasma hydrocortisone after halothane anaesthesia were recorded [52, 20, 23]. Although the trauma of an operation is a more severe stimulus than the anaesthetic, part of the adrenocortical response must be attributed to the stress of anaesthesia. The impaired removal of free plasma 17—OHCS as a result of liver impairment may be an important factor in maintaining an elevated 17-OHCS level in the immediate post-anaesthetic or post-operative period [82]. This effect on the liver may partly explain the relatively high levels obtained in the post-anaesthetic period in the Africander cattle. Struggling and excitement may have resulted in adrenocortical stimulation and consequently a higher 17-OHCS level.

The cortisol values in all three breeds remained above normal for a considerable time and only returned to near normal on the Thursday, 48 hours after anaesthesia, indicating continued ACTH liberation by the adenohypophysis. The sharp decline in the cortisol levels in the post-anaesthetic period in the Frieslands and Jerseys is in accordance with the findings of Brush [83] and others [18]. In another investigation plasma cortisol levels only returned to normal on the fifth post-operative day [51].

One must assume, if the results of the present study are considered, that halothane anaesthesia does provoke a pituitary-adrenal reaction and should therefore be considered a stress factor in the bovine at any rate. Considerable variation occurred in the magnitude of this response in individual animals.

An increase in blood sugar during and after anaesthesia has been reported in humans and animals. This hyperglycaemia could be correlated directly with the duration of anaesthesia [64].

The sudden hyperglycaemia noted in the Africander cattle was more likely to be the result of adrenalin than an adreno-cortical effect. The blood sugar level, after the drop between the immediate post-anaesthetic and 5 hour values was maintained for about 24 hours, probably due to adreno-cortical activity. The action of gluco-corticoids is slower and more persistent than of adrenalin. The magnitude of the hyperglycaemia which follows immediately upon exposure to stress, is largely determined by the hepatic glycogen reserves and depends largely upon the "energy discharge of adrenalin". Although the hyperglycaemia associated with hyperadrenocorticism is in part due to gluconeogenesis from protein, it is also partly due to inhibition of pyruvate catabolism making pyruvate available for resynthesis of glucose [85].

Various authors have demonstrated an increased potassium level in humans after prolonged anaesthesia, the increase being relative to the duration and depth of anaesthesia. The concentration ratio of potassium to sodium in bovine urine is on an average some ten times greater than that of human urine, sodium

being the principal urinary cation in man, whereas potassium may be the principal urinary cation in the bovine depending on the diet. The bovine kidney is therefore equipped to handle large quantities of potassium. Excretion of potassium by the kidney may keep the plasma levels of potassium within normal limits even though a moderate hyperkalaemia would otherwise have occurred.

In order to obtain a full picture of the changes associated with halothane anaesthesia in the bovine, it will be necessary to repeat some of the determinations after anaesthesia of varying duration especially in anaesthesia of two or three hours duration. Different findings pertaining to plasma cortisol, blood sugar, and potassium levels and leukocyte counts may be obtained when the animal is exposed to varying durations and depth of anaesthesia.

Premedicants and basal narcotics may alter the response of the adrenal cortex when used in combination with halothane. The ataractic drugs are known to cause, for instance, a fall in blood pressure similar to the effect of halothane. Used simultaneously, these drugs may have a summation effect. Induction of anaesthesia with other agents and maintenance with halothane, as it is used in clinical practice, will be the object of further study.

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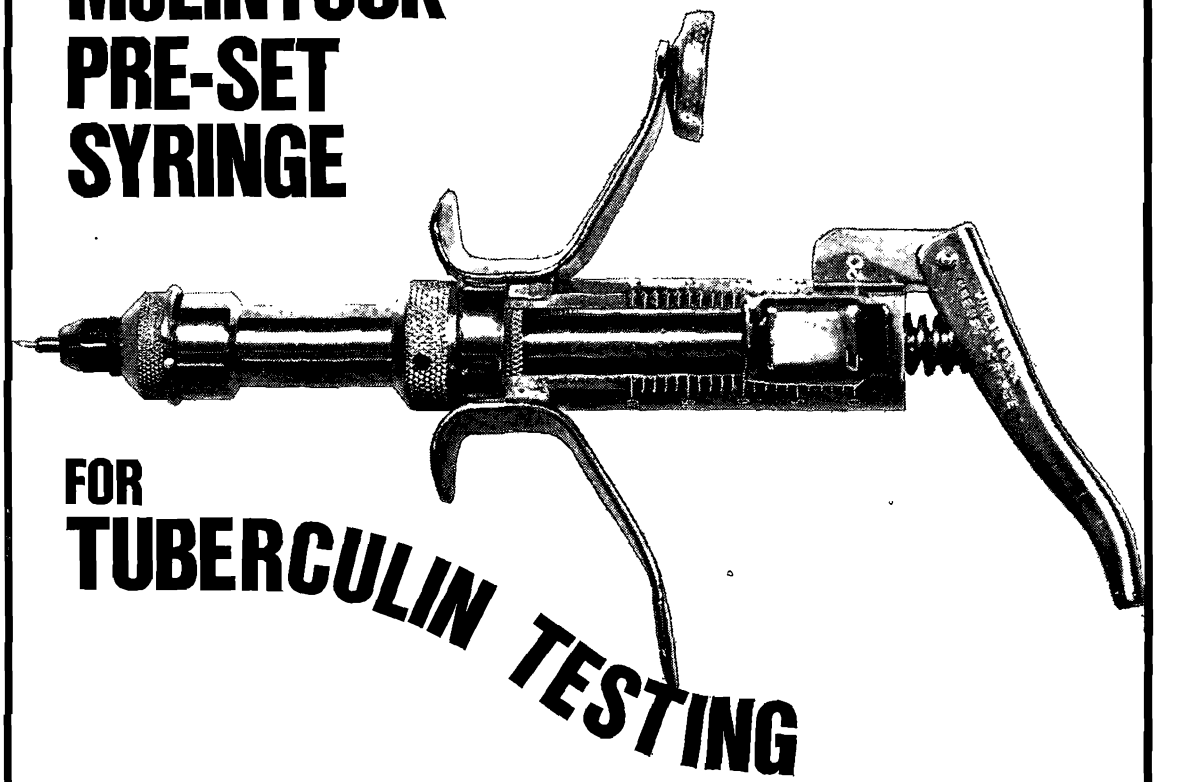

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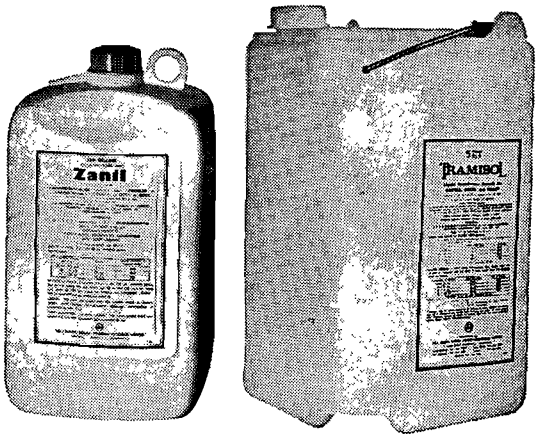

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A SEMIPERMANENT EXTRACORPOREAL VENO-VENOUS SHUNT IN BOVINES

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SUMMARY

A semipermanent veno-venous shunt applicable to bovines for extracorporeal irradiation of circulating blood is described. Although extracorporeal in position, it causes no discomfort and requires no special care.

INTRODUCTION

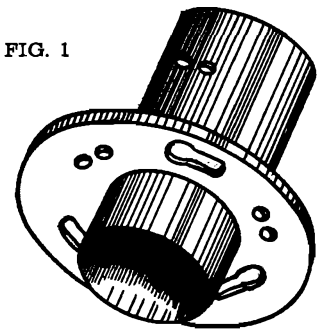
Extracorporeal irradiation of circulating blood (ECIB) has proved to be a valuable research tool, apart from its present experimental clinical application [1-10], which may well in future prove to be practical and beneficial. Extracorporeal irradiation has been used in this laboratory in studies on lymphocytes in collaboration with Cronkite*** and co-workers. In earlier studies [8, 10], tygon tubing was inserted and tied into the external jugular veins of calves, establishing a veno-venous shunt. Although a great deal of information [10, 11, 12] was obtained with those studies, the need soon arose to establish more permanent exteriorized vascular shunts in order to make further progress. The main disadvantage of the earlier veno-venous shunts was that they only permitted uninterrupted extracorporeal irradiation.

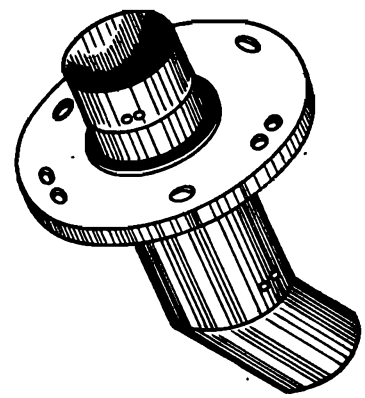
Chanana and Cronkite [13] have developed a surgical technique and an improved Teflon silastic shunt to establish a semipermanent exteriorized vascular shunt, enabling them to do extracorporeal irradiation daily for periods up to 44 days. This permitted them to do studies on homograft rejection and on lymphoma in cattle. Their shunt, however, is an arterio-venous one, which is not physio logical; other complications were also experienced.

DESCRIPTION OF APPARATUS AND TECHNIQUE

We now have a veno-venous shunt that is established under local anaesthesia with

FIG. 1





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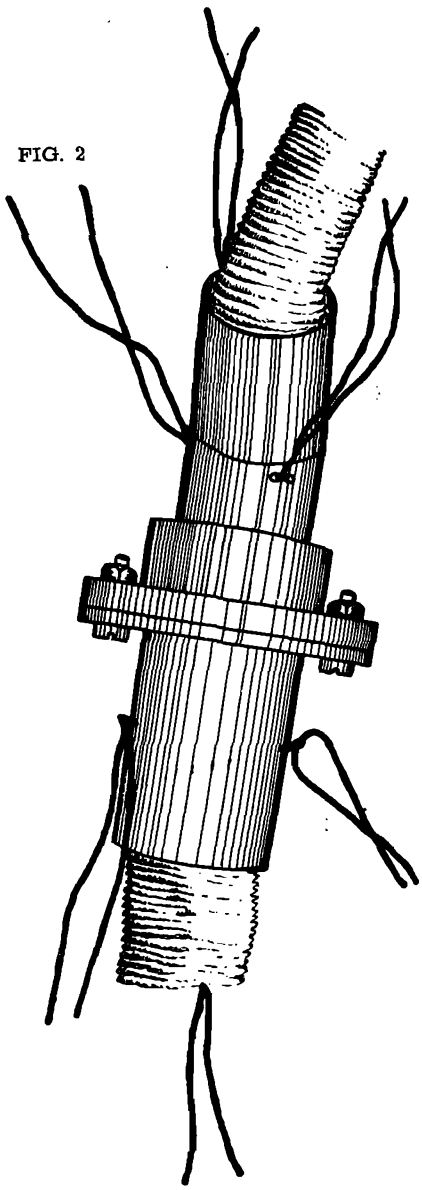
*** Brookhaven National Laboratories, U.S. Atomic Energy Commission.

minimal immobilization and discomfort to the animal. We do up to six hours extracorporeal irradiation daily. Between these sessions, the shunt is coupled up the animal living normally with other animals in the barn and

FIG. 2



requiring no special care whatsoever.

Our shunt consists of two cylindrical metal connectors, as shown in Fig. 1. A dacron vascular shunt is inserted into each metal cylinder and the end furthest away from the vein is inverted and sutured onto metal with No. 1 braided nylon, as shown in Figs. 1 and 2. The metal connectors with the dacron sewn on are sterilized by autoclaving. Two pieces of tygon tubing, $3\frac{1}{2}$ in. long and with $\frac{5}{8}$ in. inside diameter, are sterilized by immersion in 130 vol. H_2O_2 for 24 hours. The tygon tubing is split for about 2 inches on the one side and pulled over the dacron graft and metal connector with the latter pushed into the tubing through the split end.

The dacron graft when stretched, now extends for about $\frac{1}{2}$ in. outside the tygon tubing. The shunt is now ready for insertion into the animal.

Calves weighing 400 to 600 lb are used in our studies. A calf is put into a stanchion and restricted with slings as described earlier [10]. The neck is shaved and washed with germicidal soap and water. The skin is then prepped with tincture of iodine. Xylocaine 2% local anaesthetic is injected into the skin overlying the external jugular vein. Two incisions, 10 cm long and 7 cm apart, are made over the external jugular vein. The vein is exposed and mobilized through both incisions. The most cephalic and caudal ends of the vein are compromised with rubber catheters. The vein is severed $1\frac{1}{2}$ in away from each catheter and the vein in between the transcisions is mobilized, including the section under the skin bridge separating the two skin incisions. This piece of vein is discarded.

An end-to-end anastamosis is established between the cephalic dacron graft with a continuous 000 silk suture. The tygon tube is now pushed over the metal connector until its external end lies against the shoulder of the metal connector. This results in the tygon tube covering the whole dacron graft from the point of emerging from the metal connector to the site of anastomosis with the vein, as well as about 1 inch of vein.

The tygon tube is fixed to the metal connector with three No. 2 braided nylon sutures, to the fascia and surrounding muscles with about four sutures and to the skin with one suture. These sutures are very important as they prevent the shunt being torn away with force from the outside.

Care is also taken in having the correct

length of dacron grafts and tygon tubing to ensure that, after anastomosis, the vein section and graft lie freely, without tension or distortion, inside the tygon tubing.

Similarly, the caudal section of the shunt is anastomosed to the vein and fixed to the deeper tissues and skin. The two catheters compromising the ends of the vein are now released and the connectors coupled up, establishing the exteriorized shunt. The dacron-covered end of the cephalic metal connector fits firmly into the dacron lining inside the caudal connector to ensure that no blood leaks out.

The skin wounds are now closed with interrupted No. 1 braided nylon sutures up to the tygon tubing.

Two pieces of strong cloth, measuring 10×4 in. each are now fixed to the skin on each side of the shunt, 2 in. away and parallel to the shunt with interrupted No. 1 braided nylon sutures. When the free ends of the two pieces of cloth are joined together, the shunt is covered completely to prevent it from hooking onto any object when the animal walks around outside. When the extracorporeal irradiation is performed the animal is again taken into a stanchion and slings are utilized for restraint.

With the coupling-up procedure, the external jugular vein is manually compressed, just cephalic to the anastomosis, the metal connectors separated and connected with suitable plastic connectors which are connected to the external tygon tubing which passes around the radiation source. This enables connecting up with hardly any blood loss.

At the end of an extracorporeal irradiation session, the blood in the external circuit is reinfused and the two sections of the shunt connected, as described above.

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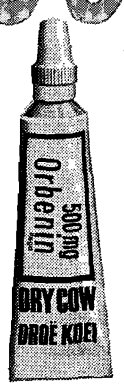

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Jl S. Afr. vet. med. Ass. 40 (4): 371-374 1970

THE BLOOD SUPPLY TO THE PERIOSTEUM OF THE CANINE FEMUR

D. G. STEYN*

SUMMARY

The coxofemoral area, the area of the shaft of the femur and the femorotibial joint were dissected in nine dogs to determine the sources of blood supply to the periosteum of the femur. The main vessels concerned are the following: *A. glutealis caudalis,, A. profunda femoris* and *A. circumflexa femoris lateralis, A. genus descendens* and *Aa. genus caudales.* Blood vessels either go to the periosteum directly or via the synovial plexus, or via muscular attachments. The insertion of the *M. adductor magnus et brevis* is particularly important in the latter respect.

INTRODUCTION

Fractures of the femur in the dog occur more frequently than fractures of any other bone in the body. Leonard[1] cited a figure of 27.1 per cent. After a fracture, healing of bone and union of the fragments are achieved by proliferation of the osteogenic layer of the periosteum and endosteum. Two advancing collars of callus are formed which bridge the gap between the two fragments to obtain union.

The periosteum and endosteum of long bones have a relatively rich blood supply. From these structures numerous small blood vessels enter the Haversian system to supply the compacta. Markowitz, Archibald & Downe[2] state that the periosteal vessels enter the medullary circulation directly, then supply the compacta. They also state that bone necrosis follows the removal of the entire periosteum. Anastomoses exist between all sources of blood supply to the periosteum.

Although the endosteum is the more important source of callus formation the periosteum also plays an important part in the healing of bone. This contribution to bone healing, however, is dependent upon on adequate blood supply. Miller, Christensen & Evans[3] state that it is chiefly through enlargement of the periosteal arteries and veins that an increased blood supply and increased drainage are obtained at the site of fracture. With intramedullary fixation much of the internal blood supply is destroyed or at least disturbed.

Various text books mention blood vessels supplying structures in the vicinity of the femur. Fitzgerald[4] determined the blood supply to the head of the canine femur. No references to the sources of blood supply to the femoral periosteum could be found. It was decided, therefore, to determine these sources.

MATERIALS AND METHODS

Nine medium to large dogs were used in this study. After the dogs had been exsanguinated under general anaesthesia, the posterior part of the body was removed just caudal to the last rib and kidneys. By placing a cannula in the distal aorta, the vascular system was thoroughly perfused with 10 per cent formalin and the specimen left in formalin for three days. Red latex was then injected under pressure and the specimen left in formalin for a further three days to allow the latex to set. The coxofemoral area, the area of the shaft of the femur and the femorotibial joint were then dissected.

OBSERVATIONS

For the sake of systematic description a classification of the blood vessels will be given at this stage according to the Nomina Anatomica Veterinaria[5]. Only the relevant vessels will be mentioned here, as elsewhere in this article.

The blood supply to the femoral periosteum is derived from two main blood vessels viz. *A. iliaca interna* and *A. iliaca externa.*

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A. *A. iliaca interna*

A. glutealis caudalis

B. *A. iliaca externa*

1. *A. profunda femoris*

"A. nutricia femoris"

A. circumflexa femoris medialis

R. obturatorius

R. acetabularis

R. ascendens

2. *A. femoralis*

A. circumflexa femoris lateralis

R. ascendens

R. transversus

A. femoralis caudalis proximalis

A. genus descendens

A. femoralis caudalis media

A. poplitea

Aa. genus proximalis lateralis et medialis

A. genus media

Aa. genus distalis lateralis et medialis

The *A. glutealis caudalis* gives off a small branch which crosses the *N. ischiadicus* to run along the medial surface of the *M. gluteus medius* and over the *M. gluteus profundus* to the hip joint where it supplies the joint capsule (Fig. 1).

The *A. profunda femoris* (Fig. 1) arises from the *A. iliaca* externa and gives off the *Truncus pudendoepigastricus*. About 3 cm from the latter's origin a distally directed vessel appears to branch off from the main stem. This vessel, which ramifies in the *M. adductor magnus* et *brevis* and ends by one branch in the *M. vastus medialis* and several in the *M. biceps femoris*, would correspond to the terminal part of the *A. profunda femoris* in man, but has been designated the *"A. circumflexa femoris medialis"* by Miller, Christensen & Evans[3]. It gives off *inter alia* the *"A. nutricia femoris"* (not listed in the N.A.V.[5]). The latter extends distally to enter the nutrient foramen on the caudal surface of the femur at the junction of the proximal and middle thirds. (An exception to the usual pattern was found in one dog in which the *"A. nutricia femoris"* was given off by the *A. poplitea*. From there it ran in a proximal direction to enter the foramen at about the middle of the femur). The continuation of the main stem would then be the *A. circumflexa femoris medialis*, according to its branches as listed in the N.A.V.[5]. The *R. obturatorius* (Fig. 2) is the first to be given off, craniomedial to the obturator foramen. It furnishes a large muscular branch to the proximal part of the *M. adductor magnus et brevis*, as well as a branch which extends lateralward to enter the trochanteric fossa and supply the caudal part of the joint capsule (Fig. 2). This branch may arise next to the *R. obturatorius* directly from the *A. circumflexa femoris medialis*. It is interpreted as representing the *R. acetabularis* of that vessel.

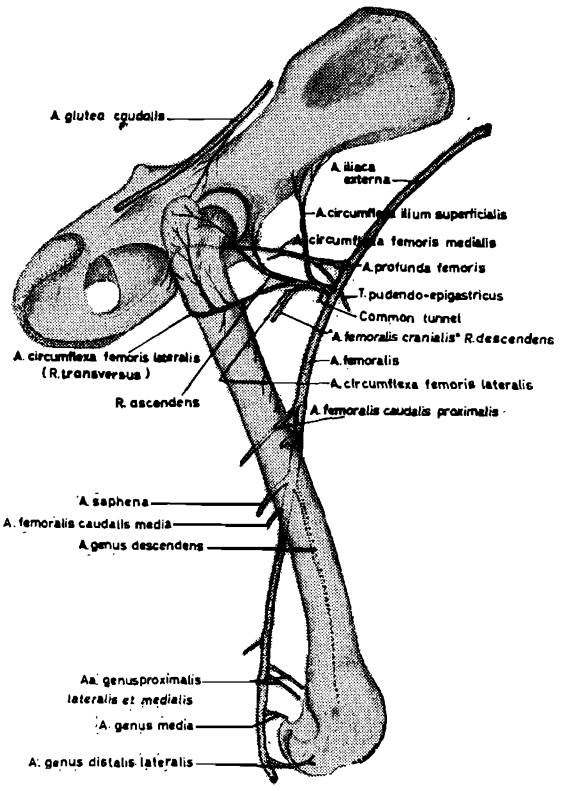

Figure 1. Vessels supplying the femur
Right lateral view.

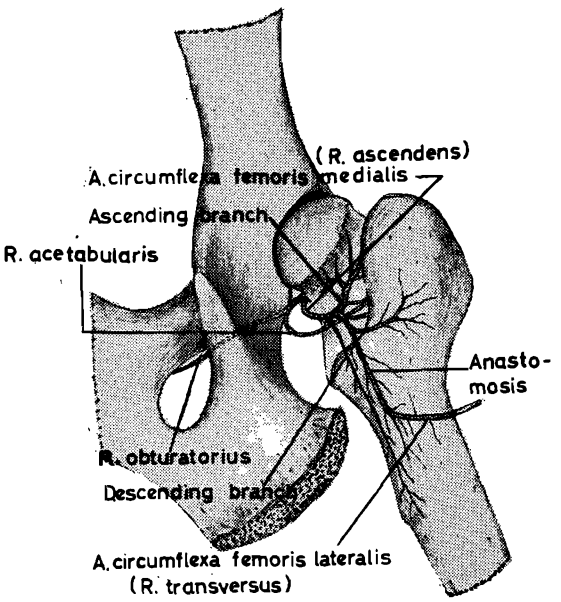

Figure 2.
Vessels around hip joint.
Dorsocaudal view.

The *A. circumflexa femoris medialis* continues crossing the surface of the *M. vastus medialis* to reach the caudal surface of the femur. It then gives off the *R. ascendens;* running proximolaterally. At the level of the junction of the trochanter major and shaft of the femur, under cover of *the M. quadriceps femores,* the *R. ascendens* divides into a secondary ascending and descending branch (Fig. 2). The ascending branch supplies the fascia and periosteum over the trochanter major. Various small branches go to the caudodorsal and caudoventral parts of the neck of the femur and forms an anastomosis with the *R. transversus* of the *A. circumflexa femoris lateralis* (Fig. 2). The ascending branch courses distally over the caudolateral aspect of the femur to supply the fascia and origin of the *M. vastus lateralis.* The *R. profundus* and *R. transversus* of the *A. circumflexa femoris medialis* are not concerned directly with the supply to the femur.

The craniolateral arterial supply in the proximal part of the thigh comes by way of the *A. femoralis* via the *A. circumflexa femoris lateralis.* What is listed in the N.A.V.[5] as the first branch of the *A. femoralis,* namely the *A. circumflexa ilium superficialis,* in the material examined always arose in common with second listed branch, the *A. circumflexa femoris lateralis* (Fig. 1).

From the *A. circumflexa femoris lateralis* the *R. ascendens* (Fig. 1) which is also described by Miller, Christensen & Evans[3] runs in a caudodorsal direction to the cranial part of the joint capsule and to the insertions of the gluteal muscles on the trochanter major.

The *R. transversus* crosses over the lateral surface of the femur in the *M. vastus lateralis* distal to the trochanter major. It continues caudally to anastomose with the *R. ascendens* of the *A. circumflexa femoris medialis.* It gives off small branches which supply the trochanter major (Fig. 2). On the cranial surface of the femur, immediately before entering the *M. vastus lateralis,* the *R. transversus* furnishes a branch to the periosteum of the femur. This branch extends distally over the cranial surface of the femur and is visible up to about the middle of the bone (Fig. 1). Small twigs of this vessel ramify in the periosteum. The *R. descendens* of the *A. circumflexa femoris lateralis* (the *A. femoris cranialis* of veterinary textbooks[5]) does not supply the femur.

The *A. femoralis caudalis proximalis* and the *A. femoralis caudalis media,* by virtue of their supply to the *M. adductor magnus et brevis,* also contribute to the arterial supply to the periosteum of the shaft of the femur.

Contrary to the order of listing in the N.A.V.[5], and in agreement with the statement by Miller, Christensen & Evans[3], the *A. genus descendens* (Fig. 1) leaves the *A. femoralis* distal to the origin of the *A. saphena.* It continues distalward, parallel to the femur, between the *M. vastus medialis* and the *M. semimembranosus* to the medial surface of the stifle joint where it divides into numerous branches to supply both femoropatellar and femorotibial joint capsules.

The *A. genus proximalis lateralis* and *A. genus proximalis medialis* leave the cranial face of the *A. poplitea* at the level of the femoral condyles. In addition to supplying the two heads of the *M. gastrocnemius,* they also furnish branches to the joint capsule. The *A. genus media* and the *Aa. genus distalis lateralis et medialis* also supply the caudal part of the femorotibial joint capsule.

Few blood vessels enter the periosteum directly. Where the joint capsule blends with the periosteum, the blood vessels of the synovial plexus are continued into the periosteum. This is the case at the coxofemoral and stifle joints. The blood vessels supplying the joint capsules of these two structures, therefore, are important sources of supply to the periosteum.

The insertion of the *M. adductor magnus et brevis* blends with the periosteum on the popliteal surface of the femur. Numerous small blood vessels enter the periosteum in this region. They run through the substance of the muscle. The blood vessels which supply this muscle, therefore, also contribute to the supply of the periosteum. These vessels include the *A. profunda femoris*, *A. femoralis caudalis proximalis* and *A. femoralis caudalis* media. At the origin of the *M. vastus lateralis* on the cranio-lateral surface of the femur similar observations were made. In this respect, however, the *M. adductor magnus et brevis* plays a much more important role.

The *"A. nutricia femoris"* by way of the anastomosis between periosteal and endosteal vessels must also play an important part in supplying the periosteum with blood.

An interesting observation was made in one case in which a healed fracture of the femur was encountered at dissection. Numerous adhesions were seen between the periosteum and the surrounding muscles. Numerous small vessels coming from the substance of these muscles entered the periosteum in the region of the fracture. In this way additional blood was brought into the area to promote healing.

DISCUSSION AND CONCLUSIONS

Fitzgerald [4] stated that the blood supply to the head of the canine femur is derived from three sources: the *"A. profunda femoris"*, *"A. femoralis cranialis"* and *A. glutealis caudalis*. These findings, when equated with the new terminology, could be confirmed in this study. In addition to the supply to the head of the femur these blood vessels also supply the periosteum of the trochanter major, various muscles, the periosteum of the shaft of the femur and the *"A. nutricia femoris"*.

The blood supply to the periosteum through the substance of muscles is of practical significance. In treating fractures by open reduction and internal fixation, pieces of bone with their muscular attachment intact can be left in position as they will probably still be viable and promote blood supply. Extensive stripping of muscular attachment from the bone, particularly of the *M. adductor magnus et brevis*, should be avoided where possible.

ACKNOWLEDGEMENTS

The author wishes to express his gratitude toward Prof. H. P. A. de Boom for his interest and guidance, Dr. W. H. Gerneke for the drawings and Mr. A. M. du Bruyn for preparing the photographs.

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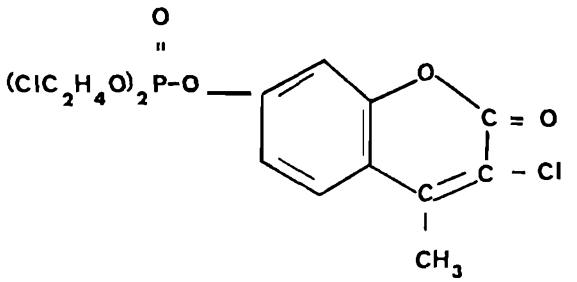
HALOXON



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J. 4790

Jl S. Afr. vet. med. Ass. 40 (4): 377-378 1970

THE DIAGNOSIS OF PREGNANCY IN THE EWE WITH AN ULTRASONIC FOETAL PULSE DETECTOR

D. K. SHONE AND J. W. FRICKER*

SUMMARY

In a flock of 309 Merino ewes, only four were wrongly diagnosed as being pregnant and one wrongly diagnosed as non-pregnant by means of the 'Doptone' (Smith Kline Instrument Company) ultrasonic foetal pulse detector. The lambing percentage in the flock was 78.6%.

INTRODUCTION

As pregnancy in the ewe can only be established with certainty by exploratory laparotomy, it rarely forms part of a clinical examination. The farmer uses the development of the udder and the presence of colostrum as indicative of pregnancy but this is not only limited to the terminal stages of the gestation period but is also unreliable. An ultrasonic foetal pulse detector has been reported to have been used successfully for the detection of pregnancy in the ewe and pig by Fraser and Robertson [1] and ewe by Hulet [2].

An ultrasonic foetal pulse detector was used to diagnose pregnancy in a flock of Merino ewes used in a study on reproduction and the results are reported.

MATERIALS AND METHODS

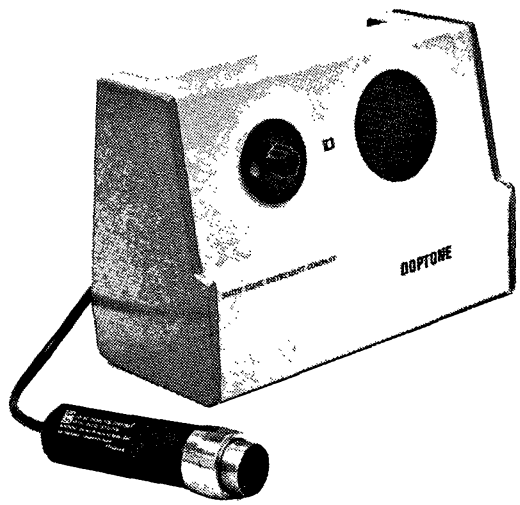
The ultrasonic foetal pulse detector: This instrument** (figure) consists of an amplifier and transducer or probe. The transducer which contains transmitting and receiving crystals transmits a continuous output signal of low intensity inaudible to the human ear. If the sound waves strike a moving object they are reflected to the transmitting source at a slightly altered frequency—the "Doppler" effect. The altered sound waves are picked up by the transducer, transmitted to the amplifier and converted into audible sound. The reflected sounds have different characteristics depending upon the type of movement. The foetal and maternal arterial sounds are readily distinguishable from the venous sounds.

The foetal pulse rate is nearly double that of the maternal pulse rate and the foetal heart beat is in addition readily recognized by the double beat. Diagnosis of pregnancy is dependent upon the detection of these very characteristic sounds.

The magnetic tape recording of the sounds audible in the examination of human subjects supplied by the manufacturers is most valuable to the novice.

The instrument operates on rechargeable batteries and is fully portable.

Examination of the ewe: The transducer head is placed in direct contact with skin devoid of wool or hair and the air excluded by a layer of transmission gel applied to the skin. The ewes were placed on their backs on a table and the transducer applied to the wool-free skin of the abdomen in the right inguinal area. The right inguinal area was selected because it was presumed that any displacement of the uterus by the rumen would be to this side. Manipulation of the skin with the left hand while the transducer was guided



The 'Doptone' Ultrasonic Foetal Pulse Detector.

* Terenure Research Station, A. S. Ruffel (Pty.) Limited, P.O. Box 38, Isando, Transvaal.

** 'Doptone' Smith Kline Instrument Company.

with the right hand enabled the sound wave to be directed to any part of the posterior abdomen.

The ewes: A flock of 309 individually numbered Merino ewes were run with 13 rams for a period of 52 days. Service dates for each ewe were established by the use of marking devices attached to the rams. The lambing date of each ewe was also recorded.

RESULTS AND DISCUSSION

The foetal pulse sound from the umbilical artery was most frequently detected while only rarely the foetal heart was heard first. The examination was stopped as soon as one or other of these sounds was heard. If no foetal pulse sound was detected within approximately two minutes, a negative diagnosis was made. It was observed that in cases of advanced pregnancy the examination became more difficult as the foetal pulse was not so readily detected and the sounds were muffled. It is presumed that this occurs because the foetus at this stage of pregnancy is in the anterior part of the abdominal cavity under the costal arch and the sound waves have a greater depth to penetrate. The wool precludes siting the transducer more directly over the foetus.

Of the 309 ewes in the flock, 243 had lambs, giving a lambing rate of 78.6 per cent. Four of the 66 ewes which did not lamb were diagnosed as pregnant. Post mortem examinations conducted on these ewes indicated that they probably had been pregnant and aborted.

In 3 of the 243 ewes which had lambs, pregnancy was only diagnosed at the second examination while in 1 ewe it was not detected et all.

The pregnancy of 301 of the 309 ewes was correctly diagnosed at the first examination, an accuracy of 97.4%. At the second examination of the ewes previously diagnosed as not pregnant, a further 3 were detected, giving an accuracy of 98.4%. If the ewes originally diagnosed as pregnant had been re-examined, the accuracy rate would no doubt have been increased by detection of the non-pregnant ewes.

Table: RESULTS OF EXAMINATION OF MERINO EWES FOR PREGNANCY USING AN ULTRASONIC FOETAL PULSE DETECTOR

Day after service examined	Number of ewes examined	Number correctly diagnosed as pregnant	Number correctly diagnosed as not pregnant	Number wrongly diagnosed as pregnant	Number wrongly diagnosed as not pregnant	Precentage of correct diagnoses
66— 70	1	1	—	—	—	100
71— 80	7	4	3	—	—	100
81— 90	33	20	13*	—	—	100
91—100	137	114	18*	4	1	96.3
101—110	85	68	17	—	—	100
111—120	44	33	11*	—	—	100
121—122	2	2	—	—	—	100
TOTALS	309	242	62	4	1	98.4

*One ewe in each of these groups died and was found not to be pregnant at autopsy.
The instrument measures 25 × 18 × 13 cm., plus case it weighs 5.5 Kg.

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Jl S. Afr. vet. med. Ass. 40 (4): 381-383 1970

PSEUDOPREGNANCY IN THE BLACK-BACKED JACKAL (*CANIS MESOMELAS* SCHREBER)

V. DE VOS*

SUMMARY

The oestrus cycle of a black-backed jackal bitch, culminating in pseudopregnancy, is described. The most salient features were: (1) ovulation was spontaneous; (2) a pro-oestrus period of approximately ten days, of which the bleeding phase lasted four days, and a true oestrus period of seven days were observed; (3) the duration of pseudopregnancy was 58 days or approximately as long as normal gestation; (4) changes in behaviour associated with the oestrus period included increased nervousness, excitability and restlessness. These abated and were replaced by maternal instincts as pseudopregnancy supervened. Abdominal enlargement and hypertrophy of mammary glands with lactation were observed towards the end of pseudopregnancy.

Attention is also drawn to the similarity of the manifestation of pseudopregnancy in the different members of the family *Canidae*.

INTRODUCTION

The term "pseudopregnancy" was first used by Hammond and Marshall in 1914 [1] for a condition in the domestic rabbit *(Oryctolagus cuniculus)* which can be induced by a sterile mating, the stimulus of intercourse resulting in ovulation and the subsequent formation of corpora lutea which develop as in true pregnancy and bring about a growth of the uterine mucosa and hypertrophy of the mammary glands comparable to that which occurs during true pregnancy. A similar condition has also been found to occur in the marsupial cat *(Dasyurus viverrinus)* [2], Kangaroo rat *(Bettonga cuniculus)* [3], hedgehog *(Erinaceus europaeus)* [4], Eastern cottontail rabbit *(Sylvilagus floridanus)* [5], golden hamster *(Mesocricetus auratus)* [6], water vole *(Arvicola amphibius)* [7], field mouse *(Microtus agrestis)* [8], gerbil *(Tatera brantsi, Tatera afra)* [5], Australian rat *(Rattus assimilis)* [9], Norway rat *(Rattus norvegicus)* [10], Multimammate rat *(Mastomys coucha)* [11], laboratory mouse *(Mus musculus)* [12], domestic dog *(Canis familiaris)* [13], silver fox *(Vulpes fulva)* [14], English red fox *(Vulpes vulpes)* [15], ferret *(Mustela vison)* [17], and the domestic cat *(Felis catus)* [18, 19]. This listing indicates a fairly wide species-range of animals which exhibit signs of pseudopregnancy, and extends through the *Marsupialia, Insectivora, Lagomorpha, Rodentia* to the *Carnivora*, with the latter two best represented.

An experiment on the domestication of the black-backed jackal *(Canis mesomelas)* in the Kruger National Park provided an opportunity of observing the manifestations of oestrus and pseudogregnancy in this species.

OBSERVATIONS

Two black-backed jackal pups, a male and a female, were reared in captivity from about seven days old. From the age of approximately six months they were allowed to wander free. The male took to the veld straightaway and sometimes went off for periods of up to two months, but always returned. The female seldom left the garden and was never away for more than three days at a time. It was therefore relatively easy to observe her.

At about ten months of age the bitch showed increasing signs of nervousness, viz.: increased calling during the night, excitement while being handled, turning voluntarily onto her back and squirming on the ground whilst uttering high-pitched squeals—which might be written "Eeeeea-eeeeeea-ee"—while emitting jets of urine. This restlessness gradually increased until attempted mountings onto the male were noticed. At this stage the moderate swelling of the vulva which had been noticed at the onset of the restless phase had increased and was associated with congestion of the vulvar mucous membrane. Shortly afterwards

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the mucous discharge became tinged with blood. This bleeding phase lasted for four days and then subsided. The male recognised the approaching oestrus of the female by smelling and licking her external genitalia and by smacking his mouth. Nevertheless he again went off alone.

As bleeding and pro-oestrum had ended and true oestrum commenced, the female was confined to the garden. As oestrus progressed her restlessness increased and her calls during the night were answered with ever-increasing frequency by wild jackals. There were abundant signs that wild jackal were aware of the presence of a female in oestrus in the garden. This stage lasted for about seven days after which the swelling of the vulva receded and the restlessness decreased rapidly. In another two days she was released from confinement.

The bitch then showed an increased attachment towards her "home" and very seldom left the premises. From about 20 days post-oestrum she started burrowing. From the onset she picked a spot in the garden in soft soil under an old dead tree trunk. At this stage it was believed that she had become pregnant in spite of precautions and, as the spot that she picked for her burrow was not thought suitable for raising her puppies, we tried to deviate her attention from it. The burrow was repeatedly filled with soil, thoroughly wetted and even treated with an aromatic insecticidal dust. Another and better hole was made and we tried to entice her to it but without avail. Another behavioural change which accompanied the digging was an increasing aggresiveness towards the male, which had returned. She would not allow him within a radius of approximately five yards of the opening of her den. On a violation of this imaginary boundary she would charge out of the hole and chase him for about fifteen yards with teeth snapping and ears pulled back flat against her head. This vigilance gradually diminished after about 55 days post-oestrum. At about 60 days the burrow-digging completely stopped and she resumed her normal behaviour uncomplicated by any pseudopregnancy drive.

From 30 to 55 days post-oestrum, proliferative changes in the mammary glands were evident. From 40 days onwards it was possible to obtain a watery milky fluid from the swol len teats. At about 41 days post-oestrum abdominal enlargement was first noticed and this increased until about the fifty-eighth day. These signs of pseudopregnancy disappeared rapidly over the next three days.

DISCUSSION

This bitch came into oestrus for the first time during the end of the autumn after the winter in which she was born, i.e. at the age of approximately ten months.

On external appearances and behavioural patterns the pro-oestrus and oestrus periods were not defined. These periods were recognized by an increasing restlessness followed in six days by a bleeding phase of four days and a period of presumed sexual receptiveness of about seven days. This points to a pro-oestrus period of approximately ten days and a true oestrus period of seven days. The latter figure corresponds with data from a publication by Van der Merwe [20].

Copulation apparently did not take place and pseudopregnancy supervened. The stimulus of intercourse would therefore not appear to be necessary to bring about ovulation and the subsequent formation of corpora lutea. This points to spontaneous ovulation and a duration of pseudopregnancy which is approximately the same as normal gestation. The normal gestation period of the black-backed jackal is considered to be 60 days [21], whilst Haagner [22] records periods of 57 and 60 days for the side-striped jackal *(Canis adustus)*, a closely related species.

The pseudopregnant bitch simulates bitches that are about to whelp or have recently whelped. This period is often called pseudocyesis [23].

The manifestations of pseudopregnancy in the black-backed jackal closely resemble those in the other members of the *Canidae*, e.g. the domestic dog [13], silver fox [14] and English red fox [15]. In these species pseudopregnancy follows ovulation in the absence of mating. Furthermore it seems that in these animals the duration of pseudopregnancy roughly equals that of gestation. However, there may be considerable variation in the dog [24], but in less diverted breeds, such as the German shepherd, the length of pseudopregnancy equals that of gestation. At the termination of pseudopregnancy these animals also show behaviour which are usually preparatory to parturition.

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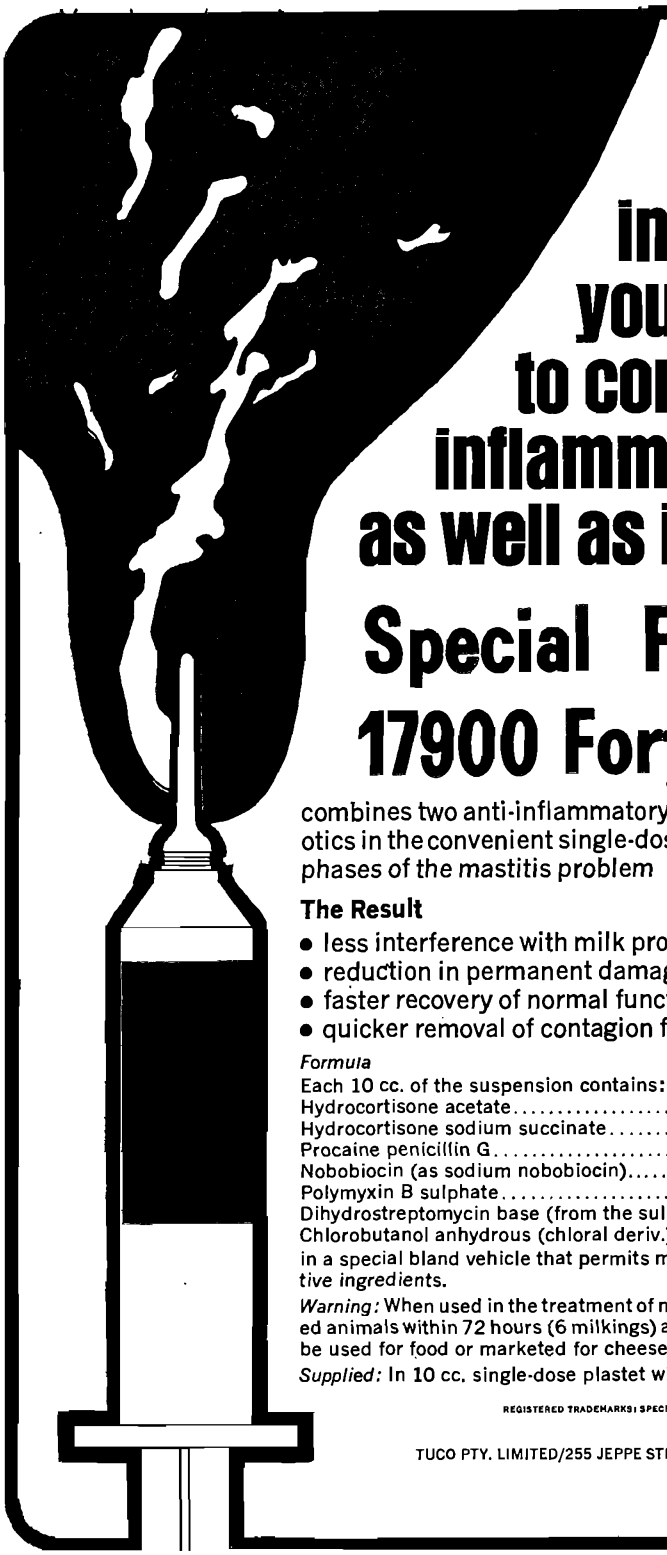

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THE PATHOLOGY OF EPHEMERAL FEVER: A STUDY OF THE EXPERIMENTAL DISEASE IN CATTLE

P. A. BASSON*, J. G. PIENAAR* AND B. VAN DER WESTHUIZEN**

SUMMARY

Nine cases of ephemeral fever in bovines were experimentally produced by intravenous injection of an infective leukocyte-platelet cell suspension. At varying intervals after the onset of the febrile reaction the animals were killed and autopsied. The following lesions were found: a serofibrinous polysynovitis in 8 cases, a serofibrinous polytendovaginitis in all the cases, a serofibrinous periarthritis around affected joint capsules in 7 cases, localized serofibrinous fasciitis in 7 cases, localized serofibrinous cellulitis of the limbs in 5 cases, focal necrosis of skeletal muscles in 8 cases and localized necrosis of the skin in 2 cases. In 5 cases a mild lymphadenitis was present in the regional lymph nodes of the affected limbs. Vascular changes accompanying these lesions consisted of swelling and hyperplasia of the endothelium, hyperplasia of pericytes, fibrinoid necrosis of the muscular coat of small arteries, perivascular cell reaction, perivascular fibroplasia and occasional thrombosis of vessels in the muscles: these appeared to be the basic lesions in ephemeral fever. They were very variable in severity and distribution. The characteristic clinical symptoms of the disease are related to the lesions described.

INTRODUCTION

Although the symptoms in ephemeral fever, or three-day-stiffsickness, of cattle may sometimes be very alarming, the mortality rate is negligible, seldom exceeding 2 or 3 per cent according to Henning [1]. In the majority of fatal cases, death is usually due to secondary complications [1]. The true pathological changes of ephemeral fever may thus often be obscured by lesions not directly related to the disease. These factors, together with the fact that the pathology has not previously been studied systematically, have been responsible for a basic lack in our knowledge of the pathogenesis of the disease.

Of the few existing reports which furnish some data on this aspect of the disease, the most comprehensive is that by Mackerras, Mackerras & Burnet [2]. They studied material from 11 natural cases which died or were killed at various stages of the disease. Compared to the clinical symptoms, the pathological changes in the cases examined by them were very mild. A neutrophilic leukocytosis, swelling of the lymph nodes, capillary engorgement of the serosae and internal organs, congestion of the mucous membrane of the abomasum, acute inflammation of the nasal mucosa and variable inflammatory changes in the joints are some of the more important lesions recorded by these authors. Only fleeting consideration is given to the pathology of the joints and no lesions were seen by them in any of the other components of the locomotor system, including the voluntary musculature.

In this paper the gross and microscopic pathology observed in material derived from experimentally produced cases of ephemeral fever in bovines are reported.

MATERIALS AND METHODS

(a) *Infective material*

A sample of citrated whole blood, collected during 1958 at the height of the febrile reaction from an experimentally produced case of ephemeral fever was used to commence this study. The original blood sample had been stored in sealed ampoules under dry ice refrigeration during the intervening period of 6 years.

A two-year-old steer, maintained in an insect-proof stable, received 3 ml of the original stored blood sample intravenously. This animal was kept under observation and the rectal temperature recorded at four-hourly intervals. At the height of the febrile

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reaction, blood was collected into 1.5 litre flasks containing 150 ml of a 10 per cent w/v solution of sodium citrate. At the same time blood smears were made, stained with Giemsa and examined microscopically to exclude possible intercurrent infections of anaplasmosis and babesiosis.

The blood was transferred into 250 ml bottles and centrifuged at 2700 rpm for 30 minutes. Thereafter the supernatant plasma was decanted and discarded, while the leukocyte-platelet fraction was removed through a No. 14 gauge needle (without a bevel) attached to a 10 ml syringe.

The cell fractions were pooled, washed twice and resuspended in a normal saline solution to give a final concentration of 1 : 5. The infectivity of this cell suspension was determined by inoculating 3 ml of the material into an ox and then observing the ensuing reaction. This infective material was stored at -76°C and used as a source of virus for challenge purposes.

(b) *Experimental animals*

Nine bovines, all females and varying in age from two-tooth to adult, were used. To ensure that they were susceptible, serum samples were collected from each animal prior to artificial infection and a serum-virus neutralization test performed as previously described [3].

Eight of these animals were then artificially infected by the intravenous inoculation of 3 ml of the infective material. Into the ninth animal, Bovine 4966, 8 ml of clear synovial fluid obtained at autopsy from another of the animals (Bovine 4976—see Table 1) was injected intravenously.

All animals were maintained in insect-proof stables, and their rectal temperatures taken at 6 hourly intervals until a fever reaction was recorded. Symptoms were recorded (Table 1). At various intervals after the onset of the fever (Table 1) the animals were slaughtered and autopsied. Specimens from gross lesions and other organs were fixed in neutral buffered 10 per cent formalin. Fixed tissue specimens were processed according to the usual routine methods and embedded in paraffin wax. Sections 4 to 10 microns in thickness were cut and stained with haematoxylin-eosin. The day of the first febrile reaction was taken as Day 1.

SYMPTOMS

The symptomatology of ephemeral fever has been reported in detail by various authors [1, 2, 4-12]. In order to assess properly the pathological changes reported in this study, some of the more prominent symptoms pertaining to the locomotor system as observed in the cases studied, are briefly recorded. Symptoms that were seen in the individual cases are summarized in Table 1.

Shivering or light tremor of the muscles of the flank, shoulder and buttocks was seen in two cases. Fluctuating swelling of the joints and tendon sheaths was noticed in three cases. This was never very marked when compared to non-affected joints. All the cases suffered from lameness of varying degree in one or more legs. In the less severely affected cases this was manifested as a disinclination to move, a stiff gait, frequent shifting of the weight from limb to limb and intermittent raising of painful legs. Frank lameness,

Table 1: LOCOMOTOR SYMPTOMS, INCUBATION PERIODS AND THE DAYS ON WHICH THE ANIMALS WERE KILLED AFTER ONSET OF FEVER REACTION.

Bovine No.	Day	Incubation period—days	Main Symptoms
4734	1	3	Lame left hind leg.
4614	1	4	Slight lameness right hind leg.
4732	3	4	Lame right fore and left hind leg.
4976	4	4	Muscle tremors, swollen fetlock joints. No pronounced lameness.
4966	4	7	Slight lameness right hind and right fore legs.
4967	6	4	Muscle tremors, swollen joints. Lame both hind legs and left fore.
4735	10	4	Marked lameness right fore and hind legs.
4957	10	4	Paresis until killed.
4969	15	5	Slight swelling of fetlock joints. Doubtful lameness.

the animal sometimes carrying the painful limb, was seen in the more severe cases. These symptoms were of a very transient nature and did not last longer than 3 to 4 days. Only one animal became recumbent shortly after the onset of the fever reaction. It remained down and was unable to get on its feet until killed for autopsy 10 days later.

PATHOLOGY

Macroscopic Lesions

The most conspicuous macroscopic changes were a serofibrinous polysynovitis, tendovaginitis, periarthritis, fasciitis, cellulitis and localized focal necrosis of the skeletal muscles. These lesions were variable in severity; being barely discernible in some and very marked in others. The lesions in both joints and muscles were invariably more severe in the limbs on which the animals were lame.

The synovial membranes most constantly and severely affected were those of the larger joints of the limbs: the stifle, followed by the hip, shoulder and elbow joints with approximate equal frequency, was most constantly involved, while the hock, carpal, tarsal and fetlock joints were less often affected. Considerable variation in the severity of the pathological changes was noticed from joint to joint in the same case. Usually two or three joints were prominently affected, while very mild or no changes occurred in the remaining ones.

Excessive amounts of turbid, straw-coloured synovial fluid was seen in the more severely affected joints (Fig. 1). Small, yellow-white flakes and plaques were visible in the fluid. Large intracapsular coagula of fibrin were also present (Fig. 1). The synovial membranes of affected joints were oedematous and invariably contained petechial haemorrhages. Sometimes villous, fibrinous strands of exudate adhered to the affected membranes. No lesions were present on the articular cartilages. In the less severely affected joints the only changes encountered were a diffuse reddening of the synovial membrane with a few petechiae and a slight increase in synovial fluid.

The lesions in the tendon sheaths (Fig. 3) closely resembled those of the synovial membranes of the joints in all respects. Usually the inflammatory reaction in the tendon sheaths was of the same intensity as that encountered in the adjacent joint. The periarticular connective tissue around affected joints was often oedematous, being infiltrated with varying amounts of slightly yellowish, serofibrinous exudate (Fig. 2).

Lesions in the skeletal muscles were almost constantly present, but were usually mild and localized; a thorough examination was necessary to locate them. They were most constant in the quadriceps group. Other muscles, less frequently affected, included the longissimus dorsi, biceps femoris, triceps, semitendinosus and semimembranosus. Muscle lesions were present in eight cases. These lesions were focal and frequently situated near the muscular attachments. In a few instances they were also located elsewhere in the muscles. In the cases examined 1 to 4 days after the onset of fever, the lesions appeared as focal, pale, well-circumscribed areas (Fig. 5) often associated with petechiae or ecchymoses. From Day 6 onwards they appeared as localized, irregular areas of necrosis, varying from one to a few centimetres in diameter and by Day 15 the more severely affected central part of the necrotic tissue was often haemorrhagic and surrounded by a developing connective tissue capsule (Fig. 6). On the other hand in the animal which became paretic and remained recumbent for 10 days before it was killed, lesions were immediately obvious and consisted of diffuse, focal areas of necrosis of almost all the muscles of all four limbs.

Changes in the fasciae varied from focal petechiae and ecchymoses, in cases killed shortly after the temperature reaction, to haemorrhages accompanied by accumulations of a serofibrinous exudate (Fig. 4) in the later stages. This fasciitis was localized and most prominent in the periarticular areas near affected joints, in the intermuscular connective tissue of the larger muscle groups of the fore and hind limbs and in the epimysium of some of these muscles. Fat necrosis was often associated with the inflammatory reaction in the fasciae and was present as small, chalky white, focal areas. The epineurium of the larger nerves in the vicinity of the affected areas often contained petechial haemorrhages.

A serofibrinous cellulitis was seen in three cases. In two of these cases it was localized to the immediate vicinity of affected joints in the posterior and lateral fetlock regions. Localized, small areas of necrosis of the overlying skin were present. In the paretic case a very extensive cellulitis involved the whole of the subcutis of the lower half of all four legs.

PLATE 1

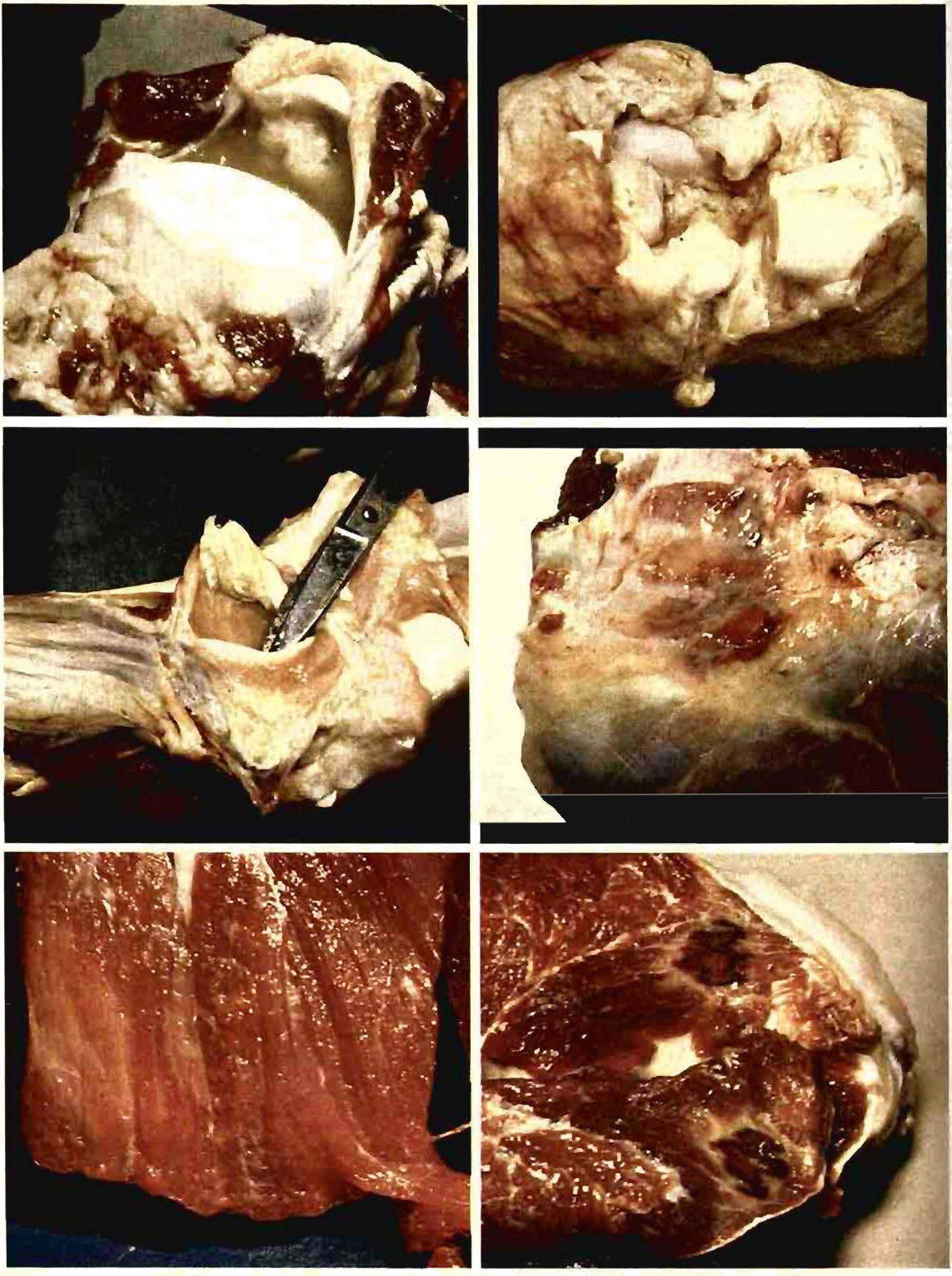

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Table 2: INCIDENCE AND SEVERITY OF LESIONS

Bovine No.	Day	Synovitis	Tendo-vaginitis	Peri-arthritis	Fasciitis	Cellulitis	Muscle Lesions
4734	1	+ +	+ + +	+ + +	+ +	−	Absent
4614	1	−	±	−	−	±	Present
4732	3	+ +	+	+	+	−	,,
4976	4	+ +	+ +	+ +	+	+	,,
4966	4	+	+	−	−	+	,,
4967	6	+ +	+ +	+ +	+	−	,,
4735	10	+	+	+	+ +	−	,,
4957	10	+ +	+ + +	+ + +	+ + +	+ + +	,,
4969	15	+ + +	+ +	+ +	+ +	+ +	,,

− = No lesions.
± = Focal haemorrhage, no exudate.
+ = Slight serofibrinous exudate.
+ + = Fair amount of serofibrinous exudate.
+ + + = Copious serofibrinous exudate.

Regional lymph nodes of the affected limbs were either mildly or markedly swollen and oedematous. Small petechiae were present in some of these lymph nodes.

The severity and incidence of the lesions observed in the locomotor system are tabulated in Table 2.

No obvious gross changes were observed in the remainder of the organs.

Microscopic Lesions

The microscopic lesions seen in the cases examined 1 to 4 days after the onset of fever did not vary significantly in appearance. They are consequently described as one group. Similarly the lesions encountered in the animals autopsied 6 to 15 days after the onset of fever are grouped together.

Skeletal Muscle

Day 1 to 4

Typical hyaline necrosis of muscle fibres was present in those focal areas of the discoloured muscle that were noticed grossly (Fig. 7). In these areas the entire or largest part of most fibres assumed a markedly swollen, homogenous and intense eosinophilic appearance. Cross striations were no longer visible. Many of the sarcolemmal nuclei of the affected fibres were pycnotic. Numerous small vacuoles were present in the necrotic sarcoplasm. The latter often was also coagulated into coarse, irregular, eosinophilic masses while the rest of the sarcolemmal tube was filled with a pink-staining, finely granular material. A similar material also accumulated between the individual muscle fibres and in the perimysium. This was interpreted as representing oedema. Focal haemorrhages of varying size were seen in most of the affected areas of muscle. In addition, on Day 3, a very light cell infiltration became evident. This consisted mainly of neutrophiles and to a lesser extent of round cells. These cells were noticed around the smaller blood vessels and in the endomysium. In some of the necrotic muscle fibres small numbers of macrophages had invaded the sarcoplasm. On Day 4 a slight degree of proliferation of the sarcolemmal nuclei was noticed.

PLATE 1

Fig. 1. Day 3: Shoulder joint. Synovitis. Joint capsule oedematous and joint cavity containing an increased volume of turbid fluid and large fibrinous coagula.

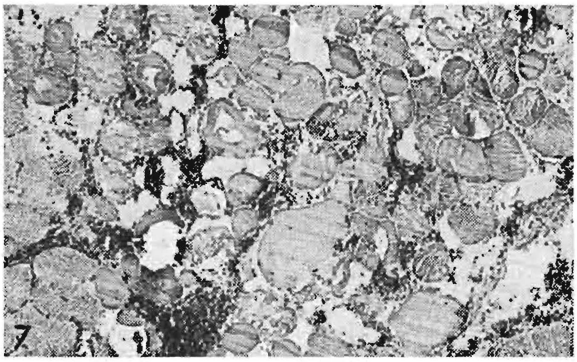
Fig. 2. Day 6: Hock joint. Periarthritis. Tissue around joint thickened by infiltration of serofibrinous exudate. Fibrinous coagula can be seen in joint cavity.

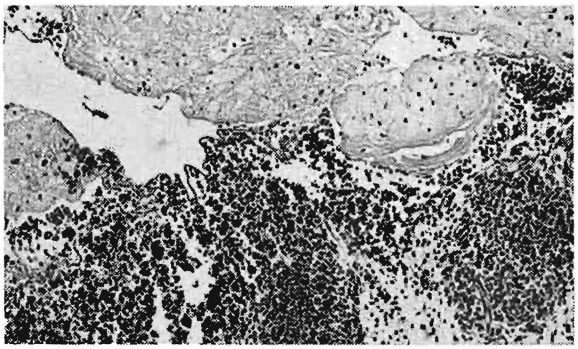
Fig. 3. Day 1: Tendon sheath. Serofibrinous tendovaginitis. Fibrinous coagula and exudate in sheath cavity.

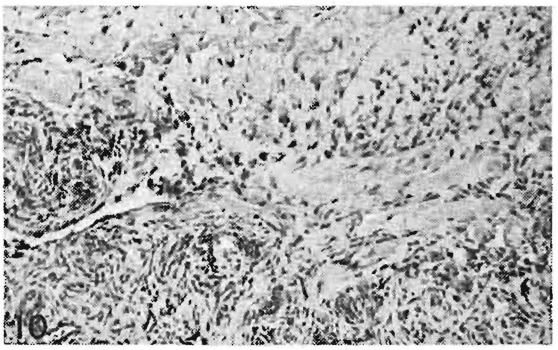
Fig. 4. Day 6: Localized fasciitis. Haemorrhage and a serofibrinous exudate can be seen.

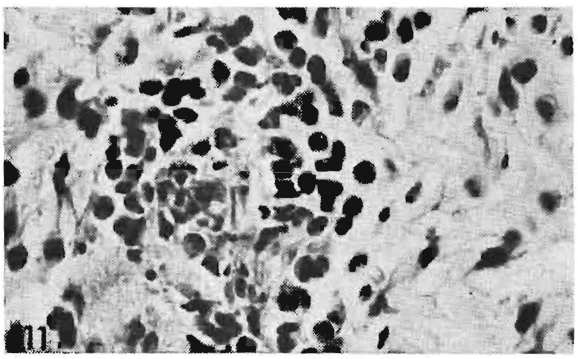
Fig. 5. Day 1: Muscle with focal areas of hyaline necrosis.

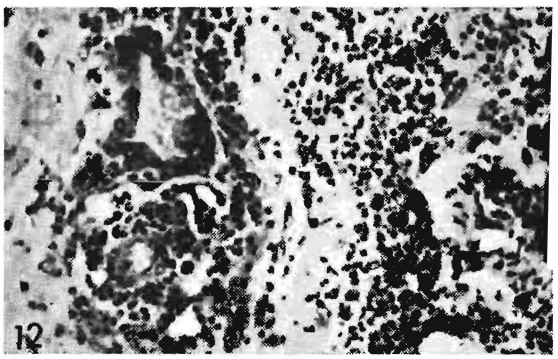
Fig. 6. Day 15: Two localized necrotic and haemorrhagic lesions in muscle surrounded by a zone of fibroplasia.

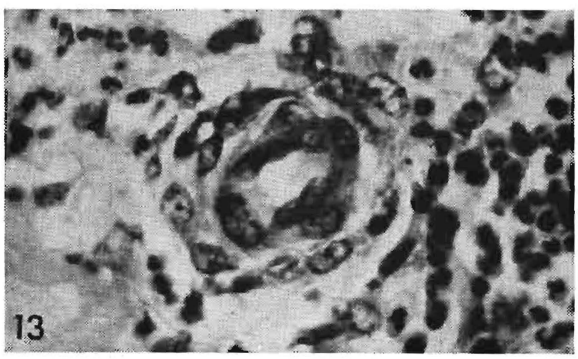


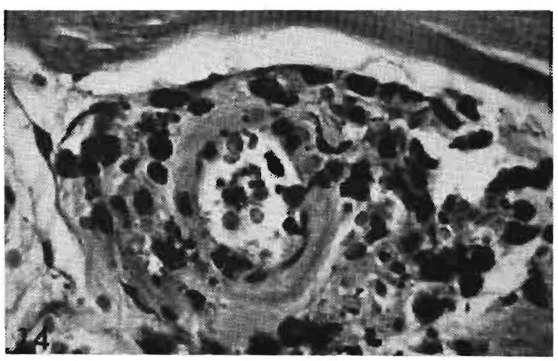












From Day 6 large numbers of round cells, predominantly macrophages, were seen between the affected fibres and within some of the sarcolemmal tubes. Sarcolemmal proliferation occurred in some cases but did not appear to be a very prominent feature, many of these cells being destroyed in the necrotic areas. Simultaneously active fibroplasia occurred in the interstitium and in focal areas replacing the necrotic muscle fibres (Fig. 8 and 19). Numerous mitotic figures were present in this mass of young granulation tissue. In most of the remaining necrotic muscle fibres calcification of the sarcoplasm was noticed as a diffuse, fine, granular, basophilic deposit.

A striking feature was the persistence of focal groups of completely necrotic muscle fibres within the connective tissue that had replaced most of the surrounding dead muscle tissue (Fig. 8). These fibres stained very eosinophilic and lacked cellular detail. No sarcolemmal nuclei were visible in them and no cell infiltration was present between the fibres. Large numbers of extravascular erythrocytes were scattered amongst these fibres as well as deposits of fibrin. Thrombosed blood vessels occurred near these foci (Fig. 19). Evidence of muscle regeneration was not present in their immediate vicinity. In the connective tissue zone which adjoined the intact muscle tissue, however, focal areas of mild regenerative changes were noticed. It was seen as groups of sarcolemmal tubes, containing a light basophilic sarcoplasm and groups of hyperchromatic nuclei centrally situated as clumps or as long chains, intermixed with the connective tissue. Regeneration was not a marked feature of the lesion.

The lesion described above corresponded to the lesion observed grossly in the muscles as a necrotic, haemorrhagic centre surrounded by a whitish grey zone of connective tissue (Fig. 6).

Joint capsules and tendon sheaths

Day 1 to 4

In the more severely affected joints, the synovial membranes had undergone a well-developed fibrinopurulent inflammatory reaction. This was characterized by a copious exudation into the joint cavity of fibrin, neutrophiles and a few mononuclear cells (Fig. 9). The fibrinous exudate adhered to the synovial membrane over large areas. At these points of attachment the synovial cells were completely destroyed and no synovial membrane could be recognized. Marked oedema of the *membrana synovialis*, the synovial villi and the *membrana fibrosa* was present. Small haemorrhages and congestion of blood vessels were noticed subsynovially and in the superficial layers of the *membrana fibrosa*. Numerous neutrophiles had infiltrated the subsynovial layer and were especially obvious around the smaller blood vessels (Fig. 12).

The synovial membrane was still intact in the joints which were mildly affected. Very little or no exudate was present on the surface of these synovial membranes. Slight oedema of the synovial membrane and villi with small scattered haemorrhages was the only other significant change.

Day 6 to 15

From Day 6 neutrophiles were no longer the predominent feature of the inflammatory reaction. At this stage the exudate consisted of large masses of coagulated fibrin containing relatively few cells (Fig. 10). Ingrowth of fibroblasts and capillaries into these coagulated masses of fibrin was in progress at the

PLATE 2

Fig. 7. Day 1: Hyaline necrosis of muscle with oedema and haemorrhage. HE X 75.

Fig. 8. Day 15: Focal group of necrotic muscle fibres surrounded by haemorrhage and connective tissue. HE X 75.

Fig. 9. Day 1: Synovial membrane showing intense neutrophilic cell infiltration especially around blood vessels. The synovial cell layer can no longer be recognized. A fibrinous exudate adheres to the membrane. HE X 75.

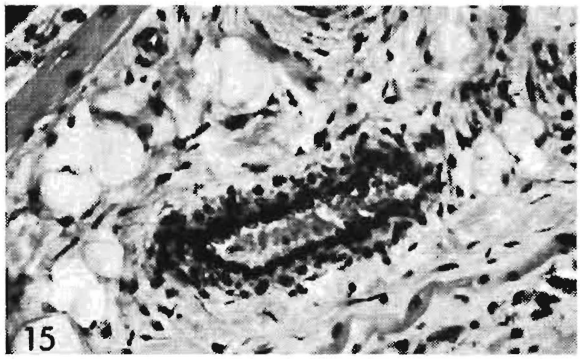
Fig. 10. Day 15: Synovial membrane with ingrowth of fibroblasts and capillaries into the fibrinous exudate. Note marked fibroplasia and hyperplasia of pericytes around blood vessels in subsynovial layer. HE X 75.

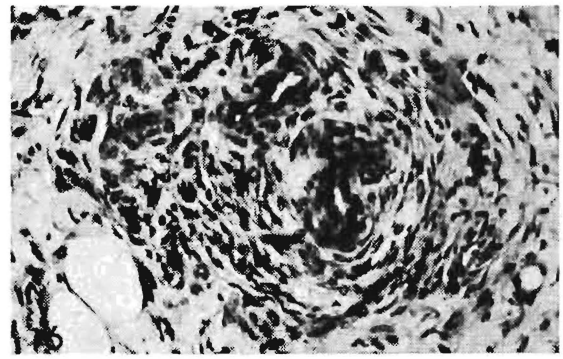
Fig. 11. Day 1: Small vessel in synovial membrane with perivascular cell infiltration. HE X 500.

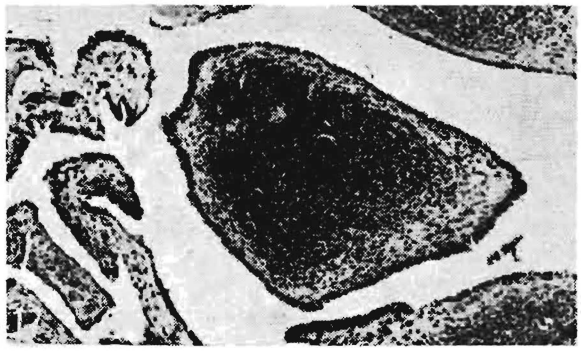
Fig. 12. Day 4: Two small vessels in synovial membrane with perivascular cell infiltration. Swelling and hyperplasia of the endothelial cells are present and diffuse neutrophile infiltration on the right. HE X 200.

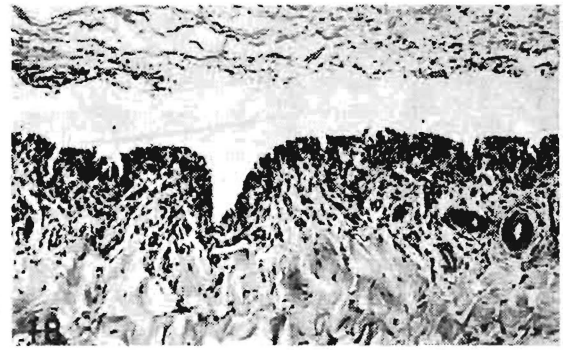
Fig. 13. Day 4: Small vessel, with swelling and marked hyperplasia of endothelial cells and pericytes, in synovial membrane. HE X 500.

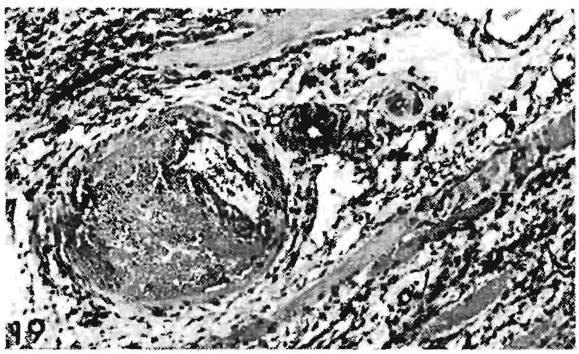
Fig. 14. Day 4: Small artery in muscle with fibrinoid necrosis and perivascular cell infiltration. Note karyorrhexis. HE X 500.

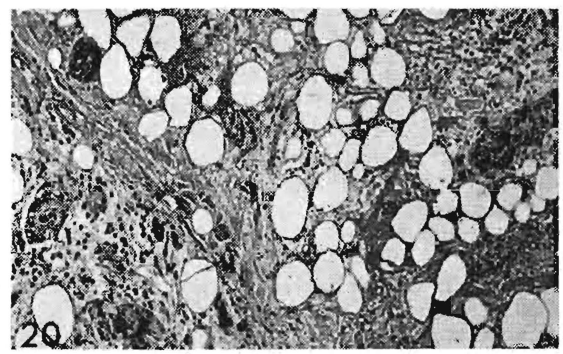


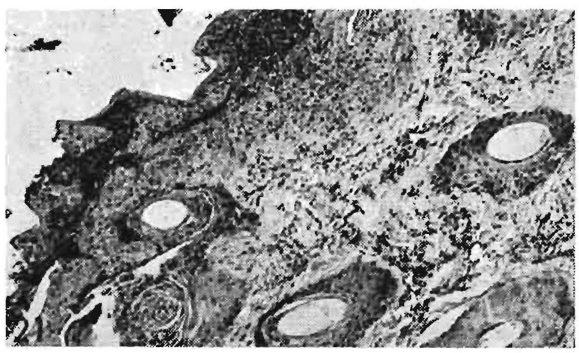


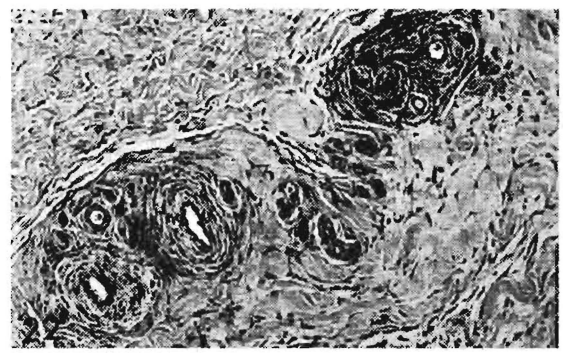












areas of attachment to the joint capsule. In places where the synovial membrane had survived; only slight hyperplasia of the lining cells was noticeable (Fig. 18).

Tendon sheath changes closely resembled those observed in the synovial membranes.

Blood vessels

Day 1 to 4

Most of the small veins and arteries in the *membrana synovialis* and subsynovial layers of the affected joint capsules had an infiltration of cells around them (Fig. 11 and 12). These cells consisted mainly of neutrophiles with a few lymphocytes and plasma cells. The perivascular cuffing was not pronounced and only an occasional vessel had an intense cell reaction around it. In some vessels, the cell infiltration was also present in the wall. Perivascular haermorrhage from such vessels was often noticed. In many of the vessels leukostasis consisting of neutro philes had occurred. Perivascular oedema was present. Infrequently, small vessels were encountered with an intense lymphocytic reaction around them (Fig. 17).

On Day 4 obvious swelling of the endothelium and hyperplasia of both endothelial cells and pericytes became evident in the synovial membranes (Fig. 13). This was not limited to vessels with perivascular cell reaction but appeared to be fairly generalized in the vessels of the synovial membranes of the more severely affected joint capsules. Only very mild or no swelling and hyperplasia of endothelial cells were seen in the capsules of joints which had mild gross changes. Similar but less pronounced changes were present in some of the small blood vessels of the muscles and exceptionally in large veins and arteries. Fibrinoid necrosis of a few small arteries in the muscle lesions was seen on Day 4 (Fig. 14). This lesion, however, was more pronounced after 6 to 15 days *(vide infra)*.

Day 6 to 15

From Day 6 the perivascular neutrophile reaction was no longer a prominent feature. This was replaced by a mild lymphocytic infiltration. Swelling and hyperplasia of the endothelium were marked (Fig. 15). The lumens of many venules, arterioles and capillaries were partially or completely occluded by endothelial cells (Fig. 16). Hyperplasia of pericytes was more pronounced (Fig. 15 and 16). From Day 10 to 15 a definite proliferation of fibroblasts followed the perivascular lymphocytic infiltration. This perivascular fibrosis was particularly pronounced around the subsynovial blood vessels, (Fig. 10), blood vessels in the muscle lesions (Fig. 16) and in the subcutis (Fig. 22). Thrombosis was observed in the muscle lesions on Day 6 (Fig. 19), 10 and 15. No thrombi, however, were detected in the blood vessels of the affected joint capsules, fasciae, tendon sheaths and subcutis.

In the muscles and subcutis partial or even complete fibrinoid necrosis of some arteries was frequently observed. The affected media of these vessels was swollen and intensely eosinophilic in appearance. This change was focal in medium sized arteries, while in the smaller ones the entire wall was often affected. In this amorphous eosinophilic material, pyknotic nuclei of smooth muscle cells could be seen. Polymorphonuclear leukocytes, a few mononuclear cells and karyorrhectic nuclear material were sometimes present in and around this material (Fig. 14). Similar necrotic changes were also seen in the adventitia of some of the affected vessels.

Fasciae and subcutis

Oedematous fluid rich in fibrin and con-

PLATE 3

Fig. 15. Day 6: Muscle. Small vein with very marked hyperplasia of endothelial cells. Hyperplasia of pericytes can also be seen. HE X 200.

Fig. 16. Day 6: Muscle. Hyperplasia of endothelial cells and perivascular fibrosis. HE X 500.

Fig. 17. Day 6: Perivascular infiltration of lymphocytes in villi of synovial membrane. HE X 75.

Fig. 18. Day 15: Synovial membrane. Hyperplasia of synovial cell layer. Fibrinous exudate present at top of figure. HE X 75.

Fig. 19. Day 6: Thrombosis of a vein. Note fibroplasia between remaining muscle fibres. HE X 75.

Fig. 20. Day 15: Fascia. Fibrinous exudate with scattered leucocytes in subcutis. HE X 75.

Fig. 21. Day 4: Skin. Focal necrosis of epidermis and scanty cell reaction in dermis. HE X 75.

Fig. 22. Day 15: Skin. Prominent perivascular fibroplasia in subcutis. HE X 75.

taining scattered neutrophiles and mononuclear cells was present in the affected areas of localized fasciitis (Fig. 20). Vascular changes similar to those present in the synovial membranes, *(vide supra)* but rather ill-defined, were seen. In addition, small haemorrhages and focal necrosis of the fat were observed.

Skin

Necrosis in focal areas of the epidermis (Fig. 21) with vascular changes in the subcutis identical to those encountered in the subsynovial layers on Day 6 to 15, (Fig. 22), were found in the two cases in which localized skin lesions had been seen grossly.

Lymph nodes

Oedema and varying degrees of neutrophile infiltration in the sinusoids were the only significant changes in the regional lymph nodes of affected limbs. In some instances there appeared to be slight hyperplasia of the reticulum cells.

DISCUSSION

The study of experimental cases of ephemeral fever that is presented here revealed the following lesions: serofibrinous polysynovitis, tendovaginitis and periarthritis, localized fasciitis, cellulitis, localized muscular necrosis and oedema and mild lymphadenitis of the regional lymph nodes. A definite correlation was found between the severity of joint and muscular lesions and lameness. These lesions amply explain the varying degrees of transient impairment of locomotion which is the most constant and characteristic clinical symptom of the disease. A pathognomonic clinical feature of ephemeral fever is the dramatic suddenness both of onset and recovery. The limited extent of the lesions, their localized distribution and relatively mild and variable nature concur with the ephemeral character and extremely low mortality of the disease.

Microscopically, the most important lesion involved the arterioles, venules and capillaries of the synovial membranes, tendon sheaths, muscles, fasciae and skin. Initially this was represented by swelling and hyperplasia of the endothelium which was accompanied by a perivascular neutrophile infiltration and perivascular oedema. This was followed by hyperplasia of pericytes and a predominance of round cells. Focal or complete necrosis of vessel walls, thrombosis and subsequent perivascular fibrosis occurred. These vascular changes probably can be regarded as the basic lesion of ephemeral fever, resulting in an increased permeability of the vessels, haemorrhages, oedema, ischaemia and necrosis of the tissues in which they occur.

Thrombosis of the vessels was invariably associated with the localized muscular lesions. Some of the latter appeared to be focal infarcts due to thrombosis of multiple, small, intramuscular vessels. The muscular lesions, described by Adams, Denny-Brown & Pearson [13], as resulting from ischaemia, are very similar to those occurring in ephemeral fever. It should be emphasized, however, that the vascular lesions were neither extensive nor generalized but were only present in association with lesions in the affected tissues.

Mackerras *et al*[2] regarded the lesions of ephemeral fever as being due not to any specific action of the virus on particular tissues, but to local anatomical conditions affected by a universal vascular lesion. According to them, the virus causes dilatation of capillaries and smaller veins which is followed by engorgement, stasis, increased permeability, haemorrhages and oedema. They did not find any evidence that the effects of the stasis were supplemented by any direct action of the virus on the endothelial cells. Some degree of swelling of the endothelium, occasional thrombosis of a small vein and a loose infiltration of scattered neutrophiles, lymphocytes and odd plasma cells in the adventitia were reported by them.

No haematological determinations were undertaken in the present study. Mackerras *et al*[2] reported a sharp, rapidly developing neutrophilic leukocytosis which coincided with the onset of fever. A neutrophilic leukocytosis was indicated by the presence of these cells in the lesions studied by us during the first four days following the fever reaction.

According to Henning[1], the most common of the rare, direct sequelae to ephemeral fever is a prolonged locomotor incapacity. A number of such atypical cases quoted in the literature [2, 7, 8, 11, 12] were summarized by him. In none of the original reports on these cases was any mention made of lesions that could account for the protracted locomotor disturbances. Bovine 4957 of the present study may be regarded as representing such an atypical case. This animal was recumbent for 10 days after defervescence until killed for autopsy. Contrary to the other cases, it had widespread

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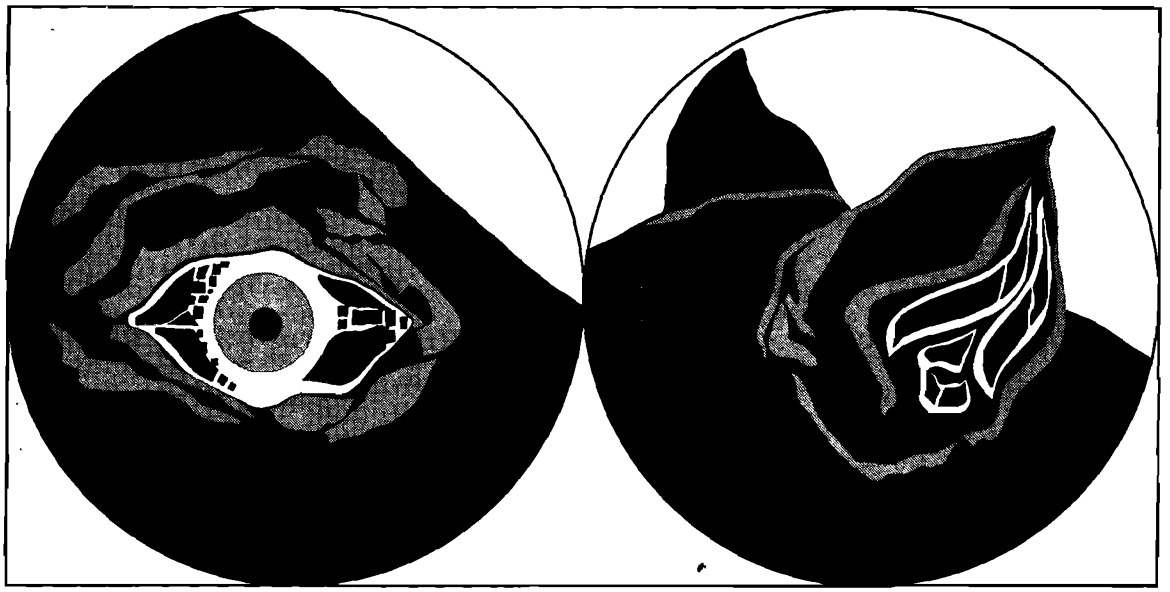

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muscle lesions and a diffuse cellulitis of all four legs. These lesions, no doubt, severely interfered with locomotion. Whether similar lesions are constantly present in cases with prolonged locomotory disturbances is unknown. No pathological study has been reported so far in the literature on these cases. Examination of representative sections from various parts of the brain, spinal cord and peripheral nerves from Bovine 4957 failed to reveal any lesions.

Tendovaginitis, muscular lesions, cellulitis and necrosis of the skin have not been reported previously. The only other report of a fasciitis in the literature is that by Theiler [14]. He noticed in one case that the intermuscular tissue, especially of the loins and of the hindquarters, was infiltrated with serous fluid. The presence of some of the lesions reported earlier [2], such as inflammation of the nasal mucosa and trachea, excess blood-stained pericardial fluid, emphysema of the lungs and pleura, patchy vascular engorgement on the serosae with fibrinous exudation and effusion and slight congestion of the mucous membrane of the abomasum, were not confirmed.

Due to its usual benign and short course, ephemeral fever is not of great economic importance. It is well known, however, that serious outbreaks, even with mortalities, may sometimes occur [1, 12]. Apart from these occasional severe outbreaks, its main significance seems to be in the interference of farming operations due to lameness of animals, especially in bulls and cows, decreased milk yield, mild loss of weight and the development of sequelae. Henning [1] states that a marked diminution in milk yield of cows in full lactation occurs. This is often associated with inflammation and oedema of and pain in the udder. The secretion from the udder may be watery or even bloody. None of the cases studied by us was in lactation and no lesions were present in the non-lactating udders. The nature of the lesions in the lactating udder, however, requires elucidation, as the decreased milk yield is one of the most important economic factors of ephemeral fever in the dairy cow.

ACKNOWLEDGEMENTS

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DETECTION OF ANTIBODIES AGAINST *STRONGYLOIDES PAPILLOSUS* BY THE INDIRECT IMMUNO-FLUORESCENT METHOD

J. L. DU PLESSIS, J. G. PIENAAR AND P. A. BASSON*

SUMMARY

In goats and sheep infested with *Strongyloides papillosus*, serum antibodies were detected by means of the indirect fluorescent antibody method using larvae of this parasite as the antigen: In this technique, these antibodies appeared to be adsorbed onto the larval cuticles and apparently were specific for this helminth. These findings lend some support to the view that cuticular antigens are antigenic. Sera from goats and sheep simultaneously subjected to the indirect fluorescent antibody and complement fixation tests showed some correlation between the results of the two tests. The complement fixation test, however, lacked specificity and manifested cross-reactions with other helminths.

INTRODUCTION

Although fluorescent antibody techniques are extensively employed in bacterial, viral and protozoal infections, they have been applied only comparatively recently and to a limited extent in helminth infestations. In this field indirect immuno-fluorescence has been used in the sero-diagnosis of schistosomiasis, trichinosis, ascariasis and fasciolasis. Reviewing the available literature on its extensive application to bilharziasis, Pautrizel[1] states that bilharzia antibodies can be detected with a high degree of specificity and sensitivity in the serum of patients exposed to this parasite. Sadun, Anderson & Williams[2] found the test reliable in the serological diagnosis of trichinosis of man. By exposing *Toxocara canis* larvae to the serum dilutions in test tubes and subsequently to the labelled antihuman globulin, Bissern & Woodruff[3] quantitatively detected *Toxocara* antibodies in human sera. Using frozen sections of rolled adult *Fasciola hepatica* as antigen, Coudert, Garin, Ambroise-Thomas, Thai, Despeignes & Pothier[4] found the indirect method sufficiently sensitive and specific in the serodiagnosis of infestation by this parasite.

The degree of immunity of sheep and goats to infestation by *Strongyloides papillosus* has been based on their resistance to a challenge dose of 300,000 infective larvae of this species placed on the skin. This number of larvae is normally lethal to susceptible lambs and kids[5]. Attempts at the quantitative detection of antibodies produced during infestation by *S. papillosus* have, as yet, not been reported. In this communication a method is described to determine quantitatively such antibodies by means of the indirect fluorescent antibody (IFA) technique. The results were then compared with those obtained by the complement fixation (CF) tests.

MATERIALS AND METHODS

Experimental animals

Twenty-eight serum samples taken at intervals from the following groups of goats and sheep were simultaneously subjected to the IFA and CF tests (Tables 1 and 2):—

Group I: Kids. Serum was obtained from one set of twins before the intake of colostrum (pre-colostral) and again nine days after birth (post-colostral). The IFA titre of the dam's serum against *S. papillosus* was 1/90.

Group II: Infested animals. The goats in this group had been exposed to natural infestation with *S. papillosus* before being reinfested artificially by placing the numbers of infective larvae, as indicated in Table 1, on their skins. The sheep O1 was infested per os. Two animals (G5 and G6) were treated with one therapeutic dose of thiabendazole followed by daily low-level dosage of this drug for one month before the last serum collection.

Group III. Worm-free lambs. These lambs were born, raised and maintained under worm-free conditions.

Control group. These animals were infested with pure strains of the nematodes listed in Table 2.

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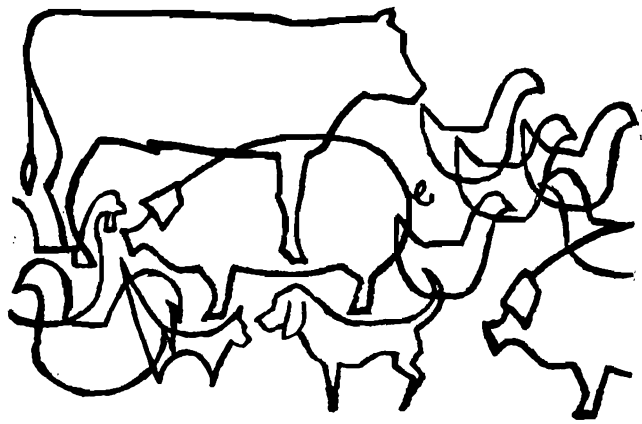

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IFA test

The antigen was prepared on microscopic slides from infective larvae of *S. papillosus* harvested from cultures prepared by the method of Roberts & O'Sullivan [6]. The larvae were placed in tapwater and inactivated by heating to 50°C for 15 minutes. The suspension was then centrifuged at not more than 1000 rpm and the larvae were resuspended in normal saline and washed. After a second washing they were concentrated by centrifugation at 1000 rpm. With the aid of a 1 ml pipette a small droplet of concentrated larvae suspension was placed inside a 1 cm diameter circle drawn on a clean glass slide. The fluid part of the suspension was allowed to evaporate partially for ½ to 1 minute and the droplet subsequently flattened out over the entire surface of the circle by means of a platinum loop. At this point the antigen could be stored at 4°C for several months.

The slides containing the antigen were fixed in acetone for ten minutes at room temperature. A few drops of three-fold serum dilutions in buffered normal saline were allowed to react with the antigen for 20 min. in a moist chamber at room temperature. After two washings in buffered normal saline, a few drops of fluorescene-conjugated rabbit anti-goat globulin (prepared by conjugating the gammaglobulins of rabbits previously subjected to the intravenous injection of alum-precipitated goat globulin and by purifying the conjugate of unbound fluorochrome by diffusion through G50 Sephadex) or fluorescene-conjugated rabbit anti-sheep globulin (Pasteur Institute, Paris), were placed in contact with the antigen for 20 min. After two further buffered saline washings, the preparations were mounted in buffered glycerine and examined under a Zeiss binocular microscope equipped with a 200 watt Wild mercury burner. A BG12 exciter filter and a darkfield condensor were used.

The absence of non-specific fluorescence of the antigen was confirmed by allowing the conjugated anti-species globulin to react with

Table 1: IFA AND CF TITRES OF SERA AGAINST **S. PAPILLOSUS***

	Animal No.	INFESTATION			IFA	CF
		No. of Exposures	No. of larvae	Duration**		
Group I: Kids						
Pre-colostral	G 1		—	—	Neg.	Neg.
	G 2		—	—	Neg.	Neg.
Post-colostral	G 1		—	—	1/90	1/8
	G 2		—	—	1/30	1/8
Group II: Infested animals	G 3	10	50 000	44 (35)	1/270	1/128
	G 4		—	—	Neg.	1/8
	G 4	1	300 000	40	1/90	1/32
	O 1		500 000 per os	42	1/270	1/64
	G 5	11	10 × 50 000 & 500 000	2	1/2430	1/64
	G 5	do	do	44 (32)	1/7290	1/128
	G 5	do	do	113 (101)	1/270	1/64
	G 6	do	do	2	1/2430	1/128
	G 6	do	do	44 (32)	1/21870	1/128
	G 6	do	do	113 (101)	1/810	1/128
	G 7	2	25 000 & 300 000	76 (69)	1/270	1/64
	G 8	2	50 000 & 300 000	81 (66)	1/90	1/64
	G 9	2	100 000 & 300 000	81 (66)	1/2430	1/64
	G10	2	25 000 & 300 000	77 (69)	1/270	1/64
Group III: Worm-free lambs	O 2		—	—	Neg.	1/16
	O 3		—	—	Neg.	1/32

*For end-points of reactions see text.

**Duration in days: Interval between first infestation and collection of serum. In parenthesis, interval in days between last infestation and collection of serum.

the antigen without prior contact with serum.

The specificity of the test was controlled by absorbing positive sera with intact infective larvae of *S. papillosus* and by testing sera obtained from sheep and goats housed under worm-free conditions but infested with a single species of nematode (see Table 2), against *S. papillosus* antigen by means of the IFA and CF tests.

The absorption was carried out by adding 1 ml of serum with a 1 : 270 IFA titre for half an hour to 500,000 *S. papillosus* larvae suspended in 9 ml of buffered saline at room temperature. After centrifugation a few drops of the supernatant fluid were subjected to the IFA test. One ml of another serum with a 1 : 90 IFA titre was similarly absorbed by 2×10^6 larvae.

Complement fixation test

Metabolic products of *S. papillosus* were used as antigen. Approximately one million larvae suspended in 15 ml of tapwater were allowed to stand at room temperature for 24 hours. After centrifugation the supernatant fluid was inactivated by heating to 60°C for 30 min. Tincture of merthiolate was added to a dilution of 1 : 10,000 as preservative. This product served as antigen.

The test was executed in tubes, the primary and secondary incubations were done at 37°C for 90 and 30 min. respectively and two units each of antigen, haemolysin and complement were used. The sensitized red cells, used at a final concentration of one per cent, and the complement each comprised two volumes, while the antigen and serum each consisted of one volume.

RESULTS

The results of the IFA and CF tests are summarized in Tables 1 and 2. It is evident that in 15 sera a positive reaction was recorded in both tests, two pre-colostral sera were negative in both tests and 11 sera were negative in the IFA test but positive in the CF test.

A rise in the antibody level was evidenced in both tests in the case of the kids, G1 and G2 as a result of passive immunity, as well as in the naturally infested goats which were re-infested artificially. A fall in antibody level following thiabendazole treatment in two of the goats was more pronounced in the results obtained by means of the IFA test.

In general, there was much greater variation between the lowest and highest titres obtained with the IFA test than between those of the CF test. Although antibodies were detected at considerably higher dilutions with the IFA test, there was some correlation between the results obtained in both tests.

While reading the IFA results, the fluorescence in positive cases was consistently observed in the cuticles of the larvae (Fig. 1).

Fig 1. Indirect immuno-fluorescence of **S. papillosus** larvae.
× 400. Agfacolor. 35 mm positive transparency, ASA 50.

With strongly positive sera there was also some fluorescence within the substance of the larval bodies. Towards the endpoint of the reaction the cuticle of the tail of the larva only adsorbed the conjugated anti-species globulins and in some cases appeared as a broken fluorescent line. The dilution at this point was taken as the final and highest titre.

During the process of spreading the larvae on the slides for the preparation of antigen many of the larvae were severed or cracked at some point in their bodies so that only portions of larvae or fractured larvae were seen. At the fracture points body contents protruded from the larvae. In both positive (Fig. 1) and negative control sera as well as preparations of antigen plus anti-species globulin only, this substance showed strong yellowish-green fluorescence. This was regarded as being non-specific and was ignored when reading the results.

In the specificity control tests, the serum (IFA titre 1 : 90) absorbed by 2×10^6 larvae was totally IFA negative, whereas the titre

Table 2: SPECIFICITY OF **S. PAPILLOSUS** ANTIGEN IN IFA AND CF TESTS IN ANIMALS INFESTED WITH PURE STRAINS OF THE NEMATODES LISTED

Animal No.	Nematode	IFA	CF
O 4	**Haemonchus contortus**	Neg.	$^1/_8$
O 5	**Haemonchus contortus**	Neg.	$^1/_{64}$
O 6	**Chabertia ovina**	Neg.	$^1/_{16}$
O 7	**Nematodirus spathiger**	Neg.	$^1/_{16}$
O 8	**Oesophagostomum colombianum**	Neg.	$^1/_{16}$
O 9	**Trichostrongylus colubriformis**	Neg.	$^1/_{32}$
O10	**Gaigeria pachyscelis**	Neg.	$^1/_{32}$
G11	**Ostertagia circumcincta**	Neg.	$^1/_8$

of the other serum absorbed by 500,000 larvae was decreased from 1 : 270 to 1 : 30. The results of the control test in which *S. papillosus* antigen was tested against eight sera from sheep and goats harbouring other parasites are shown in Table 2. All were negative.

DISCUSSION

The sensitivity of the IFA reaction was revealed by the extensive variation in the titres obtained. The serum of nine-day old kids which was negative before the transfer of passive immunity, gave relatively low titres due to the intake of colostral antibodies. In contrast, sera from goats with an immunity to *S. papillosus* acquired through natural exposure to this worm and subsequently hyperimmunized by repeated artificial infestations (e.g. G5 and G6), produced significantly higher titres. Likewise a fall in the antibody level following treatment was accordingly reflected by the IFA titres. The test apparently then indicated the level of serum antibodies detectable by immuno-fluorescence, revealing comparatively small quantities of passively transferred immunoglobulins as well as high levels subsequent to repeated immunization.

The specificity of the IFA test was shown by the absence of any cross-reactions with sera obtained from the control animals harbouring helminths other than *S. papillosus* (Table 2) as well as by the absorption of specific antibodies from a positive serum by *S. palillosus* larvae. The amount of antigen required to absorb all the antibodies was surprisingly large but at the same time demonstrated the sensitivity of the test, in that residual antibodies after partial absorption were revealed by a proportionate IFA titre

The gamma globulins which were specifically responsible for the indirect fluorescent reaction appeared to be directed against cuticular antigens of the larvae, as fluorescence was confined to this part of the larvae in the case of known positive sera and absent in the case of sera obtained from newborn kids deprived of colostrum, as well as in those originating from animals infested with other species.

As the specificity of this reaction largely depends upon the adsorption of immunoglobulins by the larval cuticle, the controversial question arises as to whether the cuticle of the nematode itself is immunogenic. Moore [7] was able to demonstrate that cuticular glycoproteins and lipids from *Trichinella spiralis* conferred protective immunity against this nematode. Hogarth-Scott [8], however, using the mixed antiglobulin reaction, was unable to show any increase in the anti-cuticular antibody level in sera from cases of ascariasis and visceral *larva migrans*, when compared with those of normal sera. The same author suggested that these anti-cuticular antibodies are naturally occurring, cross-reacting immuno-globulins adsorbed by the nematode cuticle and therefore non-specific in nature. This finding is contrary to our fluorescent antibody results in strongyloidosis, as we were unable to demonstrate any cross-reactions between larval cuticles of *S. papillosus* and sera obtained from animals infested with other nematodes. The fluorescent antibody method and the antigen preparations as applied here lend themselves to the observation and location of the antigen-antibody reaction in the cuticle of the larva. These findings support the view that cuticular antigens are antigenic and/or immunogenic. It must, however, be added that the formation of the antibodies which adhere to the cuticle may have been elicited by somatic antigens which have antigenic determinants in common with those of cuticular antigens.

Although there was some correlation between the IFA and CF titres, the CF test lacked specificity, as 11 sera devoid of antibodies demonstrable by the IFA method contained complement fixing antibodies bound by an antigen occurring in metabolic products of *S. papillosus* larvae. The cross-reactions in this case in all probability related to the nature of the antigen, because metabolic products of *S. papillosus* possibly possess cross-reacting antigens common to those of other nematodes. In this respect the IFA

technique has the decided advantage that the reaction between antibody and a particular site on the larva can be accurately located.

In view of the difficulties encountered with helminth antigens suitable for immunoserological work, the IFA method described here may prove to be a dependable and sensitive method for tracing serum antibodies in response to infestation by *S. papillosus*. The role played by antibodies detectable by this method in immunity to this worm, will be dealt with in a subsequent report.

ACKNOWLEDGEMENTS

We wish to thank Mr. S. T. Boshoff, Section Virology, for the execution of the complement fixation tests, Mr. A. M. du Bruyn, Section Photography, for the preparation of the photographs, Mr. W. J. Pienaar for growing the larvae and the Section Helminthology for their co-operation in making available facilities for culturing larvae.

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BOOK REVIEW

VETERINARY MEDICINE AND HUMAN HEALTH

CALVIN W. SCHWABE

Baillière, Tindall & Cassel, London. Second Ed. 1939, pp. XX+713; Tabs: 125; Figs. 147.

This is the second edition of a volume first published in 1964 and reprinted in 1966. The cover still depicts Cheiron the centaur, legendary father of the veterinary art and mentor and foster father of Aesculapius, the god of healing and father of Hygeia, goddess of health. The author is a veterinarian who has taught at medical schools, directed departments of public health and served on the Secretariat of W.H.O., thus personally proving the worth of veterinary science to the promotion of human health.

The first edition was reviewed in this Journal (Vol. 36(2): 287, 1965). This latest edition is the result of a successful attempt to keep the presentation up to date and to shift the emphases dictated by recent work. Thus, the section on zoonoses has been expanded to chapter length, and case studies have been expanded to lay extra stress on the epidemiology of less well understood zoonoses such as *Pasteurella multocida* infection, influenza, vesicular stomatitis and histoplasmosis. Special attention is given to comparative approaches to diseases of man of unknown aetiology. The chapter on environmental hygiene has been brought up to date, and a completely new chapter on meat hygiene has been added. Numerous other chapters have been rewritten and revised. Much of the revision and many additional contributions are the result of suggestions by acknowledged experts in their respective fields.

This book epitomizes what Rudolf Virchow once said: "Between animal and human medicine there is no dividing line — nor should there be. The object is different but the experience obtained constitutes the basis of all medicine".

In the study of medicine in its broadest sense, and for veterinarians, most of whom are concerned with human health and well-being in some form or other, this book is invaluable. Even more so than the original edition, this revised version indicates just how much wider is the field of veterinary medicine than the narrow concept which prevails within the profession, a concept largely responsible for the public image of the work and worth of the veterinarian.

Schwabe's book is not only up to date and informative, but highly stimulating and therefore highly recommended, even essential, for veterinary and medical scientists in general and epidemiologists in particular.

L. W. v/d H.

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Jl S. Afr. vet. med. Ass. 40 (4): 407-409 1969

THE DIAGNOSIS OF VIBRIOSIS BY THE FLUORESCENT ANTIBODY TECHNIQUE

B. J. H. BARNARD*

SUMMARY

The value of the direct fluorescent antibody test in the diagnosis of vibriosis has been assessed. Under local conditions it appears to be a quicker and more reliable diagnostic procedure than bacteriological examination.

INTRODUCTION

The detection of carrier bulls is a major problem in the control of vibriosis in South Africa. Due to the long distances over which specimens have to be sent to the laboratory for diagnosis, the isolation of *Vibrio* organisms is often unsuccessful. Under optimum conditions, when specimens were cultured soon after collection, the percentage of carrier bulls found positive after one, two and three attempts were 39%, 67% and 95% respectively [1]. Although methods of isolation have been greatly improved [2, 3], it is still an expensive and time-consuming diagnostic procedure.

Coons, Creech, Jones & Berliner [4] developed the fluorescent antibody (FA) technique for the detection of antigenic substances in tissue cells. Mellich, Winter & McEntee [5] reported that the FA reaction provides a highly accurate and sensitive method for the detection of *Vibrio fetus* in carrier bulls in the U.S.A..

MATERIALS AND METHODS

Preparation of antisera

Immune sera were prepared by inoculating rabbits intravenously with a mixture of three strains of *Vibrio fetus venerealis*. Injections, which consisted of 1.0 ml of the growth layer in Bacto Thiol**, were repeated once weekly for three weeks. One week after the last injection all the rabbits were exsanguinated under ether anaesthesia. The serum was separated by centrifugation and assayed for the presence of antibodies by means of agglutination tests. Sera that did not cause complete agglutination at a dilution of 1 : 10,000 were discarded.

Preparation of conjugates

Twenty mg of fluorescein isothiocyanate per gram of serum globulin was used for labelling.

The pooled serum was divided into two equal parts. Part A was fractionated and conjugated according to the procedure described by Cherry [6]. The method of Summer [7] was used for the fractionation of part B and conjugated according to the method used by Spendlove [8]. The conjugated globulin was purified by passage through a Sephadex column after the procedure of Peters [9].

Preliminary staining experiments at this stage displayed a certain degree of non-specific fluorescence with both undiluted conjugates. At a dilution of 1 : 5, however, non-specific staining was virtually eliminated, whereas specific fluorescence was retained, The conjugates, diluted accordingly, were distributed separately in ampoules in 1.0 ml amounts; half of each was stored at -20°C and the other half freeze-dried.

Staining procedure

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Suspensions of pure cultures of the following organisms were prepared: *V. fetus venerealis* (16 isolates), *V. fetus intestinalis* (3 isolates), *V. bubulus* (10 isolates), *Brucella abortus, Brucella ovis, Corynebacterium* spp., *Staphylococcus* spp. and 10 different unidentified organisms obtained from preputial specimens.

About 0.02 ml of a suspension was spread over a marked area on a glass slide, allowed to dry and then fixed in acetone for ten minutes. After drying at room temperature the smear was covered with a drop of conjugate, incubated at 37°C in a moist chamber for 30 minutes and washed with three changes of saline over a period of 10 minutes. The smear was then allowed to dry, mounted and examined with a Wild microscope using an HBO 200 source of light and a BG 12 ultraviolet filter in conjunction with a protective Zeiss filter. A dark field condensor and 10× and 40× objectives were employed throughout the experiments.

Examination of preputial specimens

Ten ml of sheath washing was centrifuged at 1,500 rpm. The supernatant was again centrifuged at 3,500 rpm and the sediment suspended in 0.2 ml of supernatant fluid. A loopful was placed on a marked area on a glass slide without stirring. By this method the bacteria settled down at the periphery of a small circle which facilitated microscopic examination. The slide was stained as described above. Bacteriological examination of the sheath washing was performed by the millipore technique described by Barnard [10].

RESULTS

Laboratory studies

Specific bright fluorescence was only obtained with *V. fetus* and not with any of the other bacteria tested. Fluorescence was slightly duller with *V. fetus intestinalis* than with *V. fetus venerealis*, but not to such an extent that it was possible to differentiate between the two types. The different methods of conjugation, fractionation and storage had no appreciable effect on brightness and specificity of staining, even after storage of the conjugates for one year. The fractionation and conjugation procedures used in the preparation of conjugate B are preferred on account of the time factor.

Preputial specimens

Sixty-eight specimens from bulls of an AI station, known to be free of vibriosis for the past three years, were all found to be negative by the FA test (Table). Twenty-six of these specimens gave positive cultures for *V. bubulus*.

Eighteen specimens from six known positive bulls were examined. Bacteriological isolation of *V. fetus* was possible on only 9 occasions, whereas 17 of the 18 specimens were positive with the FA test (Table). A further 14 specimens obtained from 12 bulls from herds known to be infected were

Table: COMPARISON BETWEEN BACTERIOLOGICAL AND FA DIAGNOSIS OF VIBRIOSIS

Origin	No. of Specimens	Positive isolation of			Positive FA	
		V. bubulus	V. fetus	% Pos. V. fetus	No.	%
21 AI Bulls	68	26	0	0	0	0
6 Positive Bulls	18	4	9	50	17	94
12 Bulls infected herds	14	0	0	0	10	71
8 Bulls infected herds	10	0.5% Formalin added to specimens			8	80

examined. Bacteriological examination was entirely negative for *V. fetus,* probably due to a delay of more than 24 hours in submission of the specimens, yet ten of these specimens gave a postive FA reaction (Table). Ten specimens, with formalin added to a concentration of 0.5%, were submitted from the same source. Eight gave a positive FA result.

DISCUSSION

The FA technique proved to be very valuable in the diagnosis of vibriosis in South Africa. Its accuracy appears to be improved by the addition of formalin which prevents the growth of contaminants. With the FA technique, time is therefore no longer such a limiting factor, as is indicated by the results obtained in the last two groups of bulls. The test can be carried out by one person and does not require elaborate equipment. A standard microscope fitted with an ultraviolet lamp can be used. The conjugates can be prepared in a central laboratory and stored for at least a year.

At this stage it is not possible to distinguish between *V. fetus venerealis* and *V. fetus intestinalis* by the FA technique. Since a bull infected with any one of these two types is capable of transmitting disease, it must be dealt with accordingly and differentiation between the two types is not of practical importance.

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RESISTANCE TO CERTAIN ORGANOPHOSPHORUS COMPOUNDS BY *LINOGNATHUS AFRICANUS* ON ANGORA GOATS IN SOUTH AFRICA

J. A. F. BAKER*

SUMMARY

A strain of *Linognathus africanus* infesting Angora goats in the Eastern Cape Province is suspected of being resistant to the organophosphorus compounds, dioxathion and dichlofenthion. Excellent control of this strain was achieved by chlorfenvinphos (Supona**).

INTRODUCTION

The presence of a rotenone resistant strain of blue, or sucking lice, *(Linognathus africanus)*, parasitizing Angora goats in South Africa, was established in 1963 in the Jansenville district of the Eastern Cape Province[1]. As these investigations also revealed "either a developed resistance or inherent tolerance" to acceptable insecticidal levels of chlorinated hydrocarbons, subsequent chemical control trials were undertaken with various organophosphorus compounds. Eradiction of this resistant strain was achieved by dipwash concentrations of 0.025% dioxathion and 0.04% dichlofenthion respectively, as adjudged by a 100 percent mortality within 24 hours and the complete absence of lice 15 or more days after treatment.

Early in 1966 reports of failures by dioxathion and dichlofenthion to achieve kill of blue lice populations were received from two farmers within this district, one of which, Farm "A", was a site on which rotenone resistance was originally demonstrated.

A series of small scale hand-dipping trials was carried out in order to investigate these reports.

MATERIALS AND METHODS

On the two suspect properties, here designated Farms "A" and "B", groups of infested goats of three, four or five in number were assessed for louse infestation and reliably marked. The animals comprising each group were thoroughly immersed singly in a 30 gallon capacity handbath, containing freshly mixed insecticidal washes. The materials employed were:

	FARM "A"	FARM "B"
Dioxathion	0.03%	0.03%
Dichlofenthion	0.06%	0.06%
Chlorfenvinphos	0.005%	0.014%
Chlorfenvinphos	0.02%	0.02%

After treatment the groups were isolated, each in its own paddock, to avoid any risk of cross infestation. Isolation continued until the termination of the experimental perod.

For comparative purposes, hand-dippings employing 0.04% and 0.06% dichlofenthion only, were carried out on infested animals on six farms in the Graaf-Reinet, Pearston, Somerset East and East London Districts respectively. These properties are referred to here as Farms 1—6.

Examinations for lice infestations were made with the animal resting on its side on a waist high board or table top under conditions of good incident light. Where moderate, heavy or gross degrees of infestations were encountered, opening of the staple formation to skin level at a number of points on the animal body served as a reasonable basis for assessment.

When lice were not readily discernible, however, the entire body surface was searched. Experience had indicated that when *L. africanus* are least numerous, either following a natural decline or a partially successful insecticidal treatment, they are found chiefly on the area comprising the upper aspect of the neck, the base of the ears, the poll and the ventral surface of the jaw. Particular attention was thus paid to these regions when post-treatment inspections were made.

A single examination approximately 20 days after treatment served to establish the degree of control obtained. This was based on the following classification:

1. The absence of living lice indicated com-

* Cooper & Nephews S. Af. (Pty.) Ltd., Box 108, East London.

** Supone: Shell Company.

Table 1: COMPARISON OF INSECTICIDAL TREATMENTS—FARMS "A" AND "B"

FARM	DISTRICT	TREATMENT	PERCENT CONCENTRATION	ANIMAL NUMBER	PRE-TREATMENT ASSESSMENT OF LICE INFESTATION	POST TREATMENT COUNT OF LICE INFESTATION
"A"	Jansenville	Dioxathion	0.03%	1	+ + + +	Nil
				2	+ + +	8 AI**
				3	+ + +	3 A*
				4	+ + + +	3 A
		Dichlofenthion	0.06%	1	+ +	>20 AI
				2	+ + + + +	>200 AI
				3	+ + +	>50 AI
		Chlorfenvinphos	0.005%	1	+ + +	1 A
				2	+ + + + +	>20 AI
				3	+ +	4 AI
				4	+ + +	Nil
				5	+ + +	5 AI
		Chlorfenvinphos	0.02%	1	+ +	Nil
				2	+ + +	Nil
				3	+ + + +	Nil
				4	+ +	Nil
				5	+ + +	Nil
"B"	Jansenville	Dioxathion	0.03%	1	+ +	Nil
				2	+ + +	6 AI
				3	+ +	1 A
		Dichlofenthion	0.06%	1	+	1 A
				2	+ + +	10 AI
				3	+ +	8 AI
		Chlorfenvinphos	0.014%	1	+ +	Nil
				2	+ +	Nil
				3	+ + +	Nil
				4	+ + +	Nil
				5	+ + + +	Nil
		Chlorfenvinphos	0.02%	1	+ +	Nil
				2	+ +	Nil
				3	+ + + +	Nil
				4	+ + + +	Nil
				5	+ + +	Nil

*A : Adults only present.
**AI: Adults and immature stages present.

plete mortality of immature and adult forms present at the time of treatment as well as sufficient residual activity to kill all emerging nymphae.

2. The presence of live lice in all stages revealed a failure to achieve complete kill. *In vitro,* the longest hatching period of *L. africanus* eggs has been shown to be eight days[1]. Under the optimum conditions experienced within the fleece, there is little reason to suggest that this period would be exceeded. Live immature forms thus indicated a lack of ovicidal action or an inability to inhibit nymphal emergence.

3. Odd adults only on dipped animals suggested reinfestation from fellow group members rather than 'on host' survival.

Assessment of lice infestation was based on the following criteria:

DEGREE OF INFESTATION	SYMBOL	NUMBER LICE PRESENT
Very light	+	1—20
Light	+ +	21—100
Moderate	+ + +	101—500
Heavy	+ + + +	>500
Gross	+ + + + +	Not possible to count

RESULTS

On Farms "A" and "B", neither 0.03% dioxathion nor 0.06% dichlofenthion achieved

Table 2: COMPARISON OF INSECTICIDAL TREATMENTS—FARMS 1—6

FARM	DISTRICT	TREATMENT	PERCENT CONCENTRATION	ANIMAL NUMBER	PRE-TREATMENT ASSESSMENT OF LICE INFESTATION	POST TREATMENT COUNT OF LICE INFESTATION
1	Pearston	Dichlofenthion	0.04%	1	+ + + +	Nil
				2	+ + + +	Nil
			0.06%	3	+ + + + +	Nil
				4	+ + + + +	Nil
2	Pearston	Dichlofenthion	0.03%	1	+ + +	Nil
				2	+ + + + +	Nil
3	Somerset East	Dichlofenthion	0.04%	1	+ + + + +	Nil
				2	+ + + + +	Nil
			0.06%	3	+ + + + +	Nil
4	Graaff Reinet	Dichlofenthion	0.06%	1	+ + + + +	Nil
5	Graaff Reinet	Dichlofenthion	0.04%	1	+ + + + +	Nil
				2	+ + + + +	Nil
			0.06%	3	+ + + + +	Nil
				4	+ + + + +	Nil
6	East London	Dichlofenthion	0.03%	1	+ + +	Nil
				2	+ +	Nil

effective control of the lice infestations present, although the degree of control achieved by dioxathion was better than that of dichlofenthion.

Chlorfenvinphos at wash concentrations of 0.014% and over gave complete control of *L. africanus,* but failed in this regard when the level was reduced to 0.005% (Table 1.)

On farms 1—6, 0.04% and 0.06% dichlofenthion enabled complete control of these lice to be maintained (Table 2).

DISCUSSION

The variation in the pattern of behavior of *L. africanus* on farms "A" and "B" towards insecticidal application of dioxathion and dichlofenthion is in sharp contrast to that previously recorded[1], and the emergence of a strain resistant to these compounds is strongly suspected. Support to this theory is given by the treatments undertaken on Farms 1—6 in which an equal or lower concentration of dichlofenthion than that employed on Farms "A" and "B" afforded excellent lice control. The superior but still ineffective results obtained by dioxathion suggest that the resistance factor present in this strain is more marked in respect of dichlofenthion than for dioxathion.

Selection pressure for the development of lice strains resistant to the organophosphorus compounds on Farms "A" and "B" is probably greater than that normally encountered owing to the stock husbandry methods employed, as five or more total immersion treatments are carried out annually. Dioxathion was regularly employed on both an experimental and commercial basis on these properties from 1959 to 1963, until superseded by dichlofenthion; thus a possible 42 or more organophosphorus treatments were given by the time a breakdown in protection was observed by the flock owners. (It must be noted that the replacement of dioxathion by dichlofenthion for goat dipping purposes at this time was occasioned solely by the superior efficiency of the latter compound against *Damalinia* spp.)

It is evident from the excellent results achieved by chlorfenvinphos that little or no cross resistance to this organophosphorus compound is present. A promising field of application for this insecticide in the control of resistant *L. africanus* strains is indicated.

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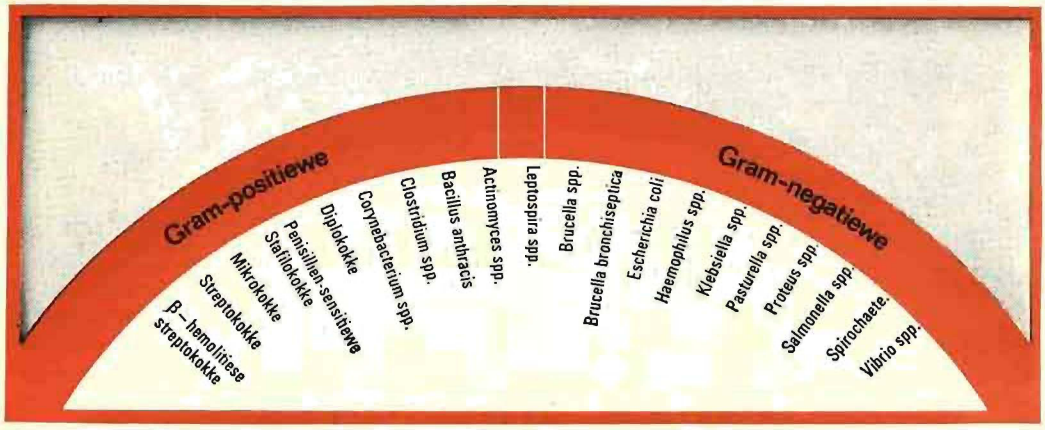
Penbritin
gareg.

Doen eintlik meer

as ander antibiotika omdat dit bakteriedodend is



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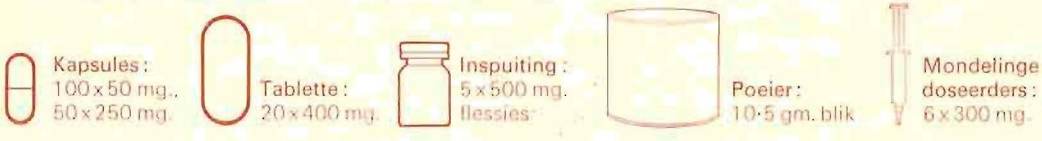
Penbritin is beskikbaar in 'n reeks van praktiese verpakkings vir groot en kleindier praktyke:



ALLEENLIK DEUR DIE VEEARTSENYKUNDIGE PROFESSIE BESKIKBAAR.

Penbritin (ampisillien) is 'n produk van Beecham navorsing.

Beecham Veterinary Products, Brentford, England.

Versprei in Suid-Afrika deur: Klipfonteinse Organiese Produkte—Korporasie Beperk, Posbus 150, Kempton Park, Transvaal.

KOP KOP VET

Jl S. Afr. vet. med. Ass. 40 (4): 417-419 1969

HEGTING EN HEGMATERIAL BY ROETINE-BUIKOPERASIES OP KLEINDIERE

A. M. LUBBE*

SUMMARY

The problems encountered with suture materials in routine abdominal operations on small animals are outlined. Efforts to overcome them resulted in the use of mono-filament nylon. Personal experience in the use of fishing nylon as a monofilament-nylon in 1500 oophorectomy operations indicated its reliability. It is also noted that the use of monofilament nylon has been scientifically evaluated, with reference to the shortcomings of catgut as a suture material.

INLEIDING

By operasies bly daar altyd die strewe tot die hoogs moontlike getal *per primam* genesings, veral waar insnyding in gesonde weefsel gedoen word, soos in oöforektomie. Een suksesvolle operasie gevolg deur postoperatiewe komplikasies skep meer probleme as wat deur vyftig ideale genesings vergoed kan word.

Van al die faktore wat wondgenesing beinvloed[1] en komplikasies veroorsaak, is hegting en hegmateriaal weliswaar net twee, maar ook twee baie vername faktore.

Die steekspanning is van groot belang: die neiging tot stywe steke gee aanleiding tot druknekrose, weefselreaksie en insny of selfs deursny van steke. Te stewige hegtingsprosedure is sekerlik een van die vernaamste oorsake van 'n hewige wondreaksie, en die mees algemene fout van die onervare chirurg.

Ten einde buikwondspanning as gevolg van buikspierspanning of verhoogde intra-abdominale druk tot 'n mate te verlig en tegelykertyd wondloslating bykans volkome te voorkom, moet afsonderlike deur-en-deur steke met nie-absorbeerbare materiaal deur die hele buikwond, ingeslote die peritoneum, geplaas word, soos deur Efron[2] voorgestel.

Die ideale hegmateriaal moet goedkoop en hanteerbaar wees en moet knoop sonder rafeling. Verder is minimale weefselreaksie 'n vereiste, sowel as 'n hoë trekspanning met dun deursnee. Dit mag nie 'n gunstige bodem vir bakteriële groei skep nie, moet bevredigend steriliseerbaar wees en moet spoedig geabsorbeer word. Sodanige hegmateriaal bestaan nie.

Die invloed van hegmateriaal op wondgenesing is vir baie jare onderskat en nie behoorlik geëvalueer nie. Die verskil van mening, selfs in die nuutste handboeke, oor die voor- en nadele van verskillende soorte hegmateriaal, dui op die gevaar van persoon like gevoelsvoorkeur en wetenskaplik onbewese uitsprake. Armistead[3] wy 'n lang hoofstuk aan die bespreking van verskeie hegmateriale en maak die stelling dat die keuse van hegmateriaal aan die voorkeur van die chirurg oorgelaat word. Die bespreking is nie net verwarrend nie, maar foutiewe stellings word gemaak. Byvoorbeeld, voorkeur word aan dermsnaar gegee, met die stelling dat nylon nie inwendig gebruik kan word nie, omdat dit meer reaksie as sy sou veroorsaak.

Naas besmetting en 'n te stewige hegtingsprosedure, is die aard van die spesifieke hegmateriaal van uiterste belang by die vermyding van hewige wondreaksies. Daarbenewens kom vorming van aseptiese sinusse en granulome voor wanneer die liggaam heg- of afbindmateriaal wil uitwerp. 'n Hematoom wat wondvlakke van mekaar skei, vertraag genesing, terwyl afsonderlike afbinding groot hoeveelhede vreemde materiaal vereis. Om die rede is dit wensliker om by wondbloeding die klein onderhuidse en spierbloedvaatjies af te draai of diatermies te brand.

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WAARNEMINGS

Die onbetroubaarheid van dermsnaar as hegmateriaal het na drie jare van praktyk 'n wesenlike probleem vir die skrywer geword. Die voorkoms van wondloslating en wondreaksies, ten spyte van sorgvuldige asepse, het dit duidelik gemaak dat dermsnaar hoegenaamd nie bevredigend is nie.

Gevlegte nylon ("Braided Nylon") te wete Abralon* is toe vir bykans vier jaar vir die afbinding van die ophangband van die eierstok en vir wond- en velhegting gebruik. Eers na 'n periode van twee jaar het daar geïsoleerde gevalle van wondsinusse voorgekom.

Nadat Abralon vir bykans drie jaar gebruik was, het gevalle van inwendige granulome, tot so groot as 'n krieketbal voorgekom. Die genoemde tydsverloop van drie jaar was besonder ontstellend. Alhoewel die insidensie van hierdie gevalle ongeveer 0.5% was, was die korrektiewe operasie omslagtig. By 'n Steekbaardteef byvoorbeeld, het so 'n groot granuloom ontwikkel dat dit die ureter geblokkeer het met gevolglige hidronefrose. Altesaam veertien gevalle is weer geopereer, met insnyding in die groeisel en slegs verwydering van die gevlegte nylonmateriaal. Tien van hierdie gevalle het volkome herstel. Verder was daar vier gevalle waar 'n aktief dreinerende sinus in die dier se sy ontwikkel het. In twee van hierdie gevalle is die hegmateriaal deur 'n laterale toegang verwyder.

Ongekompliseerde granulome het na verwydering van die vreemde materiaal totaal verdwyn. Sinusse vanaf diepliggende gevlegte nylonsteke het opgeklaar nadat hierdie steke verwyder is. Die stelling van Ormrod [4] dat gevlegte nylon weinig of geen reaksie veroorsaak nie, kan dus nie onderskryf word nie. Soortgelyke granulome kan ook met besmette dermsnaarafbinding ontstaan. (C. F. B. Hofmeyr: persoonlike mededeling.)

Op aanbeveling van prof. H. Derksen en dr G. de Mûelenaere, is besluit om enkelveselnylon te gebruik. O'Connor [5] het reeds die inwendige gebruik hiervan genoem. Eersgenoemde meen dat enkelveselnylon oor die algemeen die geskikste hegmateriaal vir inwendige gebruik is, selfs vir enterektomie, terwyl dr de Mûelenaere uitsluitlik nylon vislyn gebruik.

Vanaf Desember 1965 is daar in my praktyk al meer as 1500 roetine-kleindieroperasies met enkelveselnylon as hegmateriaal vir wond- en velhegtings gedoen, en wel in die vorm van Atlas- en Damylvislyn met deursnee 0.18, 0.20 en 0.25 mm. As die steke te styf getrek word, vind daar gedurende die eerste week 'n reaksie met serumaansameling plaas, wat na veertien dae, met of sonder aspirasie, volkome genees.

Sommige van die operasies is na 'n tyd gekontroleer, te wete:-

1. 'n kat na ses maande (dood in motorongeluk);
2. 'n hond na een jaar (laparotomie vir verdagte neoplasma);
3. 'n kat na 15 maande.

Deurgaans was die voorkoms van die steke asof dit 'n natuurlike liggaamsvesel is. Makroskopies kon hoegenaamd geen reaksie waargeneem word nie.

Slegs vir die afbind van die ophangbande van die eierstok en baarmoeder word medium kroomdermsnaar vanaf Nr 0 tot Nr 3 gebruik. Dermsnaar word gebruik omdat die afbinding styf moet wees sonder om deur te sny en die knoopspanning met gevoel beheer word.

Maltesiese kruise word gebruik om twee diktes vislyn, 0.18 of 0.20 mm en 0.25 mm op te rol; dit word in dromme telkens saam met die instrumente gesteriliseer (30 minute by 15 lb stoomdruk). Omdat die materiaal so dun is, word dit met die naaldhouer geknoop, eerste 'n dubbelslag en dan twee ekstra slae, en omdat die materiaal glad is, moet die knope met omsigtigheid gemaak word om te verhoed dat die knoop onnodig styf trek.

Ethicon enkelveselnylon of Dermalon** word nie aanbeveel nie, omdat dit 'n harde struktuur het. Ook maak dit moeilik betroubare knope, en kan die knoop beswaarlik in 'n stewige bondeltjie saamgetrek word. Vislyn egter, tot selfs 'n redelike dikte, kan in 'n behoorlike standhoudende knoop saamgetrek word.

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* Armour Pharmaceutical Company Ltd., Eastborne, England.
Ethicon Ltd., Edinburgh, Scotland.

** Davis & Geck, Div. American Cyanamid Co., Danbury, Connecticut. V.S.A.

SLOTSOM

Op grond van bovermelde ervarings, word enkelveselnylon in die vorm van vislyn vir wond- en velhegtings aanbeveel, en in die buikwand in die vorm van afsonderlike deur-en-deur steke deur die hele buikwand ingeslote die peritoneum. Notelovitz en Crichton [1] kom ook tot die gevolgtrekking dat enkelveselnylon, polipropileen of poli-etileen, die beste nie-absorbeerbare hegmateriale is, en dat nie-absorbeerbare hegtings verkieslik is bo absorbeerbares in die anterior (mens) buikwand.

DANKBETUIGING

Ek is baie dank verskuldig aan:

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2. Prof. H. P. A. de Boom, Fakulteit van Veeartsenykunde, Onderstepoort vir sy belangstelling en daadwerklike hulp in die opstel van hierdie artikel.

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LETTER TO THE EDITOR

OBSERVATIONS ON THE DURATION OF PREMUNITY FOLLOWING ADMINISTRATION OF THE BIVALENT REDWATER VACCINE

Sir,

The bivalent redwater vaccine will protect cattle against a natural infection of the European *(Babesia bovis)* and African *(B. bigemina)* forms of babesiosis. The period of protection given by the European parasite may persist for 2 years and even longer in contradistinction to that afforded by the African parasite which is in many instances less than a year.

In one instance it was observed that a successful transmission of *B. bigemina* with *Boophilus decoloratus* to a splenectomized year-old heifer (No. 9988) was followed by a very mild reaction which did not require treatment. Biological tests, conducted 8 months later, established that autosterilization had already taken place. In contradistinction, an artificially *B. bigemina* infected year-old splenectomized heifer (No. 2611) resulted in a severe reaction necessitating treatment with trypan blue. Biological tests, conducted subsequently at irregular intermittent intervals, revealed that the cow was still a carrier after 12 years.

Experience has shown that the short-lived premunity, produced by *B. bigemina,* is boosted periodically when cattle become reinfested

with infected *B. decoloratus* and *B. microplus* ticks, the chief vectors of this pathogen. On farms where systematic dipping with highly efficient acaricides is practised, it can be expected that periodic natural reinfections will be diminished, thus resulting in a progressive increase in the number of cattle susceptible to *B. bigemina*. It is anticipated that the premunity, due to *B. bovis* in the absence of natural reinfections, will eventually terminate in the same way.

Modern chemotherapeutic agents are highly effective in curing both forms of bovine babesiosis. Systematic laboratory and field observations have, however, revealed an important defect in that cattle, which have contracted a *B. bigemina* infection after exposure to infected ticks or after immunization, are liable to be sterilized after treatment, thus rendering them fully susceptible.

A phenomenon that is not fully appreciated, is that *B. bigemina* and *B. bovis* rapidly lose their virulence when they are maintained by serial passages in cattle. Such a decrease may become evident after a second or third passage in entire cattle and after six passages in splenectomized animals. This feature is accompanied by a decrease in their resistance to chemotherapeutic agents. In the case of quinuronium sulphate, cures can still be achieved when the dosage rate of 1.0 mg/kg is reduced to that of 0.125 mg/kg. In order to maintain the premunity in such circumstances, treatment should be preceded by collecting an adequate amount of blood in ci trate and to administer it a day later when the curative effect of the drug has subsided. Such a process would not be practical, particularly in instances when a large number are involved.

The source of the pathogens required for vaccine production, are cattle showing an active infection of either *B. bigemina* or *B. bovis*. In most instances such reservoirs harbour an intercurrent infection of *Anaplasma marginale* and *Theileria mutans*, both of which have a very much longer incubation period after an artificial infection than the two *Babesia* spp. Pure strains of *B. bigemina* and *B. bovis* are obtained by six rapid serial passages undertaken on days when these pathogens are first seen. Consideration of this isolation process makes it apparent that when *Babesia* spp. qualify for vaccine production, they have already undergone the modification described in the previous paragraph. Instances are nevertheless known where vaccine administration is followed by severe reactions. Whether or not this is related to tick toxicosis or a concurrent infection of a pathogen still needs to be determined. Consideration of the autosterilization and chemoprophylaxis, as practised in some forms of trypanosomiasis with antrypol, antrycide and aromatic diamidines, may yet be another avenue to be explored for the control of bovine babesioses. Such a chemoprophylactic agent, 4A65 (Burroughs Wellcome), has become available. Laboratory tests have disclosed that after its subcutaneous administration, cattle are protected for a period of 3 months against an artificial infection of *B. bigemina* and *B. bovis*. At present field trials are being conducted to determine the value of this compound in cattle exposed to infected ticks.

W. O. Neitz

Section of Protozoology, Veterinary Research Institute, Onderstepoort.

FINAL YEAR CLASS 1969

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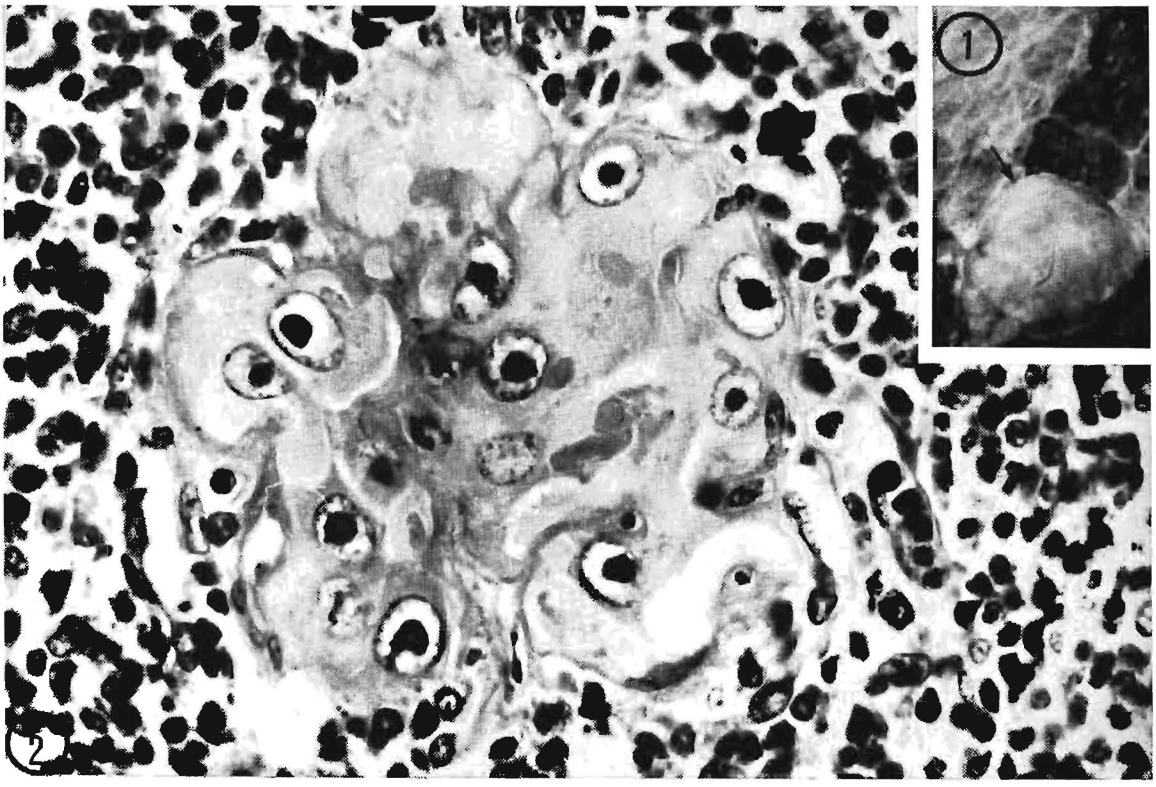

HERPES-LETSELS IN OLIFANTE

Meer as die helfte van 'n groep van 50 olifante wat onlangs in die Kruger-Wildtuin ondersoek is, het verskeie limfoïede knoppies van wisselende grootte (3 tot 30 mm) in die longe gehad (Fig. 1 pyltjie). Die alveolêre epiteel in hierdie knoppies het metaplasie ondergaan en Tipe A intranukleêre inklusies bevat (Fig. 2). Elektronmikroskopie het die aanwesigheid van 'n virus in hierdie epiteel aangetoon en die virus is vervolgens in weefselkultuur geïsoleer en as 'n herpesvirus gekarakteriseer.

Ingestuur deur: R. M. McCULLY, Armed Forces Institute of Pathology. U.S.A. (Besoekende navorser, Seksie Patologie, Onderstepoort).

P. A. BASSON & J. G. PIENAAR, Seksie Patologie, Navorsingsinstituut vir Veeartsenykunde, Onderstepoort.

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E. YOUNG & L. M. PIETERSE, Veerartsenykundige Ondersoeksentrum, Skukuza.

HERPES NODULES IN ELEPHANTS

The lungs of the majority of 50 elephants examined recently in the Kruger National Park had several lymphoid nodules varying in size from 3 to 30 mm (Fig. 1 arrow). The alveolar lining cells within these nodules were metaplastic and contained Type A intranuclear inclusions (Fig. 2). Electronmicroscopy revealed the presence of a virus in the epithelial cells. It was subsequently successfully isolated in tissue culture and characterized as a herpes virus.

Submitted by: R. M. McCULLY, Armed Forces Institute of Pathology, U.S.A. (Guest worker at Section Pathology, Onderstepoort).

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(Numbers in bold type indicate senior or sole author)