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CONTENTS/INHOUD

Content continued on page 141

In Memoriam

Feature Page **Trefferblad**

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FACTORS AFFECTING SERUM CREATINE PHOSPHOKINASE ACTIVITY IN PIGS

G. MITCHELL* AND J.J.A. HEFFRON*

SUMMARY

Serum creatine phosphokinase activity was determined in German Landrace and Landrace x Large White pigs from 11 weeks to 28 weeks of age. A very pronounced age dependence of enzyme activity was observed in both groups, peak activities occurring at 19 weeks of age for the German Landrace and at 15 weeks for the crosses. The large increase in serum creatine phosphokinase activity observed occurred during the rapid phase of growth after which enzyme levels returned close to initial values. Sedatives and the stress of handling while obtaining blood can also cause raised serum creatine phosphokinase levels and complicate the interpretation of the data in regard to the detection of the stress syndrome. Enzyme activity also displayed a diurnal variation thus introducing a further parameter in the use of creatine phosphokinase activity for detection of the stress syndrome. Certain limitations in the method of measurement of creatine phosphokinase are discussed.

INTRODUCTION

The malignant hyperthermia (MH) syndrome occurs in Landrace, Pietrain and Poland China breeds of pig in response to exercise, the anaesthetic halothane and many other stressors[1,3]. The syndrome is characterised by a rapid rise in body temperature, tachycardia, muscle rigidity and death. Transport deaths and deaths during service due to the syndrome represent significant losses to the breeding and meat industries. Muscle from MH susceptible pigs produces low quality pork, the so-called pale, soft, exudative (PSE) pork which can only be used in the cheaper processed meats. The undersirable properties of the pork are due to protein denaturation caused by excessive lactate production concomitant with an elevated carcass temperature at the time of slaughter.[4] It would be desirable to incorporate in a breeding programme a non-destructive screening test for detection of carriers of the syndrome and thereby eliminate stress-prone animals from herds.

A clinically similar syndrome occurs in man[7] and may be detected by finding elevated serum creatine phosphokinase (CPK) levels. The MH syndrome in humans is inherited as an autosomally dominant trait.[7] The mode of inheritance of the syndrome in pigs is not known though it runs in breeds which have been intensively selected for high total muscularity, food conversion and growth rate. The possibility exists, therefore, of using serum CPK values as a genetic marker for the syndrome in pigs. It is shown in this paper that several factors affect serum CPK levels in pigs, notably age, handling stress, tranquilisers, and to a lesser extent, the breed of pig. A diurnal rhythm in serum CPK levels was also noted

METHODS

Blood was obtained from two litters of pedigree German Landrace pigs (5 per litter) and one litter of Large White x South African Landrace pigs (6 animals). Males and females were equally represented in both breeds. Blood was taken from both breeds at 4 week intervals, commencing at 11 weeks of age and finishing at 28 weeks of age. The technique used to draw blood was as follows: the pigs were restrained on their backs; using 18 gauge needles, varying in length from 3.5 to 11 cm depending on the size of the pig, venipuncture of the right or left jugular vein was performed at the thoracic inlet. As diurnal variation in serum enzyme levels was possible all blood samples were drawn between 08h00 - 11h00. After withdrawal the blood was allowed to clot at room temperature for 30 - 60 min. Serum was obtained by centrifugation at 2000 r.p.m. for 5 min. in a bench centrifuge. Serum was assayed for CPK activity immediately or stored at 4°C for not more than 24 hours. In all cases it was essential to dilute the serum by 1 part to 9 parts of 0.9% NaCl just before assay as serum CPK activities in pigs are generally considerably higher than in man. CPK activity was measured using the test kit of Boehringer GMBH, Mannheim, West Germany (activated "Monotest" type). The rate of reduction of nicotinamide adenine dinucleotide phosphate (NADP) in the test kit was recorded at 340 nm on a Beckman DB-GT spectrophotometer thermostatted at 30°C. Serum enzyme activities are expressed as international units per litre of serum at 30°C (I.U./ℓ). Analytical grade NaCl in glass double-distilled water was used for dilution of serum.

RESULTS

Initially, the effect of dilution of the serum with 0.9% NaCl solution on enzyme activity was examined. Table 1 shows the results for dilutions in the range 1-5 to 1-20 of serum from six pigs having a large range of CPK activities. It is seen that in the dilution range of 1/5 to 1/15, the CPK activity is reasonably constant. Because of the great variation in serum CPK activity in pigs a 1/10 dilution of serum was found to be the most convenient for routine assays. This dilution is adequate for serum CPK levels up to about 2000 I.U./ℓ, corresponding to an absorbance increase of 0.06 per min. at 340 nm. When higher CPK activities are indicated a 1/20 dilution of serum is usually sufficient.

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Table 1: EFFECT OF DILUTION WITH SALINE OF SERA WITH LOW AND HIGH CPK ACTIVITIES.

Pig No.	Dilution				
	1/5	1/7,5	1/10	1/15	1/20
1	181	–	181	–	231
2	258	270	256	235	331
3	377	354	306	348	398
4	858	–	808	–	799
5	925	–	858	–	967
6	1 668	1 502	1 585	1 752	1 920

CPK activities expressed as I.U./litre of serum.

Although not mentioned in the test kit instructions, it was found that the blank, consisting of an equal volume of saline in place of serum, had a low apparent CPK activity. The mean value of 11 blank determinations was 3.3 I.U./ℓ. Clearly, the blank value only assumes significance when the serum CPK activity is low. The reproducibility of the assay for serum CPK activity, expressed as the coefficient of variation (S.D.÷$\bar{x}$ · 100), was 7.2%. Usually CPK activity can be determined on the serum immediately after centrifugation though it may not be possible for various reasons to do this. It may be seen in Fig. 1 that serum may be stored up to 5 days after preparation without any loss of enzyme activity. After this time, a modest decline in activity occurs.

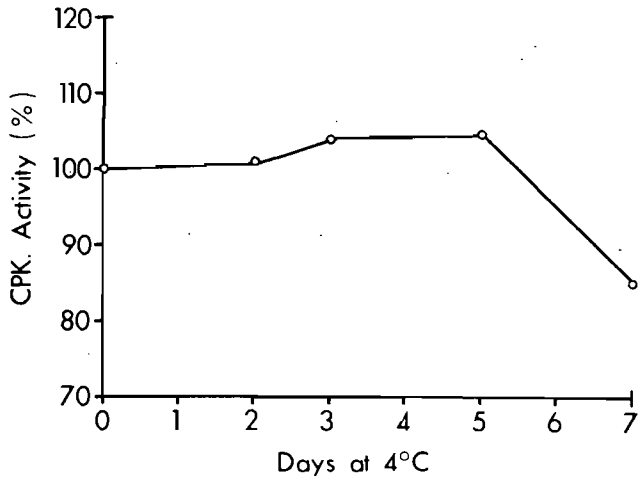

Fig. 1 Stability of serum CPK activity stored for up to 7 days at 4°C. The values, expressed as a percentage of initial values, are means for six animals.

Fig. 2 shows the absolute values of serum CPK activity expressed as a function of age for the 10 German Landrace pigs. The data are given as means± S.E.M. Serum samples were obtained at 11, 15, 19, 23 and 28 weeks of age. It is seen that a great increase in CPK activity occurred during the 11th to 19th week period of growth, after which the activity returned gradually to the initial value. Using the 11th week value as reference a four-fold increase in serum enzyme activity occurred at 19 weeks of age. A similar experiment was carried out on six Landrace x Large White pigs at the same ages and at 5 and 36 weeks of age. Serum was obtained in exactly the same manner as for the German Landrace pigs. The variation of serum CPK activity, illustrated in Fig. 3, was similar to that observed with the German Landrace pigs. En-

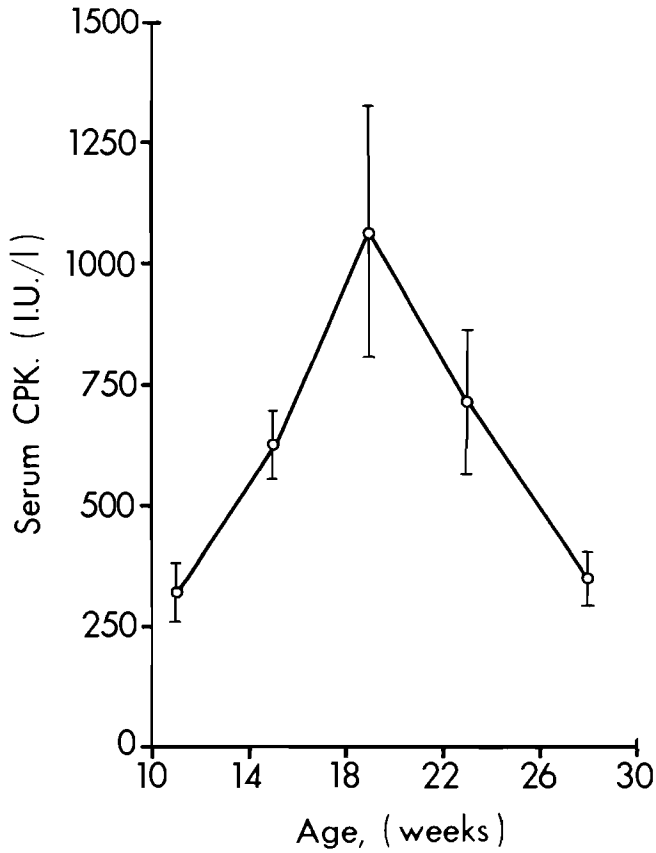

Fig. 2 Variation of serum CPK activity with age in 10 German Landrace pigs. Values are means± S.E.M.

zyme activity remains constant until 11 weeks of age after which it rises sharply to a maximum at 15 weeks of age. Activity declined gradually to a constant level after 28 weeks. The 5 and 11 week CPK levels were not significantly different from the 28 week level. In the crossed pigs an eight-fold rise in enzyme activity occurred at the peak compared with the initial age values. The peak value for the German Landrace pigs at 19 weeks of age was significantly lower than the peak value for the crosses ($P < 0,02$), while the values

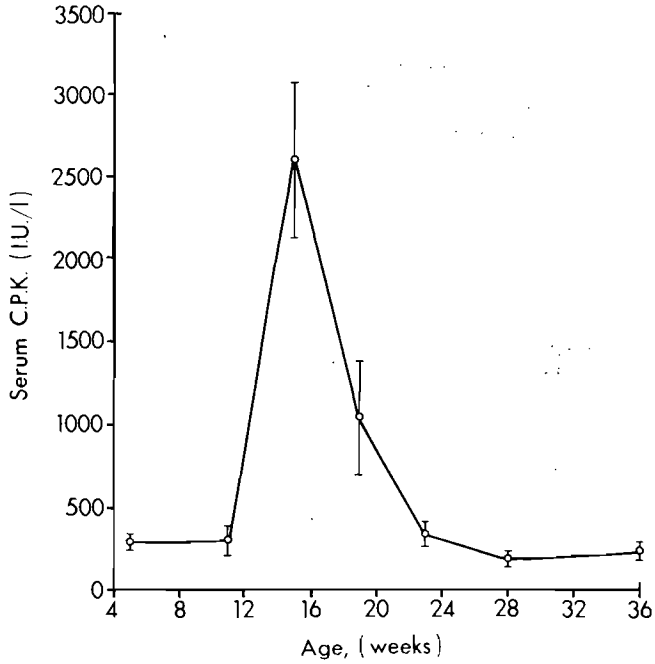

Fig. 3 Variation of serum CPK activity with age in six Landrace x Large White pigs. Values are means± S.E.M.

were the same at 11 weeks of age. The CPK activity was however, significantly higher in the German Landrace pigs compared with the crosses after 28 weeks of age, ($P < 0,02$). In both breeds of pigs studied there were no statistically significant differences in serum CPK activities between the sexes at any age. Fig. 4 shows the correlation between serum CPK level (c.f. Fig.3) and growth rate for the six Landrace x Large White pigs over the 11 to 28 week period. The body weights were recorded monthly while the CPK levels were measured at 4 week intervals as described.

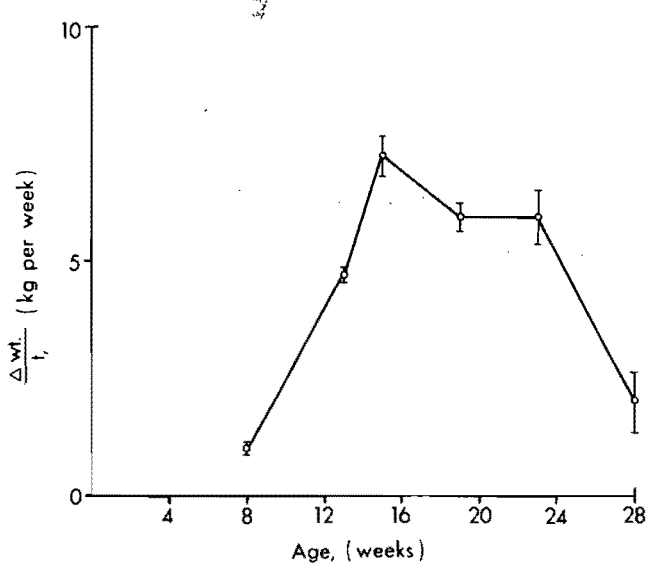

Fig. 4 Variation of growth rate with age in six Landrace x Large White pigs.

Growth rate is expressed as change of weight in kilograms divided by time in weeks at each stage of growth. Growth rate was greatest during the 11th to 15th week period reaching a maximum at 15 weeks of age. The rate then dropped slightly and finally fell off considerably during the 23 to 28 week period.

At the start of the experiment it was decided to examine serum CPK activity for possible diurnal variation. Blood samples were taken from three German Landrace pigs over a period of 24 hours at 08h00, 14h00, 20h00 and 02h00 and then serum CPK analysed at each time point. The diurnal variation is shown in Fig. 5 plotted as mean activity relative to the value at 08h00 set at 100. This was essential because there was great variation in the absolute CPK activities of the individual pigs. It is seen that maximal CPK activity occurs at 14h00 being 48% higher than the 08h00 value. Enzyme activity was least at 02h00 and intermediate at 20h00. The stress of struggling during blood sampling also constitutes a variable when determining CPK activity in pigs as shown in Table 2. Blood samples were taken at zero time, 1½ hour later from the same group of German Landrace pigs and analysed for CPK activity. It is seen that a two to four-fold increase in CPK activity occurs over the period of one hour. These measurements were made when the pigs were 30 weeks of age.

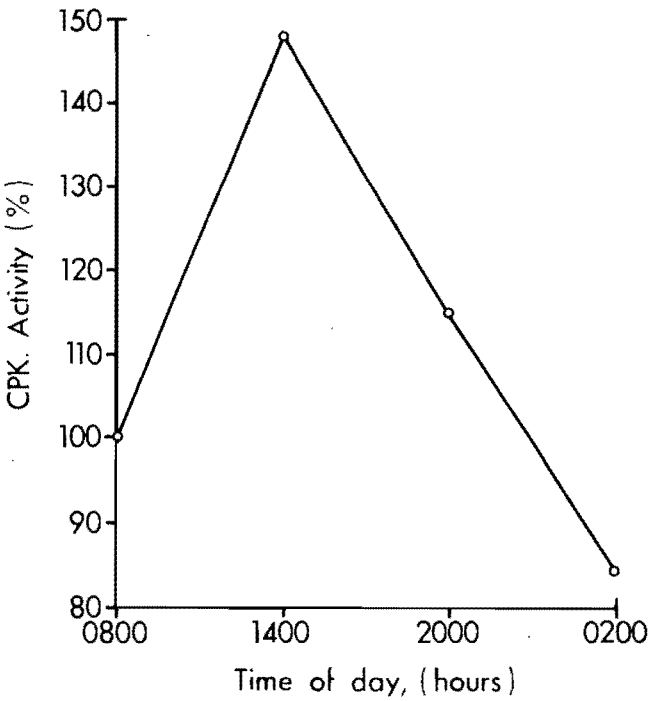

Fig. 5 Diurnal variation of serum CPK activity in three German Landrace pigs. Mean values, expressed as a percentage of the 0800 activity, are given.

Table 2: EFFECT OF HANDLING STRESS AND AZAPERONE ON SERUM CPK ACTIVITY OF PIGS

Agent	Pig no.	Serum CPK Activity – – I.U./l.		
		First Sample	30 min. Sample	60 min. Sample
Stress	1	760	2 273	2 625
	2	635	334	1 621
	3	677	1 872	334
	4	718	618	1 421
Azaperone*	1	836		1 588
	2	334		418
	3	250		418

* Azaperone injected intramuscularly at a dose of 1.5mg./kg.

A two-fold increase in serum CPK activity occurred over a period of one hour in response to a standard dose of the sedative azaperone (Table 2). Blood samples were taken from three pigs for CPK determination after which they were given an instramuscular dose of azaperone (1,5 mg/kg). Blood was taken one hour after administration of the drug for CPK analysis.

DISCUSSION

Raised serum CPK activities are indicative of a variety of myopathies'. The value of serum CPK in detecting sub-clinical myopathies with special reference to the malignant hyperthermia/stress syndrome in pigs is clear. In man, myopathy is indicated by elevated serum CPK values, the normal range being 0-50 I.U./ℓ. Raised enzyme levels are not specific for the type of muscular dystrophy seen though it is believed that dystrophies of neurogenic origin do not result in raised serum CPK levels[5]. It is well established that the human malignant hyperthermia syndrome can be detected in susceptible individuals by finding raised serum CPK levels. The evidence for using serum CPK levels in pigs for a similar purpose is not compelling since the limitations inherent in the measurement of serum enzyme levels are not appreciated. It is known that serum CPK levels do not vary with age in man,[6], and it is easy to obtain blood samples from humans without imposing stress. It is

shown in the present paper that these two factors are the main sources of error in using serum CPK levels as an indicator of the MH syndrome in pigs. Normal ranges are difficult to establish for these reasons, and also because there is further variation caused by diurnal rhythm and sedation. Breed differences would appear to be of lesser importance if CPK levels are measured early in the pig's life than at the baconer stage. Thus, if serum CPK values are to be used for detection and 'breeding out' of the stress syndrome from otherwise commercially desirable pigs, the limitations described here should be taken into account. The large increase in serum CPK activity observed up to the maximum at 15 to 19 weeks can be correlated to peak protein anabolism which occurred at 15 weeks of age in the pigs tested. During fat anabolism between the ages of 19 to 28 weeks the growth rate remains relatively high while the serum CPK activity falls to the initial age values. The extent of stress and the activity of the pig also raise the CPK levels. In both cases muscular activity is responsible for the leakage of intracellular muscle CPK into the blood. While azaperone ostensibly raises the serum CPK levels the increase may be due to the stress involved during blood sampling, and not to instrinsic drug effects. It is clear from the results that azaperone cannot prevent the leakage of CPK from the muscles into the blood even though it is known that it stabilises the phosphocreatine and adenosine triphosphate levels in the skeletal muscles of the pig[8]. In view of these results the value of serum CPK levels in predicting the presence of the stress syndrome in pigs cannot be in the absolute levels of the enzyme. It is clear that establishment of normal or 'base' values of serum CPK activity for pigs in general is meaningless. Raised serum CPK levels in individual pigs may only be of value if compared with mean levels for the same breed and at the same age. It is also clear that, due to the very great variation in serum enzyme levels in the 15 to 19 week age period, serum CPK should be measured on or before the 11th week of age or at 28 weeks of age when enzyme levels have stabilised again. Furthermore, the stress of handling is likely to be least when the pigs are at the weaner stage. Enzyme levels obtained under these conditions provide 'normal' values for a group of pigs against which raised levels may be meaningful.

ACKNOWLEDGEMENTS

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THE CHOLERETIC ACTION OF GENEBILE* IN A DOG

A. IMMELMAN**, C.J. ROOS*** AND N.C. OWEN**

SUMMARY

A procedure for cannulating the bile duct of the dog is described. An intramuscular injection of Genebile increased the bile flow rate. A possible mechanism for this increase is suggested.

INTRODUCTION

The use of Genebile in cattle[2] has been described and critical trials carried out in sheep[4] and goats[3]. Its effect in the dog has not previously been evaluated.

This communication describes the cannulation procedure of the bile duct in a dog and the collection of bile before and after administration of Genebile. The bile was analysed to investigate the possible mode of action of this compound as a choleretic.

MATERIALS AND METHODS

A clinically normal Alsation bitch, 3 years old and weighing 26 kg was used. The diet consisted of a commercial ration containing 20% crude protein, fed once daily. Water was available *ad libitum*.

Cannulation Procedure

After 24 hours fasting the dog was prepared for surgery. Anaesthesia was induced with thiopentone and maintained, after intubation, with 2% Halothane B.P. in oxygen in a closed circuit. A right paracostal incision exposed the liver, pyloric part of the stomach and the duodenum. The gallbladder was located and manual pressure applied to it to facilitate identification of the common bile duct. The latter was then ligated immediately distal to the point where the duct from the right lateral lobe of the liver entered it, and transected distal to the ligature. Care was taken not to injure the portal vein closely associated with the duct.

A polythene tube of suitable diameter was inserted in an antegrade direction into the lumen of the duodenum through the distal part of the common bile duct and its sphincter. The method illustrated in figure 1 was employed to secure the tube firmly in the lumen of the duct.

Partial cholecystectomy was performed and a polythene tube secured in the lumen of the remaining part of the neck of the gallbladder and the cystic duct, again using the method illustrated in figure 1.

One end of each of the polythene tubes was brought to the exterior through small stab incisions in the different layers of the abdominal wall, care being taken to leave as little as possible of the tubes in the abdominal cavity and not to cause kinking of the tubes. They were anchored to the peritoneum, subcutaneous tissue, and to the skin externally by using a slight modification of the method shown in figure 1.

The shaft of a large-bore hypodermic needle served as a connection between the tubes externally. This arrangement allowed the collection of total bile flow as desired on certain days, while on other days bile flowed continuously into the duodenum thus obviating the problems associated with continuous bile loss from the body.

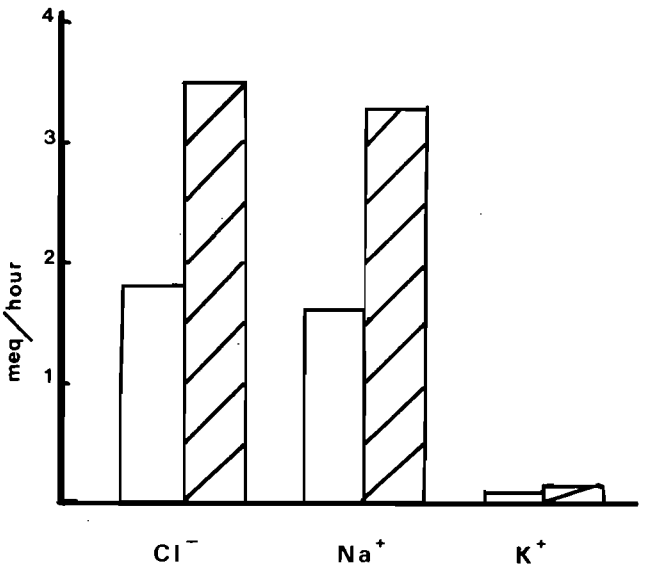

Fig. 1 Method used to secure artificial tubes in ducts.

After the operation the dog remained clinically normal but showed some inappetance, consuming about half the amount of food eaten prior to the operation. She was allowed a 72 hour recovery period before experimentation began.

Pilot trials conducted with previous dogs had shown that normal bile flow was greatest in the morning, decreasing to a minimum in the afternoon. As this is probably related to the mid-morning feeding regimen followed routinely, it was decided to collect samples hourly over a 6 hour period before feeding the animal.

Initially a 6 hour series of control samples was taken by disconnecting the two polythene cannulae and collecting the bile coming directly from the tube inserted into the neck of the gall bladder. The following day Genebile was injected intramuscularly at a dose of 10mg/kg body weight and a further series of samples collected. The samples from these 2 days were compared (Trial 1). This procedure was repeated twice with a 72 hour rest period between each trial (trials 2 and 3).

Sample Analysis

After noting the flow rate per hour, the samples were analysed for sodium and potassium by flame photometry, chloride by mercurimetric chloride titra-

* Diethano-Lamino Salt of 4- (4 Methoxy-Naphthalene -(1) -4-Oxy-Butyric Acid.

** Dept of Physiology, Faculty of Veterinary Science, University of Pretoria, P.O. Onderstepoort, 0110.

***Dept. of Surgery, Faculty of Veterinary Science, University of Pretoria.

tion (Merckotest), bilirubin by the van den Berg reaction and the osmolality determined by freezing point depression (Knauer ostometer).

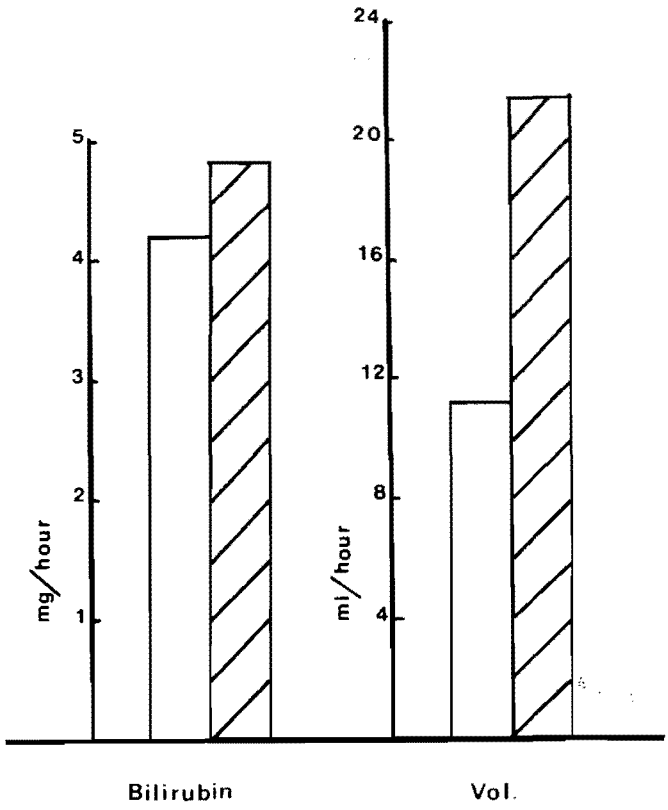

Fig. 2 The effect of treatment on bile composition and flow rate. The height of each column represents the mean value for the three trials.

□ Before treatment
□ After treatment

RESULTS

The mean flow rates per hour before and after administering Genebile are shown in figure 2. The results clearly indicate that the mean rate of bile under control conditions (11,2ml/hour) was considerably less than the mean flow rate after administering Genebile (22,7ml/hour).

The mean values for the excretion of bilirubin and electrolytes into the bile per hour are compared in figure 2.

The results show that the amount of bilirubin excreted per hour increased slightly after treatment, while the amounts of sodium and chloride excreted per hour increased markedly. The excretion of potassium was also somewhat elevated.

The mean values obtained for the individual trials were analysed statistically (Student t test) and are set out in Table 1. In all cases there was a highly significant increase in flow rate as well as sodium and chloride excretion while the excretion of potassium was unaltered in one trial, but significantly increased in the other two trials. Bilirubin excretion remained unaltered during two of the trials but was significantly increased in the other.

DISCUSSION

The results obtained suggest that the cannulation procedure employed in this trial is suitable for the study of factors affecting biliary excretion. In some preliminary trials where the cannula was passed retrograde fashion into the common bile duct of dogs and sheep (unpublished data) the bile flow has been very variable.

The results indicate that Genebile has a choleretic action in that the bile flow rate was consistantly elevated after intramuscular injection at a

Table 1: ANALYSIS OF BILE COLLECTED BEFORE AND AFTER INTRA-MUSCULAR ADMINISTRATION OF GENEBILE (10MG) KG BODY WEIGHT

	TRIAL I			TRIAL II			TRIAL III		
Analysis	Control	After treatment	Significant levels	Control	After treatment	Significant levels	Control	After treatment	Significant levels
Flow rate (ml/hour)	7±3,2	13,9±1,7	P<0,01	15,2±2,7	26,8±6,4	P<0,001	11,3±3	25,9±4,4	P<0,001
Bilirubin excretion mg/hour	1,09±0,23	1,65±0,26	P<0,01	5,83±1,6	6,70±2,64	N.S.	5,63±3,24	6,01±0,99	N.S.
Cl excretion meq/hour	0,82±0,29	1,76±0,39	P<0,001	2,51±0,41	4,61±1,04	P<0,001	2,08±0,60	4,13±0,70	P<0,001
Na excretion meq/hour	1,1±0,59	2,11±0,58	P<0,05	2,29±0,51	3,94±0,73	P<0,001	1,76±0,55	4,04±0,78	P<0,001
K excretion meq/hour	0,044±0,029	0,063±0,015	N.S.x	0,089±0,021	0,154±0,024	P<0,001	0,077±0,032	0,164±0,051	P<0,01
Osmolality Average	299,6±6,1	304,7±5,7	N.S.	292,7±4,7	301±10,9	N.S.	306,1±4,5	302,6±4,7	N.S.

x N.S. = not significant

dosage of 10mg/kg body weight. It may be postulated that the drug stimulates a sodium pump mechanism and that chloride follows passively down an electrochemical gradient. As the osmolality remains unaltered, passive water movement along with the sodium chloride would seem to explain the findings. Such a mechanism is in agreement with that postulated by Diamond [1] (1969) for the isotonic reabsorption from the gallbladder. The results suggest that the drug does not actively stimulate bilirubin excretion, the increased excretion in trial 1 possibly being secondary to the increased water movement into the bile. A similar situation may hold for potassium excretion.

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BOOK REVIEW

BOEKRESENSIE

ÜBER KAPSELKÖRPER UND GLEITDRUCKSTRUKTUREN — EIN BEITRAG ZUR SESAMBEINFRAGE

F. PREUSS AND A. WÜNSCHE

Supplement 21 to Zentralblatt für Veterinärmedizin. Paul Parey, Berlin. 1974. Price R10.

The authors analyse a 130 odd publications on the question of sesamoid bones – their origin, structure and function. Their views are further based on their own dissections which cover the domesticated animals, laboratory animals and birds. Sesamoid bones in the digital joints of the pig are described for the first time. They consider true sesamoid bones to develop in synovial capsules of joints, bursae and tendon sheaths. Three groups are recognized *viz.*: those in close proximity to joints, *e.g.* the patella and fabellae; those associated with bursae and tendon sheaths, *e.g.* the peroneal sesamoid in man; those not associated with synovial capsules and which are not true sesamoid bones, *e.g.* the ossified tendons of certain birds. The primary function is thought to be protection of the joint capsule, stabilization of the joint, and improvement of the effectiveness of muscular action.

Until such time as it would be possible to devise and introduce suitable experimental methods, conflicting views will be rife. This is an almost philosophical treatise on corresponding and conflicting views held by various authors since 1888 in regard to sesamoid bones. It makes interesting reading but its appeal will probably be limited.

J.M.W. LE R.

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A METHOD FOR THE *IN VITRO* STUDY OF DRUG TRANSFER ACROSS RUMINAL EPITHELIUM

W.L. JENKINS* AND L.E. DAVIS**

SUMMARY

A simple method for the *in vitro* study of drug diffusion across ruminal epithelium is described. The characteristics of the isolated membrane were defined by studies of ketone production from butyrate, histological studies, phenolsulphonthalein penetration and permeability to pentobarbital, antipyrine and tetraethylammonium. The preparation was found to be suitable for studies of less than 12 hours duration; after that time the integrity of the membrane as a barrier was lost due to degenerative changes. The method had a primary advantage in that experimental variables could be rigorously controlled.

INTRODUCTION

The presence of a complex, voluminous, hollow organ such as the reticulo-rumen as a component of the gastrointestinal tract might well be expected to influence the absorption, distribution and excretion of any drug administered by practically any route. In addition, posology in ruminants presents a real problem as up to 20% of the body weight may be attributable to the ruminal contents[35], while the reticulo-rumen may or may not constitute a distribution compartment for a drug. Notwithstanding the importance of these basic considerations, there have been very few studies conducted concerning the absorption of drugs from the reticulo-rumen and the diffusion of drugs from the plasma into the ruminal fluid. This lack has been emphasized by Jones [16], Dobson [7] and Stowe [34].

The physico-chemical factors which govern the passage of drugs across biological membranes have been well delineated [4 5], and the roles which these intrinsic properties play in the diverse ways in which solutes move across membranes have been extensively reviewed by Schanker[28 30]. Moreover, the practical application of these principles to the absorption, distribution and excretion of drugs in monogastric species has been fairly well documented[10 11 26 27 29]. However, once again these basic premises have not been fully evaluated with respect to the reticulo-rumen in which the keratinized stratified squamous epithelium represents a composite series of cellular membranes.

During the last 25 years a wide variety of experimental approaches have been adopted to study absorption from the rumen. Reviews of the methods which have been used to date have been presented by Annison and Lewis[1], Annison[2] and Dobson and Phillipson[8]. Most of these procedures have had both advantages and disadvantages and were employed principally for the study of absorption of nutrients and water from the rumen, and the metabolic functions of the rumen. Notwithstanding these important contributions, there remains a paucity of information associated with drug transfer across this biological barrier. Controlled observations on the effect of concentration gradients, pH differences, plasma-protein binding, and anatomic differences in epithelium from various areas of the rumen on the transfer of drugs have not yet been carried out.

The object of this work was to develop and validate an *in vitro* system which could be used to study distribution of drugs across ruminal epithelium under rigorously controlled experimental conditions.

MATERIALS AND METHODS

Experimental Procedures

The experimental approach was based on utilizing a carefully defined system which permitted the study of the flux of specifically selected compounds across a delineated surface area of ruminal epithelium. The viability and integrity of this biological barrier under the imposed experimental conditions was established to satisfy the necessary criteria[17] for such studies on solute transfer.

Sheep and goat viscera were not readily available on a regular basis in the vicinity of Columbia, whereas cattle were slaughtered daily at a local abattoir. Thus the tissues employed were generally of bovine origin. The unavoidable consequence of this situation was that there was no control over the nutritional status of the donor animals, although it is well known how great a role the dietary intake plays in the structure and function of rumen epithelium.

Rumen wall from the latero-dorsal area was collected from a local abattoir. The viscera became available about 10 to 15 min after slaughter and the section of rumen wall which was of interest was then cut free and placed in oxygenated Locke-Ringer's solution at about 311 to 313K (38 to 40°C) for transport to the laboratory within 15 minutes. The whole rumen wall was thoroughly washed in warm Locke-Ringer's solution and was then placed in Krebs-Ringer's phosphate (KRP) solution (at 311 to 312K) through which oxygen was continuously bubbled. The

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ruminal epithelium was carefully dissected free of the underlying muscularis layers while the tissue was bathed in oxygenated KRP. Sections were then cut and affixed to thistle tubes with twine. The total capacity of these thistle tubes was about 30 ml and the mean surface area was 9,01 cm^2. The stem lengths were cut to about 11cm.

Twenty-five ml of pH 7,4 KRP solution were introduced into the thistle tube. The tube was then placed in 500 ml KRP solution which had been warmed to 311,5K (38,5°C). This solution contained the drug under study at the concentration and pH desired. Thus, the system was based upon the transfer of the compounds studied from a very large fluid compartment, which constituted to some degree an infinite volume and an essentially constant drug concentration, across a fixed surface area of ruminal epithelium into a much smaller fluid compartment. This arrangement greatly facilitated analysis of the results.

The final arrangement of a single system is illustrated in Figure 1. A thin plastic sheet covering the beaker prevented excessive evaporative loss. A clamp on the stem of the thistle tube allowed adjustment of the level of the inside solution and this precluded hydrostatic pressure differences. An 18 ga.-120 mm filling needle with a connecting tube facilitated sample collections from inside the thistle tube. Thin polyethylene tubing was used to carry the gases into the solutions. Oxygen was continuously bubbled through the inside solution at a fairly rapid rate which allowed oxygenation and agitation of this solution. Nitrogen was bubbled through the outside solution at a slow rate which allowed mixing of the solution. The series of eight sets was placed in a constant temperature water bath at 311,5K (38,5°C). The gases were supplied to each system through two multiple outlet glass manifolds (Fig. 2.)

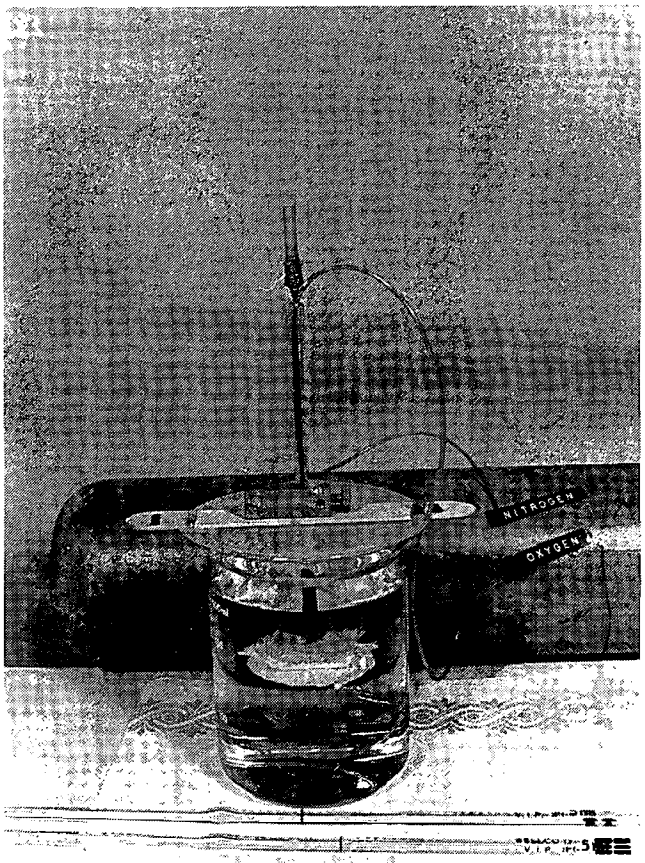

Fig. 1 A single in vitro system.

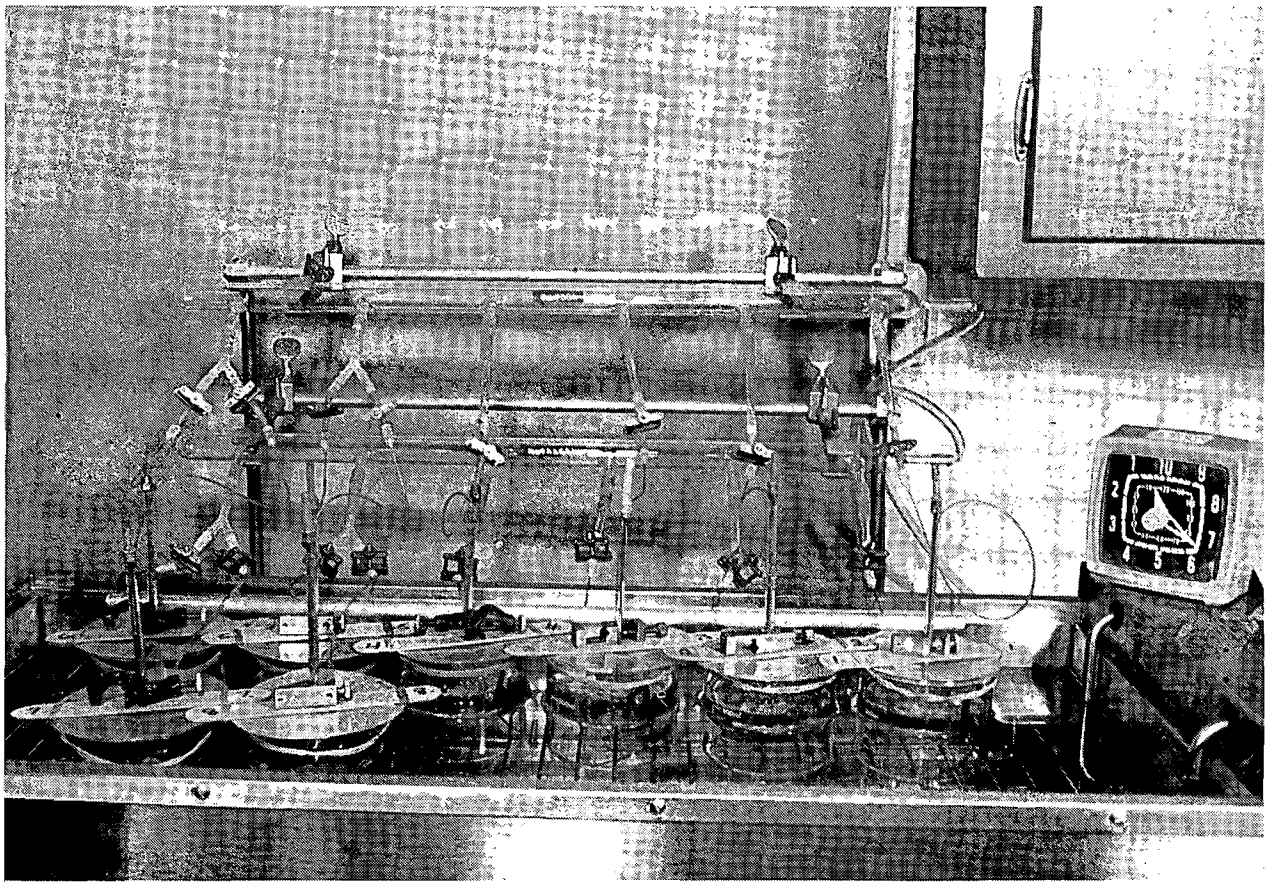

Fig. 2. The complete experimental arrangement

Ketone Body Production Studies

The ability of the ruminal epithelium to convert butyric acid to ketone bodies as a function of time was investigated to define the viability of the *in vitro* system.

A series of eight systems was set up as described above with pH 7,4 in both inside and outside compartments. At hourly intervals a system was removed, the epithelial disc excised, blotted with filter paper and then minced with scissors. The minced tissue was placed in a 50 ml beaker containing a marble and 10 ml pH 7,4 KRP solution with 0,02 mole butyric acid. This was incubated in a Dubnoff shaking incubator for 3 hours under an oxygen atmosphere at 39°C and at a rate of 120 cycles per minute. Following incubation 3 ml of the incubation medium were transferred into a Lester and Greenberg microrefluxing still and the total ketone bodies were determined by the method of Procos[23]. Epithelial tissue which had been placed in water at 353K (80°C) for 20 minutes served as the blank. The tissue was removed from the beaker, placed in an oven at 373K (100°C) overnight and weighed 12 hours later. The ketone body production (measured as acetone) by the isolated ruminal epithelium was calculated as u moles/ml fluid/g tissue (dry weight).

Permeability coefficients

Pentobarbital, antipyrine or tetraethylammonium were placed in the outside solutions and their passage into the inside solution was studied over an extended period of time. The permeability coefficients were calculated for each drug using the equation[30].

$$Pt = -1n \frac{(1 - C1)}{C2R}$$

where C1 represents the drug concentration in the fluid being entered, and C2 that in the outside solution. R is the ratio C1 ÷ C2 at the steady state[6]. On substituting in the equation the observed concentration ratios, and plotting the values of the right side of the equation against time, a straight line is obtained. The permeability coefficient P is given by the slope of the line, - 1n (1-C1/C2R)/t.

Drug Metabolism Studies

Since it is more convenient to study radioisotopically-labelled drugs it was necessary to rule out the possibility that the drugs underwent biotranrformation within the system. Accordingly, 4g minced ruminal epithelium or 20 ml ruminal fluid were added to ^{14}C-labelled pentabarbital, salicylate or antipyrine solutions and incubated in a Dubnoff shaking incubator for 3 h at 312K (39°C) and 120 cycles per min. The media were centrifuged and placed in a vacuum oven at 323K (50°C) until the fluid volume was reduced to 0,5 ml. Each solution was then chromatographed on thin-layer plates. The plates were examined under ultra violet light (254 nm) and sprayed with various reagents. The chromatograms were also submitted to radio-assay. In all cases the drugs migrated as a single spot thus demonstrating that they were not transformed by the system.

Phenolsulphonphthalein Studies

In a series of six studies utilizing bovine tissue and four studies with caprine tissue, the passage of phenolsulphonphthalein across ruminal epithelium as a function of time was investigated. The experimental procedure was as described above but the outside solution (pH 7,4 KRP) contained 0,001% (M/V) phenol red. Aliquots were collected from the inside solution every 2 hours up to 24 h in the case of the bovine studies and every hour up to 7 h in the case of caprine tissue. The end point of the experiment was the time of the first appearance of traces of phenol red in the inside solution. The method described by Austin[3] was used for the determination of phenol red concentration.

Histological Studies

To investigate the histological changes which occurred within the ruminal epithelial tissue during the experimental procedure, the tissue was removed at various time intervals, placed in 10% buffered formalin with Bouin's fluid and submitted for histopathological investigation.

RESULTS

Ruminal Epithelium Viability

The production of total ketone bodies (measured as acetone) by isolated ruminal epithelium which had been incubated in pH 7,4 KRP solution containing 0,02 mole butyric acid is depicted in Figure 3. There was a dramatic reduction in activity over the . first two hours followed by a more gradual decline until about 6 h, at which time, the residual activity was barely detectable.

Permeability to Pentobarbital, Antipyrine and Tetraethylammonium as a Function of time

The results of these studies are shown in Figure 4 for pentobarbital (n=8), Figure 5 for antipyrine (n=8) and Figure 6 for TEA (n=4). There was an alteration in the permeability of the ruminal epithelium between 12 and 14 h in each case. The slopes of the lines continued to increase, indicating greater permeability with the passage of time. The increment was greater for TEA than for pentobarbital and antipyrine.

Permeability to Phenolsulphonphthalein

In the case of bovine tissue (n=6) phenol red was detected at 16,7± 0,4 h, while with the goat tissue (n=4) no trace of the dye was detected by 7 h. These results suggested that viable ruminal epithelium was impervious to the strong sulphonic acid phenolsulphonphthalein.

Histological Studies

Over a 14 h period the cellular changes in bovine rumen epithelial tissue specimens were evaluated as follows:

0 Hour: The epithelium was approximately 5 to 7

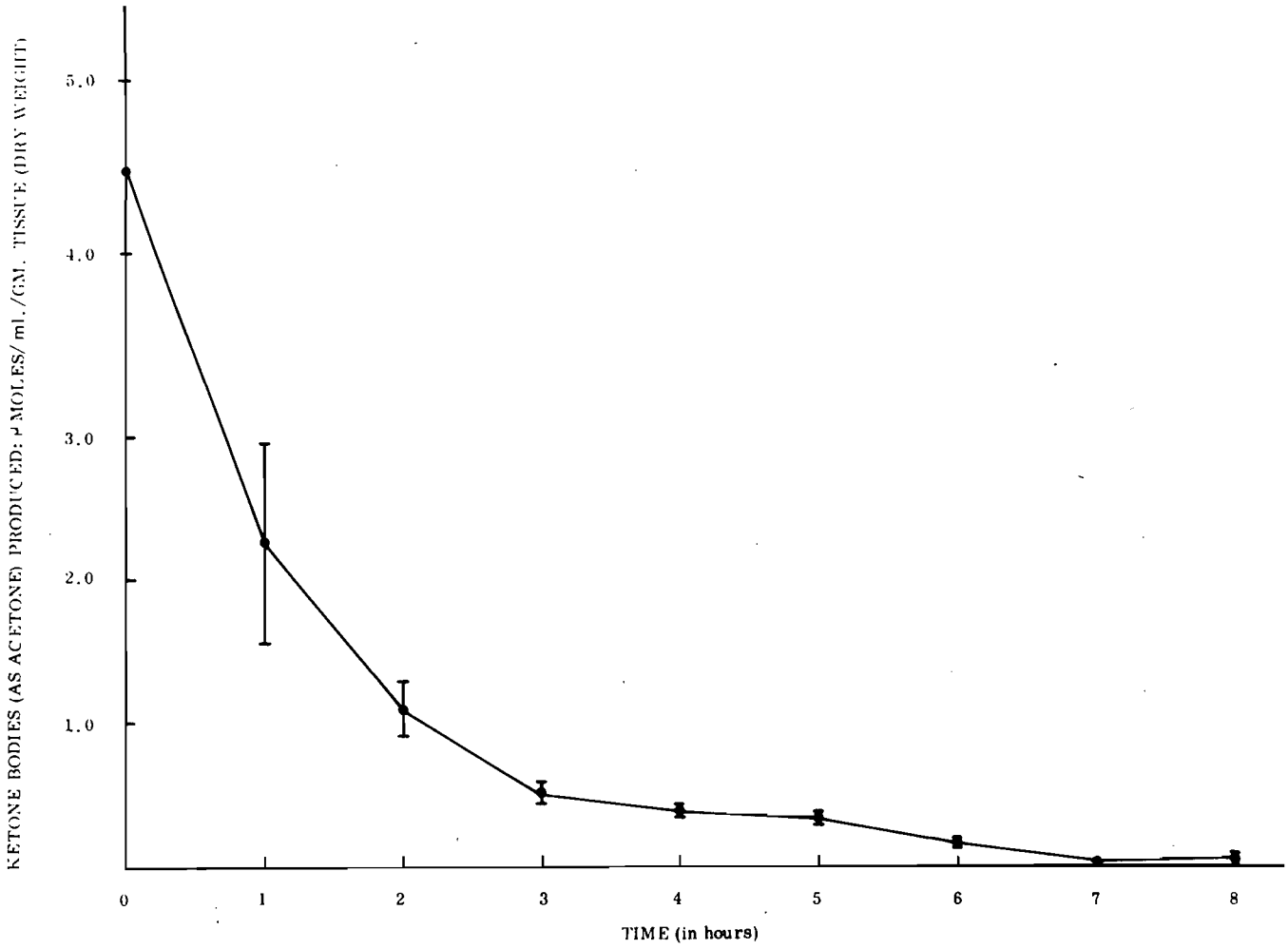

Fig. 3. Total Ketone Bodies (as acetone) produced by isolated ruminal epithelium as a function of time. N= $\frac{2}{3}$*

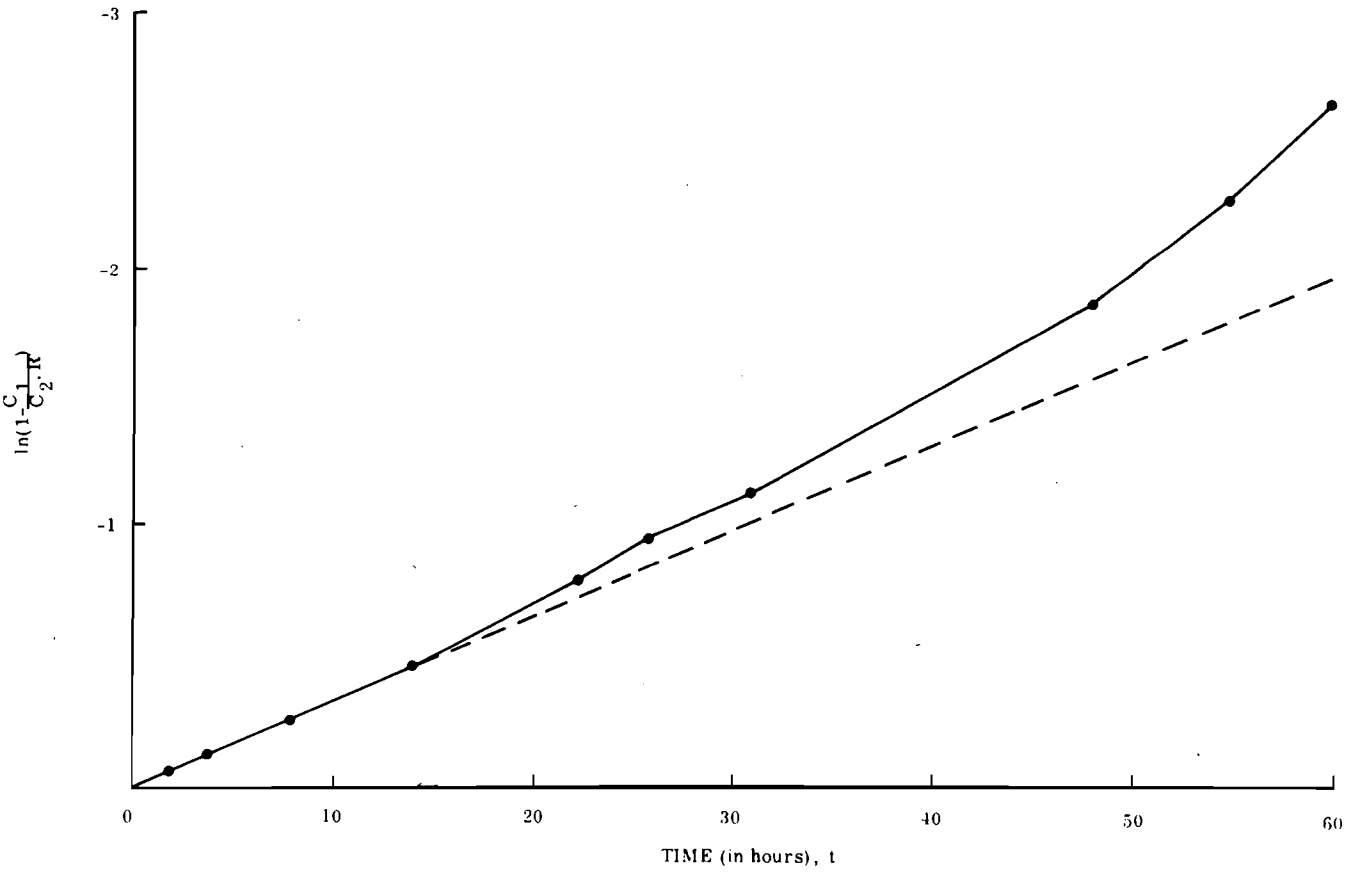

Fig. 4. Permeability of ruminal epithelium to pentobarbital.
C1= concentration in inside solution, C2= concentration in outside solution, R= C1/C2 at steady state. Slope of line, — 1n (1-C1/C2R)t, represents the permeability coefficient. Note increasing permeability after 12 hours.

cells in thickness. There was a discontinuous layer of stratum corneum on the surface. Below this layer, the stratum lucidum (also discontinuous) was approximately 1 to 2 cells thick. For the most part these cells were swollen and contained a clear, non-stainable, cytoplasm with fairly well defined nuclei. The lamina propria consisted of a fine network of collagenous fibres. The submucosa was loose and contained open and collapsed vascular channels with distinct endothelium (Fig. 7A).

1 Hour: The morphology was essentially the same. However, the superficial half of the squamous

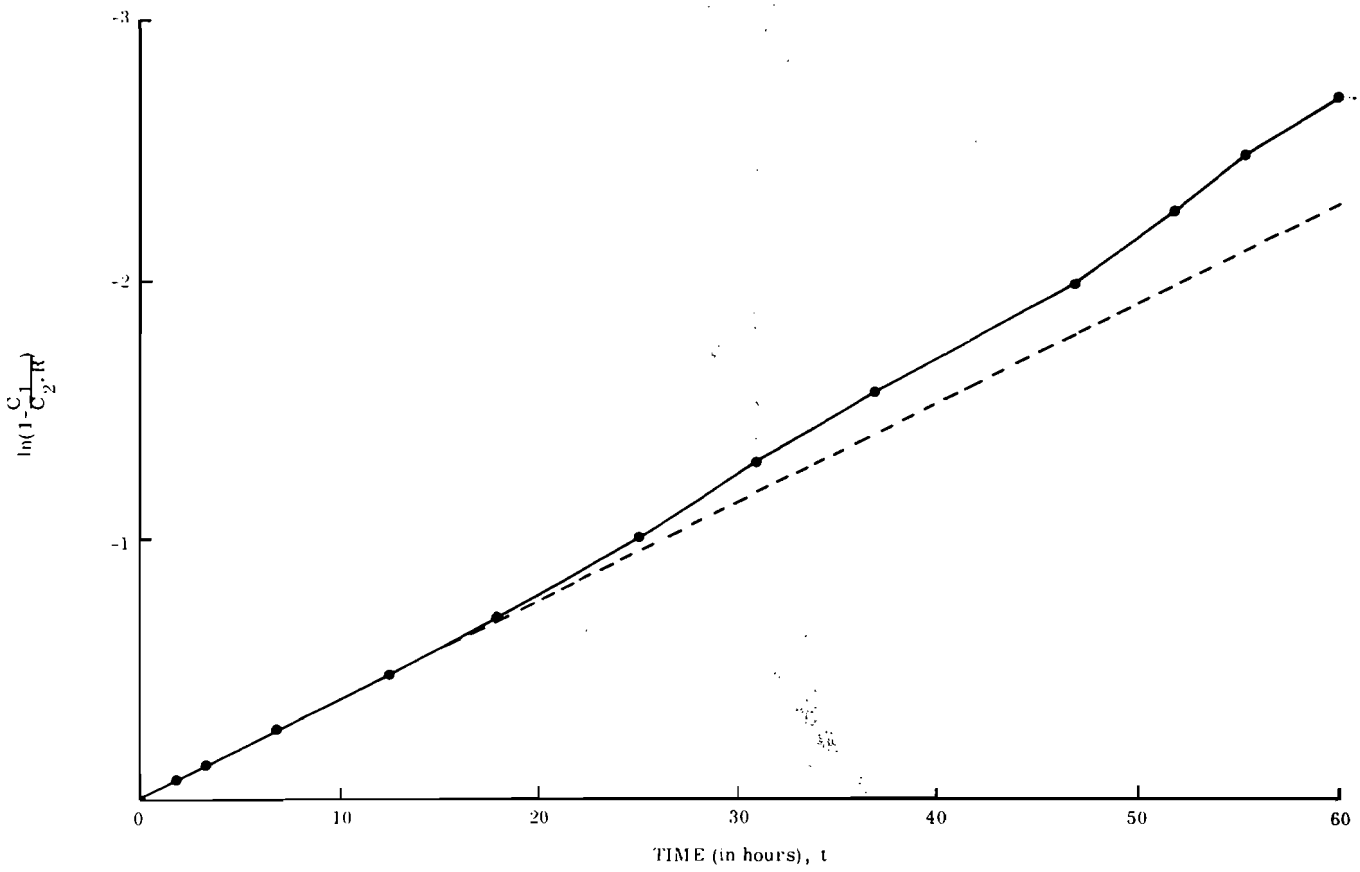

Fig. 5 Permeability of ruminal epithelium to antipyrine. Terms are same as described in legend for Fig. 4.

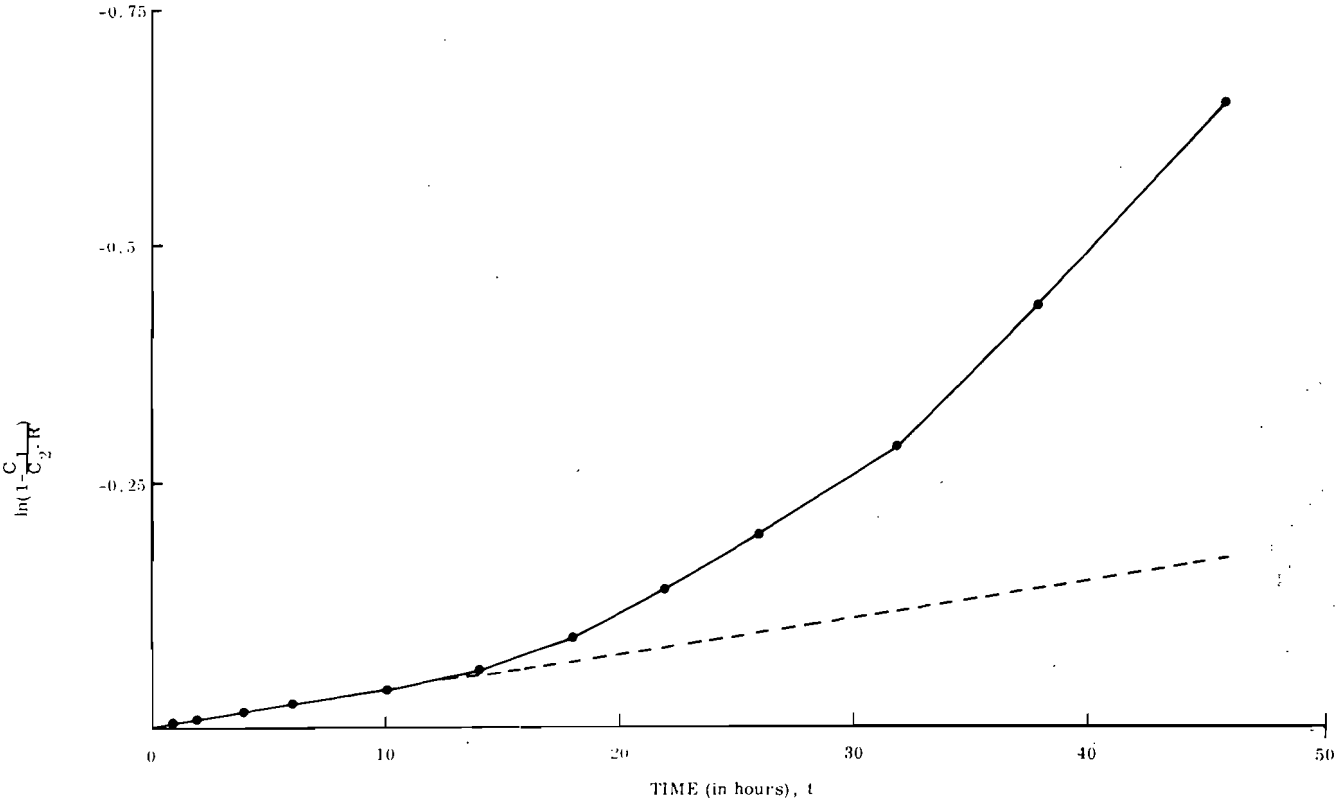

Fig. 6. Permeability of ruminal epithelium to tetraethylammonium. Terms are same as described in legend for Fig. 4.

layer revealed hydropic degeneration. Between this hydropic area and the subadjacent deeper staining layers there was a sharp line of demarcation.

3 Hour: There were foci in which the lamina propria blended imperceptibility into the epithelial layers making it difficult to discern the basalar layer of the epithelium in these foci. In other areas the basalar layer was still well delineated and there was a faint line of demarcation between the epithelium and the lamina propria.

4 Hour: Appearance was very similar to the 3 h specimen (Fig. 7B).

8 Hour: The washed out appearance of the epithelium had become more pronounced. There were more foci in which the lamina propria and epithelium seemed to blend one into the other. For the most part the basalar layer of the epithelium was the only layer which still retained good hematoxylin differentiation in most areas. The lamina propria was still quite clear (Fig. 7C).

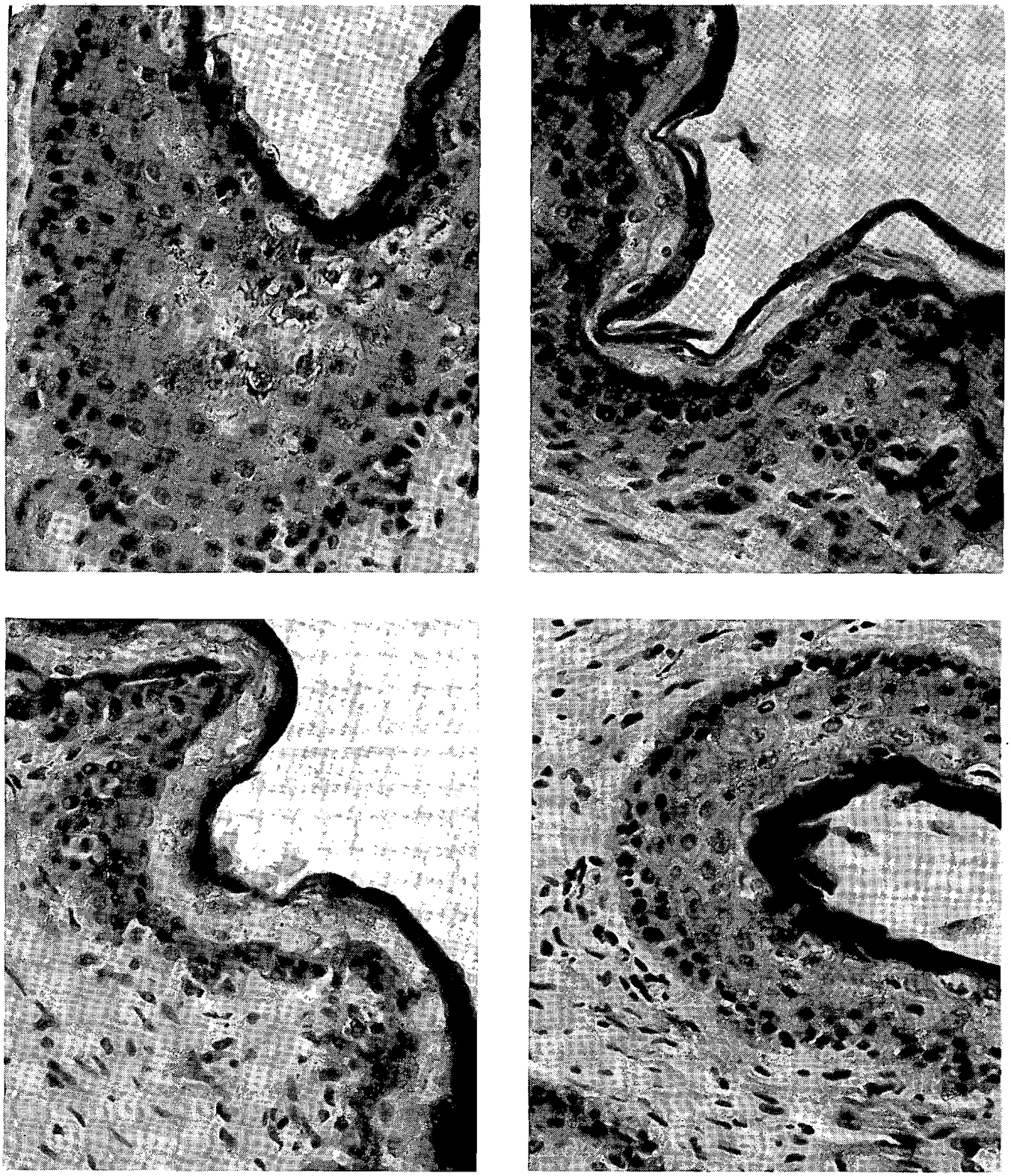

Fig. 7. Histological appearances of ruminal epithelium at time of removal from the *in vitro* system.
A. 0 Hour B. 4 Hours C. 8 Hours D. 14 Hours.

14 Hour: The cells of the epithelium presented hydropic degeneration to the depth of the basalar layer. There were keratinohyaline granules in these cells to this depth. In addition, there was a considerable increase in the size of the cells of the stratum germinativum. In focal areas the basalar columnar cells were undergoing pyknosis of the nuclei and vesiculation of the cytoplasm. Evidence of focal subepithelial oedema was also present and the collagen fibres of the lamina propria were forced aside by fluids. The collagen fibres were becoming indistinct at this stage (Fig. 7D).

DISCUSSION

The definition of the *in vitro* system by the variety of approaches used, fulfilled to a large extent the criteria for solute transfer across biological membranes proposed by Levine and Pelikan[17].

The ability of the ruminal epithelium to convert butyric acid to ketone bodies, first described by Pennington[22], proved to be a fairly useful measure of the viability of the biological membrane under the conditions of study. There was measurable enzymatic activity present up to 6 h, although about 80% of the activity disappeared within the first 2 to 3 hours. This demonstration of metabolic activity did not really represent a true reflection of the functional capacity and integrity of the cellular membranes within the epithelium. This is especially true as the site of B-hydroxybutyrate dehydrogenase activity has been localised in the mitochondria[13].

A better criterion might have been the ability of the membrane to transport sodium against an electrochemical gradient[8]. Stevens[31] showed that the tissue potential decreased with time and that this was due to a decreasing tissue current, which in turn was associated with a decrease in net sodium transport. This change occurred over about 7 h from the initial collection. Similar isolated ruminal epithelium systems have been used with success by a number of workers while investigating transport and metabolism of volatile fatty acids[12 32 33]. Thus by observation and inference, it seemed that the ruminal epithelial cells remained metabolically functional for about 6 h under the applied experimental conditions although there was a continual decrement of activity with the passage of time.

The measurement of the period of time during which the biological integrity of the ruminal epithelium could be regarded as being intact was carried out by evaluating the permeability coefficients for pentobarbital, antipyrine and tetraethylammonium. It was evident from these results that the permeability of the epithelium changed between 12 and 14 h. After 14 h the permeability coefficient increased gradually with time for the nonionized lipid-soluble compounds, pentobarbital and antipyrine, which would be expected to cross the epithelium by lipid diffusion.

A far greater increment occurred in the case of TEA, a relatively small (MM 130 daltons and molecular diameter 0,68nm) and highly polar molecule, which would be expected to traverse a lipoid-sieve barrier by diffusion through aqueous pores. It was concluded from these studies that the lipoproteinaceous architecture of the cell membranes became disrupted at about 12 h and that the structure of the aqueous channels seemed to degenerate somewhat faster than the lipid and protein components.

Additional evidence for loss of membrane integrity at about this same time interval was provided by the results of the studies on the permeability of ruminal epithelium to phenolsulphonphthalein. Phenolsulphonphthalein is a strong sulphonic acid with a pKa less than 1,0 and molecular mass of 354. This molecule is completely ionized at all physiological hydrogen ion concentrations. Phenol red has been shown to cross most biological membranes to a very limited extent and the percentage absorption from the gastrointestinal tract has varied between 0 and 2 per cent[4 5 19 21 24 25 26]. Austin[3] achieved quantitative recovery of phenol red from the reticulorumen of calves but Williams and Mackenzie[36], using acute preparations, found some absorption from the rumen in sheep. Utilizing our *in vitro* system we observed that the mean time for the first appearance of traces of phenol red was 16,7 h. This finding once again suggested an alteration in permeability due to architectural collapse of the membrane following which the diffusion of a relatively large polar molecule became possible.

The histological appearance of the ruminal epithelium initially conformed very closely with the descriptions of Dobson *et al.* [9]. Moreover, the pattern of cellular degeneration which occurred with the passage of time was anticipated[13]. It does seem that if the previous findings concerning the definition of the *in vitro* system are taken into account, the critical difference between the 8 hour and 14 hour specimen was that the progressive hydropic cellular degeneration finally involved the basalar layer of columnar cells during this time interval. This may represent the initiation of the degeneration of the most important area of the epithelium as regards its efficacy as a barrier. Studies of the ultrastructure of ruminal epithelium have revealed the presence of intercellular spaces throughout the epithelium and penetrating down to the basal cell borders[15 18]. This may be the explanation for the permeability alterations at about 12 h.

The results of the drug metabolism studies using both epithelial mince and rumen liquor revealed that no biotransformation of pentobarbital or antipyrine occurred. This finding validated the *in vitro* results obtained with the ^{14}C-labelled compounds. TEA has been shown not to be metabolized by any species or in any biological system[20].

The *in vitro* experimental system proved to be a very useful model for evaluating the transfer of drugs across the ruminal epithelium over periods of less than 12 h. Due to the simplicity of the system it is possible to define rigorously the variables which control the rate of transfer of solutes across this membrane. There are some rather obvious disadvantages to the system such as absence of blood flow and inability to control the actual surface area exposed to the solution due to varying degrees of papillation.

ACKNOWLEDGEMENTS

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Jl S.Afr. vet. Ass. 46(2) 161-163 1975

CASE REPORT GEVALVERSLAG

CHRONIC FLUORIDE POISONING IN SHEEP

I. ZUMPT*

SUMMARY

Chronic fluoride poisoning in sheep and cattle was diagnosed on a farm in the Vredenburg district, Cape Province, and confirmed by laboratory analysis. The source of the poisoning was pastures contaminated with rock phosphate dust containing 2,1 to 3,3% of fluoride emitted from a fertilizer factory.

Tabulated analyses of blood, liver, bone, pasture and water are given.

INTRODUCTION

During April 1972 the author was called to a farm in the Vredenburg district to investigate ill thrift in sheep. The farm consists of 380 hectares of poor coastal sandveld and is situated 4 km north-east of a superphosphate fertilizer factory. Sheep are kept on established mixed lucern-clover pastures and seasonally on harvested wheat land; a few dairy animals are kept for cream production.

For the last few years the farmer had complained that dust originating from the factory affected the growth of wheat and sheep. At 2 years of age his sheep showed severely worn incisor teeth, in some cases they were worn right down to gum level. Local farmers attributed this excessive wear to the mastication of contaminated plant material, assuming that the factory dust was abrasive.

CLINICAL EXAMINATION

The flock of 150 sheep was in poor to very poor condition with protruding hipbones and sharp vertebral columns. Young lambs were well developed and of strong build. Adult animals were cachectic and slightly anaemic, with poor muscular development. The incisors were pitted, lustrous, brown-gold in colour and showed irregular, severe wear with abnormal spaces between one another. The molar teeth were irregularly worn and had sharp protruding edges and corners. In all cases the upper Molar 1 was the longest and the lower Molar 1 the most worn-down of all (Fig. 1). Cattle also showed similar dental changes, but to a lesser degree.

AUTOPSY

Autopsy confirmed the dental changes described above. All incisor teeth were extremely loose in their sockets. Carcases were cachectic, slightly anaemic and pale in colour with no fat deposits. Light exostoses were observed on the lower jaw, nasal bones, costo-chondral junctions and femur. Longitudinal section of the long bones revealed a pale brown bone marrow with the extremities of the cavity filled with a snow-white lardy content. All bones were of a chalky-white colour.

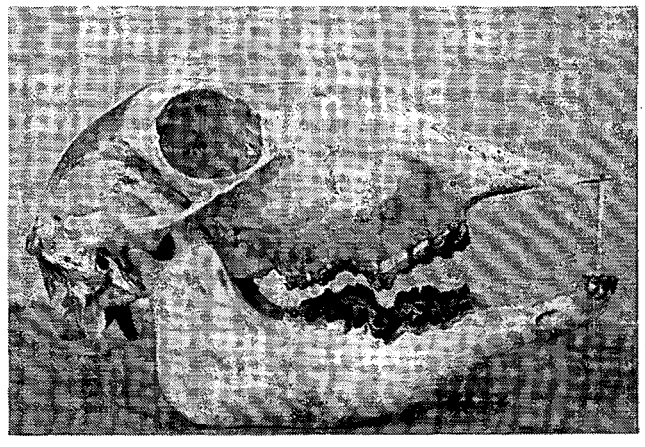

Fig. 1: Irregular wear and pigmentation of the teeth of a sheep.

METHODS AND RESULTS OF SPECIMEN ANALYSES

A variety of specimens was taken for laboratory examination. The following are the biochemical and other examinations used and the results obtained:-

(a) Blood: Blood from three adult sheep was collected in heparin for haematological examination and various biochemical tests. The results and methods used are reported in Table 1.

Methods used:

P:Varley, H. 1962. *Practical Clinical Biochemistry*. 3rd Ed. Method of Gomorri.

Mg and Ca:*Analytical Methods for Atomic Absorption Spectrophotometry* 1971. Perkin-Elmer, Norwalk, Connecticut, U.S.A.

Na and K:*Flame Photometric Methods*. Evans Eletroselenium L.T.D. Halstead, Essex, England.

BUN:*Urastrat: Urea Nitrogen Assay system. Paper chromatography. Warner-Chilcott* Laboratories.

TPP:King E.J. & Wootton 1964 *Micro-Analysis in Medical Biochemistry*. 4th Ed. Biuret method.

Haematocrit and White Cell Count:Benjamin M.M. 1961 *Outline of Veterinary Clinical Pathology*. 2nd Ed. Wintrobe method.

* Regional Veterinary Laboratory, Stellenbosch, 7600.

(a) **Blood:**

Table 1: BLOOD FROM THREE ADULT SHEEP WAS COLLECTED IN HEPARIN FOR HAEMATOLSGICAL EXAMINATION AND VARIOUS BROCHEMICAL TESTS. THE RESULTS AND METHODS USED ARE REPORTED IN TABLE 1.

		Blood Plasma						Whole Blood	
		mg/100 ml.					g/100 ml.	Haematocrit	White Cell count
Id. No.	P	Mg	Ca	Na	K	Bun	TPP	%	%
1.	3,8	2,0	8,6	307	17,0	20	7,7	36%	1%
2.	4,4	1,9	8,3	292	15,8	28	6,8	34%	1%
3.	5,5	1,9	8,3	304	17,0	25	7,2	35%	–

Methods used:

P:	Varley, H. 1962. Practical Clinical Biochemistry. 3rd Ed. Method of Gomorri
Mg and Ia:	Analytical Methods for Atomic Absorption Spectrophotometry 1971. Perkin-Elmer, Norwalk, Connecticut, U.S.A.
Na and K:	Flame Photometric Methods. Evans Eletroselenium L.T.D. Halstead, Essex, England.
Gluc.:	Gluco Strate™ General Diagnostics Division Warner-Chilcott Laboratories 1970.
BUN:	Urastrat: Urea Nitrogen Assay system. Paper chromatography. Warner-Chilcott Laboratories.
TPP:	King E.J. and Wootton 1964 Micro-Analysis in Medical Biochemistry. 4th Ed. Biuret method.
Haematocrit and White cell Count:	Benjamin M.M. 1961 Outline of Veterinary and White Cell Clinical Pathology. 2nd Ed. Wintrobe method. –

TABLE 2: LIVER SAMPLES FOR DETERMINATION OF THEIR NUNERAL CONTENT WERE COLLECTED FROM TWO SHEEP. THE RESULT AND METHODS USED ARE GIVEN IN TABLE 2.

	p.p.m. (on a dry matter basis)					
Indent. no.	Cu	Fe	Zn	Mn	Mg	K
1. Liver	10	426	92	9	540	543
2. Liver	12	512	93	10	545	590

1. Methods used as described in: *Analytical Methods for Atomic Absorption Spectrophotometry*. 1971. Perkin.-Elmer, Norwalk, Connecticut.
2. Ashing of sample: Wet ashing using HNO_3, H_2SO_4, $HC10_4$

(b) *Liver Analyses:* Liver samples for determination of their mineral content were collected from two sheep. The results and methods used are given in Table 2.

(c) *Bone Fluoride Analysis* (dry fat-free basis; specimens from one animal)
Femur: 3 320 p.p.m. Rib: 2 400 p.p.m.

(d) *Pasture Fluoride Analysis* (dry matter basis)
Representative specimens of young wheat from three fields were collected and evenly mixed. This sample contained 232 p.p.m. fluorides.

(e) *Water Fluoride Analysis:* Water samples from three drinking troughs were collected and analysed for fluoride content.
The details are given in Table 3.

Table 3

Water Trough	Source	Fluordie p.p.m.
1	Voëlvlei *	0,4
2	Voëlvlei *	0,4
3	Farm well	1,8

* Water piped from Voëlvlei dam (one of Cape Town's storage dams).

DISCUSSION

The results of the biochemical tests confirmed that the animal examined suffered from a chronic fluorosis and hypocuprosis.

There were no gross blood abnormalities, but blood calcium and the haematocrit tended to be low. A

definite hypocuprosis existed, and the bones contained abnormally high levels of fluorides.

The source of the fluorides was further investigated; the factory emitted a fine rock-phosphate dust containing between 2,1 to 3,3% of fluorides (regular analyses by the factory). This fine dust was clearly visible on the pastures over an elliptical area 4 km in length which covered the major part of the farm.

It was also found that the prevailing seawinds are broken and deflected by a chain of low hills. The affected farm lies in line with the factory and a gap in that chain. The only reasonable explanation is that wind which is not deflected causes the factory dust containing fluorides to be deposited onto the farm.

It is the general consensus of opinion that contamination of pastures with industrial fumes and dusts to an extent of 25 to 50 p.p.m. fluoride (on a dry matter basis) can be considered to be potentially dangerous to grazing stock[3]. Other sources[1] maintain that levels in excess of 100 p.p.m. are likely to cause disease. In this case the pasture sample contained 232 p.p.m. of fluoride. With few exceptions[1 3] plants do not absorb appreciable quantities of fluoride, so that this poisoning was probably caused by the rock-phosphate dust deposited onto the pasture.

Chronic fluorosis is caused by the ingestion of small amounts of fluoride over a long period resulting in the deposition of fluoride in association with phosphate in the bones, and in the teeth only in the formative stage before eruption[1].

The characteristic dental lesions are due to hypoplasia of the enamel, and the excessive mobilization of calcium and phosphates results in osteomalacia. The normal ivory colour of bones is changed to a chalky white, and signs of aplastic anaemia are noticed. The excessively irregular wear of teeth makes proper mastication impossible and animals grow poorly.

Maximum permissible fluoride values in feedstuffs are difficult to assess. Values of 2000 to 4000 p.p.m. (dry matter basis) are regarded as abnormally high, although values of 20 000 p.p.m. have been found[3].

The two values recorded in this investigation of 2400 and 3320 p.p.m. have to be regarded as high.

The sheep on this farm showed neither lameness, fractures, pain on movement, degenerative arthritis nor gross enlargement of joints[1 2 3]. The absence of these pathological changes indicated that the animals on this farm ingested only minute quantities of the toxic fluoride over their entire life span. It is unknown to what extent the low liver copper values can contribute to this condition. Similar observations have been reported from England[2].

The cattle showed less marked teeth changes. This was probably due to the fact that the bulk of their ration was purchased from elsewhere and only a limited time was spent on the contaminated pastures.

ACKNOWLEDGEMENTS

Professor T.F. Adelaar is thanked for his interest and help in compiling this report. Bone and pasture analyses were done by the late Mr. G. Truter .

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Jl S.Afr. vet. Ass. 46(2) 165-169 1975

CAT BITE TRANSMISSION OF *YERSINIA PESTIS* INFECTION TO MAN

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HAZEL D. BUBB****

SUMMARY

The transmission of bubonic plague from the kitten of a domestic cat to a man by means of a bite on a finger is described. The human case was complicated by the development of a secondary meningitis, followed, after specific therapy, by protracted recovery. The kitten showed swollen lymph nodes of the head and neck, frothing at the mouth and nostrils, and signs of an acute infectious disease which had a fatal termination. *Yersinia pestis* was isolated on about the 8th day from the cerebrospinal fluid of the man. The foster mother of the kitten exhibited signs of spinal and cerebral meningitis but recovered following treatment; her serum contained plague antibody levels of 1:512 and 1:1024 on the 22nd and 34th days respectively after the first evidence of illness. Three litter mates of the kitten also died. The outbreak occurred on a farm in the Graaff-Reinet district of the eastern Cape Province, which is situated about 160 km from the nearest known natural plague focus.

INTRODUCTION

Plague is a specific infectious disease caused by the plague bacillus, *Yersinia pestis* (formerly known as *Pasteurella pestis*). The illness in man takes the form of an acute fever which may be rapidly fatal before characteristic features appear or may cause swelling and suppuration of regional lymph nodes. These typical swellings usually result from infected flea bites and are called buboes, hence the common form of the disease is referred to as bubonic plague[9]. Transmission of bubonic plague to man by means other than the bites of infected fleas is less common. A small proportion of cases of bubonic plague may be traced to entry of the bacilli through abraded skin of the feet from infected floors, or of the hands in the performance of an autopsy, or in handling or skinning infected animals. Primary septicaemic plague may

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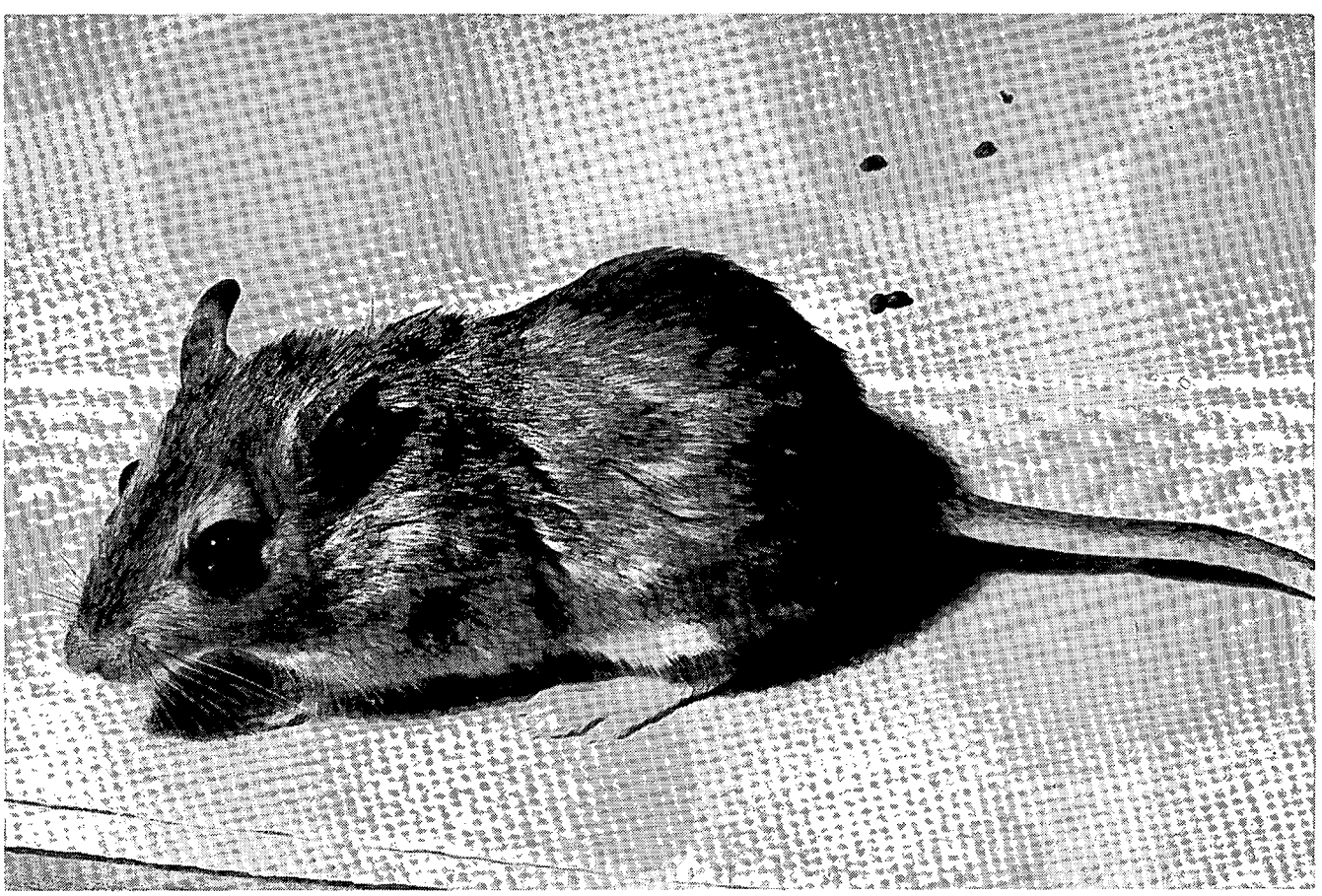

Fig. 1: The gerbil, *Tatera brantsii*, one of the small wild-living rodents responsible for the maintenance of natural plague foci in parts of South Africa.

Fig. 2: The gerbil, *Desmodillus auricularis* also plays an important role in the formation of permanent wild rodent reservoirs of infection in this country.

also result from the entry of the organisms through mucous membranes, especially those of the mouth, throat and conjunctiva[4]. Primary pneumonic plague occurs rarely and is transmitted directly from man to man by droplet infection. Plague is primarily a disease of rodents although several other mammals, including cats, are susceptible[2, 3], and is a typical zoonosis. In rodents it exists in two forms: wild rodent plague existing in nature independent of human populations and their activities; and domestic plague, intimately associated with man and the rodents living with man, with a definite potential for producing epidemics in man.

The WHO Expert Committee on Plague[11] recently emphasized that the permanent character of wild rodent plague in numerous natural foci in various parts of the world still requires the constant attention of health authorities. In the Republic of South Africa the Public Health Act 36 of 1919 and its regulations list plague as one of the six formidable infectious diseases which must be immediately reported, even if only suspected in man, rodents, cats, dogs or other animals. In a natural focus ecological conditions ensure the persistence of the aetiological agent for considerable periods of time, and epizootics and periods of quiescence alternate without the introduction of infection from outside. In natural foci the infection is maintained permanently by hosts, termed permanent reservoir hosts, which are relatively resistant. They pass the infection to less resistant animal hosts and cause epizootics (murine plague) which affect some domestic rodents and thus cause outbreaks of plague in man[10] particularly in times of famine or unusual meteorological conditions[1]. Pollitzer[7], however, contends that the presence of such reduced susceptibility of the host colony is a *sine qua non* for the continuation of infection.

Several known natural and temporary or probable plague foci exist in southern Africa[11]. In South Africa the gerbils *Tatera brantsii* and *Desmodillus auricularis* associated with the fleas *Xenopsylla philoxera* and *X. piriei* respectively form the permanent wild rodent reservoirs, although other species and subspecies of small mammals including rodents are at risk of infection and other species of fleas have to be regarded as actual or potential vectors[7]. Human infection in this country has in most cases not been directly derived from the wild rodents or through their fleas, but from the semi-domestic *Praomys natalensis* (the multimammate mouse) with a mixed flea fauna derived from wild and domestic sources and the domestic *Rattus rattus* (the black rat) which lives in close contact with man in his dwellings and its associated fleas acting as intermediaries between the primary gerbil reservoirs and man[7]. Plague first gained a foothold in South Africa in 1900 when large amounts of forage were imported from South America during the South African War, and Pollitzer[7] states that there is no doubt that the natural foci of infection became established here through a spread of infection from the commensal or domestic to the wild species of rodents during the present century.

It is the purpose of this paper to record the transmission of plague from a cat to a human by means of a bite.

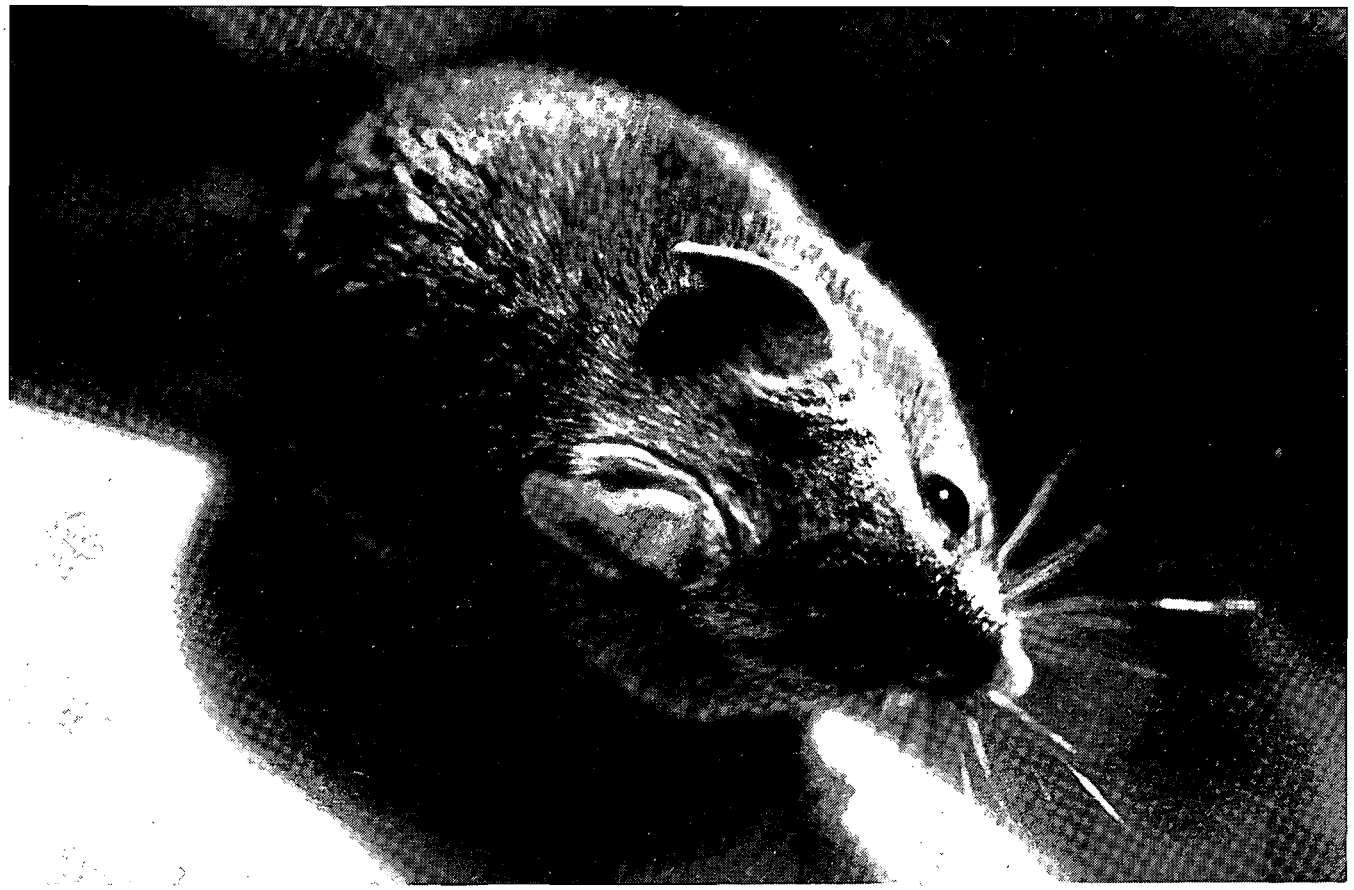

Fig. 3: The semi-domestic multimammate mouse, *Praomys natalensis* forms an important link in the transference of fleas and infection from wild rodents living in natural plague foci to "domestic" rodents and man.

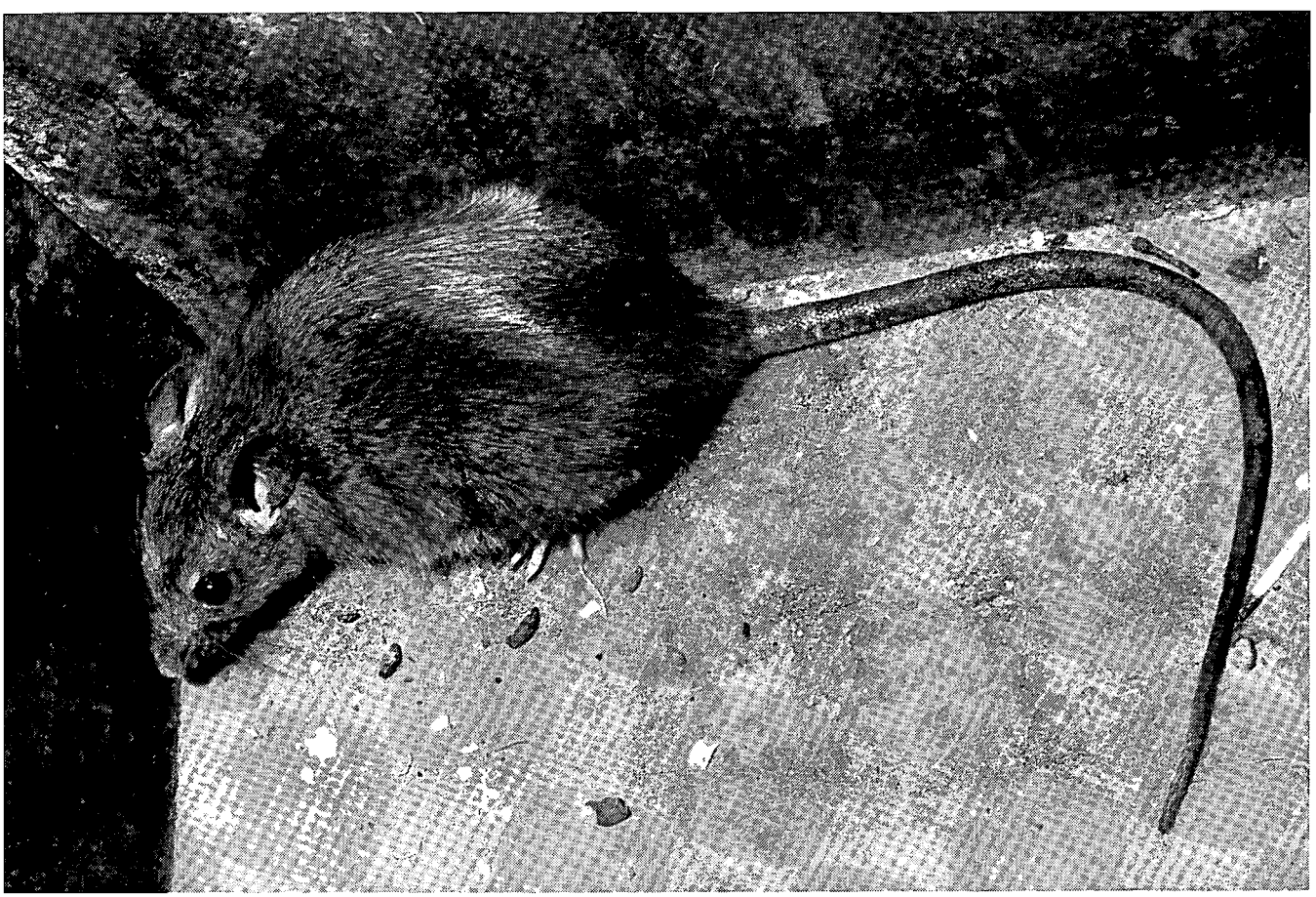

Fig. 4: The black rat, *Rattus rattus* lives in close contact with man in his dwellings.

CASE REPORT

On a farm situated in the Graaff-Reinet district of the eastern Cape Province a two-year-old female domestic cat was seen on approximately 9.11.72 to have a changed habitus; she was dull, inactive, refused to eat and for most of the day lay with her nose resting on the ground. This animal, subsequently named "Pestie", was at that time suckling her own kitten of about 4 weeks of age and had partly adopted four other slightly older kittens from another cat which had also partly adopted Pestie's offspring, i.e., two lactating females were feeding five kittens between them. During the next 2 days the clinical signs shown by Pestie had progressed to a quadriplegia and although she remained inappetent until evidence of recovery on the sixth day of illness was noticed when she appeared brighter and ate some food, she continued to suckle the kittens during the whole period.

On 11.11.72 the four older kittens were noticed to be ill. Two of these died 4 days later and the other two on the 7th day of the disease but none of these sick kittens was examined by us. The farmer and his wife reported that apart from the more general signs associated with an acute infectious disease such as malaise and inappetence, the head and upper part of the neck of the kittens appeared swollen. This appearance may have been the result of enlarged regional lymph nodes. The two kittens which died first had haemorrhaged from the mouth and nose, while the other two which had suffered from the more protracted disease showed frothing from the mouth and nostrils from which strings of mucous were suspended for several days before death. Pestie's own kitten and the other adult cat did not contract the disease. The cats had the run of the house and slept in the kitchen until a week before human illness. Thereafter they slept on the veranda. Of four other cats belonging to labourers on the farm one died during this period.

Veterinary advice was sought and given telephonically soon after Pestie was noticed to be ill. The owners feared that she and the sick kittens might have been suffering from rabies and wished to know whether they could be treated with sulphadiazine administered orally in tablet form. Pestie and the sick kittens were thereafter treated daily with this sulphonamide; in addition some erythromycin syrup was given. It was during the process of administration of a sulphonamide tablet to one of the kittens which had shown frothing from the mouth and nostrils, that the left thumb of the 58 years old farmer sustained superficial laceration from the animal's teeth. The wound healed within a few days. The farmer was bitten on 11.11.72 and examined by one of us (B.J. Te W.N.P.) on 20.11.72 after he had been ill for 3 to 4 days, i.e., the length of the incubation period was somewhere between 3 and 7 days. The condition was characterized by sudden onset with few prodromal signs and symptoms which were of short duration. The latter consisted of malaise, headache, nausea and painful left axillary lymphadenopathy. On the day of the initial examination the bite wound had healed and apart from a fever of 39,5°C the most prominent clinical sign was the presence of a painful swelling about the size of a hen's egg in the anterior region of the left axilla which was unlike that of a secondary lymphadenitis following a wound infection in that it was surrounded by extensive oedema. Treatment with tetracycline was prescribed and instituted but during the course of the following few days the patient manifested signs of mental confusion.

After hospitalization a lumbar puncture was performed on 25.11.72 and a specimen of the cerebrospinal fluid was taken for pathological and bacteriological examination. *Yersinia pestis* was isolated from this fluid. More specific antibiotic and other treatment was instituted and the patient made a very slow and protracted recovery during the course of the following 8-10 weeks[5].

In order to determine the source of the infection Pestie was clinically examined by one of us (D.J.T.) on 1.12.72 and 5 ml blood was taken for serological and bacteriological examination. Apart from being weak and in poor condition she appeared healthy on this day, and her temperature was within normal limits. Serologically, however, she had a titre of 1:512 for plague antibodies but the bacteriological culture was negative. A throat swab taken a few days later also proved negative when examined for the presence of *Y. pestis*. A second serological examination on 12.12.72 revealed a rise in plague antibody titre to 1:1024. She was not bled again and this may or may not have been the final titre.

Once a definite diagnosis of plague had been established all the necessary public health measures, including rodent control, were carried out on the farm by the appropriate authorities. Pestie was placed in isolation and was treated with streptomycin administered intramuscularly while the other cats and the dogs on the farm were regularly dusted with insecticidal powder to control flea infestation. No further animal or human cases were diagnosed on the farm or in its vicinity.

DISCUSSION

Pollitzer[7] discusses in some detail the role played by a variety of animals as hosts of infection to the plague bacillus and quotes the experience of many authors in this respect. He states that while cats may suffer from plague under natural conditions it has been debated whether they can contract infection by feeding, and whether their infection occurs frequently enough to be of importance for the spread of the disease. Various authors are quoted who had reported that under natural conditions cats could contract infection by feeding only if their buccal or intestinal mucosa was traumatized by bone fragments. Cats were also not believed, in general, to be very susceptible to experimental infection with *Y. pestis*, although in various South American countries high mortalities have been noted in cats during plague outbreaks in humans. Unequivocal proof that these animals were suffering from *Y. pestis* infection, however, seems to be lacking. In Argentina instances were often noted where the presence of plague in cats seemed to be responsible for human cases[7].

It is of interest to note that during the "Black Death" of London in 1665 when the recorded human deaths from plague in the city and suburbs exceeded 7000 per week, and the total plague mortality for the year was about 20% of the population, one of the measures promulgated by the Lord Mayor and

Aldermen of the City of London to prevent spread of infection was that "the streets are to be kept clean and no hogs, dogs or cats, or tame pigeons, or conies (rabbits) be suffered to be kept within any part of the city"[9]. Recently Sloan considered that the decision to destroy the dogs and cats was an ill-judged measure and probably led to an increase in the rat population[9]. In view of the apparent lack of resistance of at least five of eight cats described in this paper (if it may be assumed that they had all suffered from plague though there is little doubt that at least two of them were infected) as well as the recent studies by Rust, Cavanaugh, O'Shita and Marshall[8] who have shown that cats and dogs are quite susceptible to experimental plague infection, one wonders whether the edict was indeed ill-judged at that time. Moreover, cats, like dogs, are apt to be dangerous as far as the transmission of plague is concerned as they may bring not only plague — infected rodent fleas into the houses[7] but some have the habit of bringing their actual prey caught elsewhere into dwellings (no doubt diseased rats are easier to capture). While cats are useful in keeping dwellings free from mice their value for purposes of rat control has been questioned[7]. Both the cat and the dog fleas (*Ctenocephalides felis* and *C. canis* respectively) may carry the plague bacillus although they have proved rather inefficient plague vectors under experimental conditions. They occur not only on their specific hosts but also to some extent on other species of mammals including rodents and are prone to attack man[7].

In view of the persistence of infection in natural plague foci in southern Africa in which diverse biotic and abiotic factors condition the ecology of local rodent reservoirs and flea vectors and cause distinct seasonal appearances of epizootic plague, sporadic human cases will continue to occur.

The farm on which the present isolated outbreak occurred lies approximately 160 km from the nearest natural focus of plague although the disease was shown in 1925/6 to have spread among spring hares (*Pedetes capensis*) to within 48 km east of Graaff-Reinet[6]. It was not determined how the cats in the small localized outbreak under review initially contracted the infection but various possibilities do exist. One is that the disease was contracted from wild rodents although no unusual outbreaks of mortality had been observed in these at the time. The Rodent Control Group, however, were able to determine that there had been mortalities from 6 months to a year previously. The Namaqua gerbil (*D. auricularis*) and the semi-domestic multimammate mouse *P. natalensis* as well as *Otomys (Myotomys) unisulcatus* (karoo rat), *Rhabdomys pumilio* (striped mouse) and *Mus musculus* were trapped on the farm shortly after plague was diagnosed. Wild rodent nests were found very near the farm house. It is possible that the disease could have been present in wild form in the district and remained undetected since the early 1900's although no infection of fleas or rodents was found on the farm. Another possibility is that infected rodents could have been introduced onto the farm in 1970 when a quantity of maize for sheep feed was imported from the Orange Free State, parts of which are enzootic areas. A third possibility is that wild carnivores, *e.g.* red lynx, and rodents may have migrated into the area carrying the infection with them.

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We thank Prof. L.W. van den Heever, Faculty of Veterinary Science, University of Pretoria and Dr. Margaretha Isaäcson, South African Institute for Medical Research, Johannesburg for revising the manuscript, Dr. G.J. Krynauw and Mr. P.J. van der Merwe of the State Health Services, Port Elizabeth for providing some of the epizootiological data, and the Directors of the Medical Ecology Centre, Johannesburg and S.A. Institute for Medical Research, Johannesburg for supplying the photographs.

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Jl S.Afr. vet. Ass. 46(2) 171-179 1975

CHLAMYDIOSIS IN A BEEF HERD*

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AND M.M. HENTON***

SUMMARY

Chlamydial infection in a large beef herd is illustrated and discussed. The pre-natal and post-natal losses that occurred during 1972 are highlighted. Total pre-natal losses for the nine calving herds comprising 2 915 animals varied between 3,7% and 12,4%. Between 1,2% and 11,4% of the calves born alive in the nine herds died before weaning with 70% of the losses occurring within the first three weeks of life. Chlamydial organisms were demonstrated in pre-natal and post-natal losses from all nine herds. Clinical manifestations and lesions involving the intestinal, respiratory, nervous, skeletal, reticulo-endothelial and urinary systems were observed in chlamydia-infected calves. Invariably at autopsy in chlamydia positive cases there was some degree of a fibrinous inflammatory process present. Serological evidence showed that chlamydial organisms had been present on both farms prior to the 1972 investigations.

INTRODUCTION

Chlamydial infections of cattle have been recognised as a cause of abortions, intestinal infection, encephalomyelitis, polyarthritis, pneumonia, conjunctivitis, orchitis and a seminal vesiculitis syndrome.

Sporadic bovine encephalomylitis (SBE), the first recognised chlamydia-induced disease of cattle, was described by McNutt and Waller[15], and in 1953 Wenner and co-workers[33] clearly showed the causative agent to be a chlamydial organism. The disease is seen primarily in cattle less than three years of age. Whereas the morbidity is low, the mortality rate is high. The dominant feature in the genesis of SBE is a generalised chlamydial infection. The primary injurious effect of the chlamydial agent is on the blood vessels throughout the body and the lesions in various organs are associated with the vascular damage. The brain is only one target organ, but the brain lesions, which usually are evenly distributed, lead to the nervous symptoms that dominate the clinical picture of the disease.

Storz *et al.* [31] were the first to isolate the causal agent of epizootic bovine abortion (EBA). The disease affects both beef and dairy cattle and has been reported as being more widespread and severe among the former. The abortion rate can be as high as 60%[31] or even 75%[14], particularly in herds containing a high proportion of first calf heifers. However, in areas where the disease occurs for the first time cattle of all ages are susceptible[14].

The foetuses are usually aborted during the last trimester of pregnancy although in some outbreaks pregnancy continues to full term and the affected calves may be stillborn or born weak and die later[25 11].

The onset of an outbreak of EBA in a herd is sudden. No clinical illness, except an occasional febrile reaction is observed in cows that subsequently abort.

The presence of chlamydial agents in faeces of apparently normal calves was detected by York and Baker[38]. Subsequently chlamydial strains have been isolated from the faeces of apparently normal calves in various countries[25 35]. Omori *et al.*[18] and Popvici[19], however, isolated chlamydiae from the faeces of claves with mild pneumonic or pneumo-enteric symptoms and Storz and associates[28] recovered chlamydial agents from calves as young as two days of age suffering from diarrhoea. Experiments were conducted by Storz *et al.*[27] and Eugster[5] using colostrum-deprived and colostrum-treated calves inoculated with chlamydial strains of differing virulence. The watery, mucoid and bloody diarrhoea observed by them led to dehydration and frequently to the death of the affected calves. Information on the relationship of bovine age to intestinal chlamydial infection is limited, but a predominance of detectable infection in younger animals seems to exist[25]. A perfectly balanced host-parasite relationship must be the basis for the clinically quiescent intestinal chlamydial infection in ruminants. A shift in favour of the infectious agent may result in some of the clinical manifestations of overt chlamydia-induced disease of cattle and sheep. Storz[25] further reports that ewes experiencing chlamydial abortions and lambs and calves with chlamydial polyarthritis also had intestinal infections.

Experimentally infected calves continued to shed chlamydiae in the faeces for over 6 months and naturally infected sheep excreted the agent intermittently over several years[25]. According to Storz the continuation of the infectious chain must be tightly linked to the infectious faecal excretions and infection appears to be either by inhalation of ingestion [25].

Littlejohn *et al.*[13] identified chlamydial agents as causing transmissible serositis and Storz *et al.*[29] described a polyarthritic disease in young calves that was caused by chlamydial agents.

A systemic infection evidently precedes infection of the joint tissues in the pathogenesis of chlamydial polyarthritis and the spontaneous disease picture can readily be reproduced experimentally in calves or lambs by oral, systemic or intra-articular routes[25].

Bovine chlamydial pneumonia was first recognised in Japan in 1952[18]. Calves as well as older cattle are susceptible and the clinical symptoms and lesions de-

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pend on the degree of involvement[34]. In the following paper the implication of chlamydial agents in a large beef herd is discussed with the emphasis being on the pre-natal and post-natal losses that occurred during the 1971 breeding and 1972 calving seasons.

HERD HISTORY

The herd under review is the Johannesburg City Council's crossbred beef herd which is run on two large sewage effluent disposal farms. Farm 1 is 40 km north and Farm 2 is 25 km south of Johannesburg. The herd size as at October 1972 is given in Table 1. Both farms are fairly intensive and the cattle on each farm are run on four distinct sections — each section falling under a trained supervisor. Accurate records are kept of all deaths, stillbirths and observed abortions and these are all submitted for post mortem examination.

TABLE 1: TOTAL CATTLE POPULATION – OCTOBER 1972

Farm 1	3 577
Farm 2	4 620
Total:	8 197

On Farm 1 the heifers were bred from July, 1971 until the end of August and the cows from the beginning of September through to the end of November. On Farm 2 the breeding seasons started 1 month later.

After the breeding seasons 1 295 cows and heifers on Farm 1 and 1 620 cows and heifers on Farm 2 were diagnosed as being in calf, all breeding with the exception of some heifers on Farm 2 having been done by artificial insemination. These heifers and cows were run as four separate herds on Farm 1 and as five separate herds on Farm 2.

A. PRE-NATAL LOSSES

The break-down of the pre-natal losses for the individual herds and farms for the 1972 calving seasons is given in Table 2. Total pre-natal losses in terms of diagnosed pregnancies amount to 7,5% and 8,4% for Farms 1 and 2 respectively with the total observed losses (i.e. the observed abortions and stillbirths) being 6,1% and 6,4%

B. POST-NATAL LOSSES

In Table 3 the number of calves born alive in the various herds during the 1972 calving season is given together with the percentage deaths that occurred from birth until weaning at 210 days. The age groups of calf deaths are illustrated in Figure 1.

Fig. 1: Age Grouping of Calf Deaths

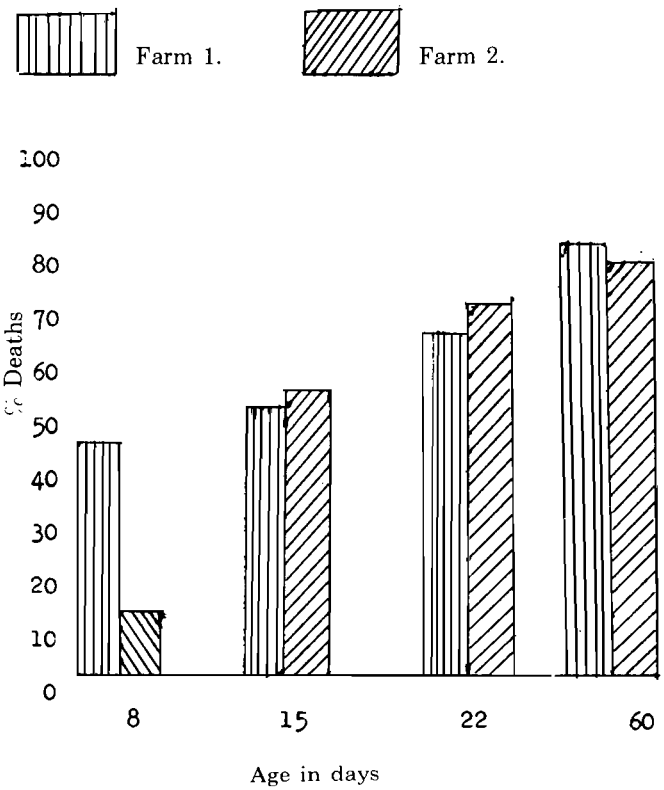

Table 2: PRE-NATAL LOSSES FOR VARIOUS HERDS AND FARMS FOR THE 1972 CALVING SEASON

Farm	Section	Herd	No.	Total	% Abortions	% Stillborn	% "No Record Cows"*	Total Pre-natal Loss
1	A	Cows	1	428	3,3	3,5	2,1	8,9
	B	"	2	508	2,2	3,2	0,8	6,2
	C	"	3	62	3,2	3,2	1,6	8,0
		Heifers	4	297	2,0	4,0	1,4	7,4
	Total			1 295				
	Average				2,6	3,5	1,4	7,5
2	M	Cows	5	472	2,5	4,2	3,6	10,3
	R	"	6	303	2,3	2,6	2,0	6,9
		Heifers	7	177	1,1	7,3	4,0	12,4
	S	"	8	298	3,7	6,0	0,3	10,0
		Cows	9	370	0,8	2,4	0,5	3,7
	Total			1 620				
	Average				2,2	4,2	2,0	8,4

* Diagnosed in calf but having no recorded live, stillborn or aborted calf.

The highest incidence of scours and calf deaths occurred amongst calves from herd 9 on section 'S' of Farm 2. During previous years this section consistently had the lowest scour and death rates of both farms. The other herd which calved down on section 'S' namely the heifer herd No. 8 had been bred on another section for a restricted 6 weeks period immediately prior to the cow breeding season. The cow herd had been bred entirely by artificial insemination whereas this was supplemented to a limited extent by natural service in the heifer herd.

Table 3: TOTAL NUMBER OF CALVES BORN ALIVE AND LOSSES ON BOTH FARMS DURING THE 1972 CALVING SEASON

Farm	Section	Herd	Herd No.	Total Calves Born	Total Calf Deaths	% Calf Deaths
1	A	Cows	1	380	14	3,7
	B	"	2	476	8	1,7
	C	"	3	57	2	3,5
		Heifers	4	274	7	2,6
	Totals			1 187	31	
	Average %					2,6
2	M	Cows	5	423	5	1,2
	R	"	6	284	23	8,1
	R	Heifers	7	155	11	7,1
	S	Heifers	8	272	10	3,7
	S	Cows	9	361	41	11,4
	Totals			1 495	90	
	Average %					6,0

The pre-natal and post-natal losses for these two herds are given in terms of diagnosed pregnancies in Table 4. The problems of calf disease and loss became progressively worse as the calving season progressed. As can be seen 3,4% of the calves born to the heifer herd and 11,1% of the calves in the cow herd died.

Table 4: PRE-NATAL AND POST-NATAL LOSSES WHICH OCCURRED DURING 1972 ON FARM 2, SECTION "S"

Total	Herd	% Abortions	% Stillbirths	% No Record Cows	% Calf deaths *	Total losses *
298 Heifers	8	3,7	6,0	0,3	3,4	13,4
370 Cows	9	0,8	2,4	0,5	11,1	14,8

* Expressed in terms of diagnosed pregnancies

As the problem became more apparent in the cow herd certain steps were taken. These included division of the herd into three groups according to calving sequence, the lessening of stress factors and the use of a broad spectrum antibiotic in an attempt at block therapy in the new-born calf. The scours incidence, age grouping of affected calves and percentage deaths in the four herds are given in Table 5.

The heifer herd in which the relatively high scours incidence developed slowly, gave no cause for alarm. However, the disease problem soon became evident in the cow herd and after approximately the first month of calving the still pregnant cows were removed from the 1 hectare overnight camp where calving had begun and placed in a nearby camp of 5 hectares in extent. Despite this and vigorous treatment* of affected calves the disease incidence worsened in herd B. Eighty-two per cent of calves had started scouring before they were two weeks old and 45% scoured at least twice after showing varying periods of remission. The age incidence was highly suggestive of colibacillosis involvement and numerous faeces swabs were submitted for bacteriological examination. The antimicrobial sensitivity pattern of the *Escherichia coli*

*1. Kalftabs, Milborrow & Co. (Pty) Ltd.
2. Neftin, A.S. Ruffel (Pty) Ltd.

Table 5: THE SCOURS INCIDENCE AND CALF MORTALITY RATE ON SECTION "S" FARM 2.

Herd	Total calves born	% Calves which first scoured aged (days) 8	15	22	Total % scours	% Repeat scours	% Deaths
Heifer	272	3	24,4	44,4	50,7	3,0	3,7
Cow A	162	10,5	59,3	71,6	74,1	16,7	11,7
B	108	35,2	82,4	85,2	85,2	45,4	18,5
C	91	6,6	30,8	34,1	34,1	6,6	2,2

isolates was determined and cognisance taken hereof in the choice of antidiarrhoeal drugs.

After a further month of calving herd C was started by moving the still pregnant cows to a new large veld camp adjacent to the pastures. The previous two calving camps had been far removed from the pastures and the very young calves had been held back whilst their dams went out to graze. Due to the proximity of the pastures to the camp used for herd C contact between dam and calf was possible at all times.

The number of calves that had died in the other herds in the first 3 weeks of life showing advanced joint lesions suggested very early it not intra-uterine chlamydial infection. It was therefore decided to use antibiotic block therapy in all the calves born in herd C in an attempt to reduce the apparently asymptomatic chlamydaemia. To this end Proterciclene† was administered intramuscularly at the rate of 8 mg/kg on day 1 and 2 of life. Of further significance in this herd is that the calving incidence was considerably slower than in either herds A or B.

C. POST-WEANER LOSSES

During the past few years a number of cases of chronic lymphocytic interstitial nephritis has been observed in young stock (aged between weaning and 16 months). Six of these animals have either died or been destroyed and have shown a complete bilateral chronic lymphocytic interstitial nephritis. In addition slaughter for examination has revealed a noteworhty incidence of renal involvement ranging from small focal areas of lymphocytic infiltration to pronounced chronic lymphocytic interstitial nephritis. The incidence has been higher in feedlot animals from Farm l and out of a random examination of 116 animals over 33% showed lesions of varying severity. On both farms kidneys from 215 animals were examined of which 55 showed lesions, *i.e.* 25,6%.

The more chronic involvement there was the more likely it was that clinical evidence had been noted. Invariably the most marked signs were loss of weight and general unthriftiness. In the few kidney function tests done the B.U.N. values appeared normal. In all cases the livers of the affected animals were normal except for the odd lesion of fascioliasis. Numerous attempts at establishing the nephritis aetiology, aimed primarily at leptospiral or feed involvement, have proved fruitless.

MATERIAL AND METHODS

All recovered aborted foetuses, stillborn calves and calves which died after birth were submitted for post mortem examination. Selected specimens from these pre-natal and post-natal losses, as well as from live animals were submitted for laboratory examination. These specimens comprised brain, lung, liver, mesenteric lymph nodes, placenta, faeces and serum samples. Although numerous possible aetiological agents were investigated the emphasis was on *E. coli* and *Chlamydia psittaci* involvement.

In order to demonstrate the presence of chlamydial elementary bodies impression smears were prepared from foetal and calf lungs, spleen, kidney and several cotyledons. These were heat fixed and stained by the Gimenez[8] and modified Ziehl-Neelsen[24] methods.

For the isolation of chlamydia organisms the methods described previously by Rake[20], and by Storz and McKercher[30] were used throughout with slight modifications. Foetal or calf lungs, kidney, spleen and liver material were pooled, homogenised in Ten Broeck grinders and diluted with Bovarnick buffer[3] to which vancomycin[10] (100 mg/ml) and streptomycin[25] (100 mg/ml) were added. Tenfold dilutions were made and 0,2 ml was inoculated into 7 day embryonated eggs. These were incubated at 37°C and candled daily. Yolk sacs from embryos which died between the 4th and the 11th day as well as those which were still alive on day 12 were harvested and impression smears made and stained according to the Gimenez[8] method.

Faecal swabs, intestinal content, foetal and calf brain, liver and spleen were streaked onto blood-tryptose-agar and McConkeys agar plates, incubated at 37°C and examined after 24 h for the presence of organisms belonging to the Enterobacteriaceae. *E. coli* isolates were typed by the slide agglutination technique using antisera prepared from all known *E. coli* O and K serogroups.

The possible presence of Brucella organisms was investigated by making cultures from foetal stomach content, cotyledon and lung tissue on blood-tryptose-agar containing 600 units polymyxin B sulphate, 2 500 units of bacitracin and 10 mg cyclohexamide per 100 ml of media[36]. Cultures were incubated at 37°C in 10% CO_2 for up to 6 days.

For the isolation of *Vibrio fetus* organisms a small quantity of material from foetal liver, lung and stomach content as well as placental tissue was plated onto brilliant green-cystine-heart-agar and thioglycolate-blood-agar plates[6 23]. These were incubated at 37°C in McIntosh jars from which $\frac{2}{3}$ of the atmosphere had been evacuated and replaced by a gaseous mixture of 95% nitrogen and 5% CO_2. Plates were examined for growth after 3 and 7 days. The same procedure was followed for the isolation of mycoplasmas except for the fact that media advocated by Chalquest[4 16] and Sheppard[22] were used.

For the isolation of other pathogenic bacteria cultures were made from the same material on blood-tryptose-agar and incubated aerobically and under anaerobic conditions at 37°C.

When suitable material was received this was also processed for the presence of viral agents*. The presence of bluetongue, Rift Valley fever and mucosal disease viruses was investigated. During the latter phase of study cryostat sections of duodenum, ileum and colon from calves were also examined by immunofluoresence [17] for the presence of corona and reo viruses.

In an attempt to throw some light on the epizootiology of the disease outbreak blood was collected from selected cow and calf groups and the chlamydial antibody levels determined[26 7] and statistically analysed**.

The animals, all from section S, were bled 2 months after the last calf was born. Thirty paired dam and

† Meds. (Chloramphenicol, Succinate of Rolitetracycline). MEDS Veterinary Laboratory (Pty) Ltd.

* These examinations were done by Drs B.J. Erasmus and B.J.H. Barnard of the Dept. of Virology and Dr. A. Theodoridis of the Dept. of Reproduction, Veterinary Research Institute, Onderstepoort.

** Statistical analyses done by K.C.S. Sandrock, Forward planning Branch, City Engineers Department, Johannesburg.

calf samples from the heifer herd, 30 paired dam and calf samples from the cow herd 'B' where the calf had a history of "repeat" scouring, 30 paired dam and calf samples from the cow herd 'C' where the calf had no history of scours and finally 30 from cows whose calves had died were taken.

After the presence of the organism had been established serum samples that had been collected in 1971 for another study and stored were examined for chlamydial antibodies. The sera consisted of paired dam and calf samples taken within 5 days of partus from animals from all three calving sections on Farm 1 and from section 'S' on Farm 2.

All the above serum samples were also checked for leptospiral antibodies by using the micro-agglutination technique and for Brucella agglutinating and complement fixing antibodies [36].

RESULTS

A. PRE-NATAL LOSSES

At no time during the gestation period were any indications of systemic disease recorded amongst cows or heifers that subsequently aborted. The same applies to those which had stillborn calves. Some cows had retained placentas but the majority went through an uncomplicated *puerperium* period.

In Tables 6 and 7 the results of the post mortal material which was examined for chlamydial organisms and Enterobacteriaceae is summarised. The foetal age from which material was submitted ranged from 190 days to full term.

Chlamydial organisms were demonstrated in calves emanating from all nine calving herds. Of interest is that 7 of the 15 positive demonstrations from the 38 stillborn calves submitted came from calves where death appeared due to straightforward dystocia. During the same season 19 placenta specimens from dams that gave birth to apparently normal, viable calves were examined. Eleven of these were positive for chlamydia on smear examination. All these calves survived to weaning.

The post mortal picture of chlamydia positive abortions varied from a relatively negative one to the more common one where there was an abundance of clear pleural and peritoneal fluid together with subcutaneous blood-tinged oedema. Many of the foetuses aborted near term and the stillborn calves showed lesions that had apparently resulted from difficult parturition. Hepatopathy as described by Storz[25] was invariably absent. Some livers appeared swollen but the mottled appearance and coarsely nodular surface described were not a feature. Nor was there gross lymph node enlargement.

B. POST-NATAL LOSSES

1. Clinical observations

1.1 Calves: The predominant picture was that of diarrhoea. In herd 6 and particularly in herd 9 the scours incidence was extremely high with many affected calves recovering. Many of the calves that died showed apparently irreversible, persistent scours. Others had two, three and rarely four scouring attacks interspersed by periods of apparent recovery. Many of

Table 6: TOTAL MATERIAL SUBMITTED FOR LABORATORY INVESTIGATIONS

	Farm 1					
	Abortions			Stillbirths		
Specimen	Complete	Organ/s	% of Total	Complete	Organ/s	% of Total
No.	11	1	36,4	5	7	26,7
Chlamydia identification	9(3⁎)	1	30,3	2 (1 dystocia)	2	8,9
% positive of specimens examined	83%			33%		
	Farm 2					
	Abortions			Stillbirths		
Specimen	Complete	Organ/s	% of Total	Complete	Organ/s	% of Total
No.	6	2	23	3	23	38,2
Chamydia identification	6(2⁎)	1	20	3(6 dystocias)	8	16,2
% positive of specimens examined	88%			42%		

⁎ Chlamydia organisms isolated.
Other demonstrations via direct smear examination.

Table 7: SPECIMENS SUBMITTED AND EXAMINED FOR ENTEROBACTERIACEAE

		E. coli isolates		
Specimen	n ( )⁎	Pathogenic	Non-pathogenic	Salmonella isolates
Faecal swab	76	13	14	–
Liver	15(3)	(1)	5(1)	–
Spleen	7	–	2	–
Brain	5(2)	(1)	2	–
Intestinal content	2	1	1	–

⁎ Totals given in brackets were specimens from foetal material. All other specimens were from post-natal losses.

these calves in fact recovered completely clinically. The faeces of affected calves varied from a watery, mucoid to a doughy consistency with the colour ranging from clear to white to a wide range of yellow and green combinations.

Five of the 31 calves that died on Farm 1 and 13 of the 90 from Farm 2 showed central nervous system involvement that ranged from difficulty in maintaining balance, circling movements, wild running and falling, apparent non-recognition of surroundings, apparent blindness, high stepping gait to a progressive paralysis which in the two calves thus affected started in the hindquarters. Invariably these calves had scoured before eliciting nervous symptoms. In some cases the calf had made an apparent recovery from the attack of enteritis, in others the scours was still persistent at the time that central nervous system involvement was observed. The shortest interval from onset of scouring to nervous signs was 2 days. Two of the five calves from Farm 1 and eight of the 13 from Farm 2 were between 10 and 19 days old when they died. Two further calves from herd 9 on Farm 2, showed mild involvement of the central nervous system following on enteritis and then recovered.

Although joint lesions in the form of fibrin flakes and plaques were an almost constant finding in chlamydia positive cases few calves showed any clinical signs of joint involvement. Only one calf showed evidence of acute polyarthritis. This calf from herd 9 scoured when 6 days old for a period of 6 days and when 26 days old suddenly developed acute polyarthritis. Two other calves developed chronic arthritis which necessitated slaughter. From specimens of these joints, chlamydial organisms were isolated.

In four chlamydia positive carcases the only recorded history was that of a weak calf following either a normal or a dystocia birth. Four apparently healthy calves were simply found dead and one positive calf showed marked respiratory distress. Of interest is one calf that was born weak with an extensive alopecia of the head and loin area that survived for 26 days and on post mortem showed typical joint lesions plus a chronic lymphocytic interstitial nephritis. Two other calves that died aged 3 and 4 months also showed a chronic lymphocytic interstitial nephritis on autopsy.

Calves born with permanently contracted tendons have been recorded on both farms for a number of years as has the odd case of hydranencephaly. However, during 1973 a frightening increase in hydranencephaly cases has been noted on Farm 1 of the nine cases one had arthrogryposis as well. Noteworthy is the isolation of a chlamydial agent from the brain of one of the calves born on Farm 1 during the 1973 calving season with hydranencephaly. Hydranencephaly has also been noted in animals that have lived for a number of months and in fact in two animals that survived to 7½ and 8 months.

1.2 Other Stock: During 1972 two first-calf heifers on Farm 1 developed nervous signs following calving with one of them showing, in addition, a severe metritis and kerato-conjunctivitis.

Chlamydial elementary bodies were demonstrated in lung and uterus smears of another first-calf heifer that died after having developed a metritis and vulvovaginitis following parturient trauma. This animal came from the same herd as the above two as did another first-calver which developed chronic arthritis following calving. However, no attempt at chlamydial demonstration was made from this or the other odd cow that developed a chronic arthritis particularly of the hip joint.

Kerato-conjunctivitis amongst the young stock especially on Farm 2 is very prevalent at certain times of the year. No laboratory attempts at establishing the aetiology have, however, been done. In addition the odd young animal with symptoms suspicious of SBE has been seen. However, paired serum samples of one of these failed to show a rising chlamydial complement fixation titre.

2. Laboratory Results: Data pertaining to the *E. coli* and salmonella investigations are summarised in Table 7. Forty *E. coli* isolates (16 of which were classified as pathogenic strains) were made from the specimens examined. In contrast, no samonella organisms could be isolated.

Table 8 reflects the calf specimens submitted together with the chlamydia identifications. As in the case of the losses that occurred during the pre-natal period chlamydial organisms were demonstrated in calves from all nine herds.

3. Serology: Tables 9 and 10 reflect the chlamydial antibody levels of the respective cow and calf groups bled during 1972. Comparison of the actual and expected antibody level frequencies of the four cow groups showed similar frequencies of occurrence except that the heifer sample showed a slightly higher than expected number of low level titres (< 1:8).

Table 8: SPECIMENS SUBMITTED AND EXAMINED FOR *CHLAMYDIA PSITTACI*

	Farm 1			Farm 2		
Specimen	Entire calf	Organ/s	% of Total **	Entire calf	Organ/s	% of Total **
No.	4	10	45	17	33	55,5
Chlamydia indentification	4(1*)	4	25,8	9	12(1*)	23,3
% positive of specimens	57				42	

* Chlamydia organisms isolated

** % of total number of calves that died that were examined

However, the heifer herd calf group (Table 10) showed a significantly higher incidence in the high antibody level class interval (> 1:8) than either of the other two calf groups. No correlation was found between the specific dam and calf antibody levels.

DISCUSSION

The demonstration of diagnostically significant antibody levels in cows and calves bled on both farms in 1971 indicates the previous presence of chlamydial

Table 9: CHLAMYDIAL ANTIBODY LEVELS OF COW GROUPS FROM SECTION 'S', FARM 2, 1972

Antibody levels	Heifers	Cows/calves scoured	Cows/no calf scours	Cows/calves died
1:8	10	3	5	4
1:8 to 1:15	5	4	3	8
1:16 to 1:23	5	5	5	1
1:24 to 1:39	6	10	8	8
1:40 and above	4	7	7	8
n*	30	29	28	29

* number of determinations possible. 30 cows per group were bled.

Forty-nine of the 65 cow and 56 of the 66 calf serum samples collected during 1971 showed chlamydial complement fixing antibody titres higher than 1:16. Levels higher than 1:85 were demonstrated in cow and calf samples from all four sections.

No agglutinating antibodies to the seven commonly occurring leptospiral species in South Africa could be demonstrated. All serum samples also proved to be negative for brucella agglutinating and complement fixing antibodies.

organisms. That these organisms were present prior to the investigations of 1972 is further indicated by the occurrence of similar disease entities and loss during previous years. The low abortion losses in particular being indicative of an enzootic condition.

From examination of Table 2 it is seen that the total pre-natal losses for the nine herds varied between 3,7 and 12,4%. For the purpose of discussion let us assume that the majority of "no record cows" in fact either resorbed or aborted their foetuses although it is

Table 10: CHLAMYDIAL ANTIBODY LEVELS OF THREE CALF GROUPS FROM SECTION 'S', FARM 2, 1972

Antibody levels	Calf Groups		
	Heifer herd	Scours	No scours
1:8	10	27	23
1:8 to 1:15	7	1	1
1:16 to 1:23	2	1	–
1:24 to 1:39	7	–	–
1:40 and above	1	–	–
n*	27	29	24

* number of determinations possible. 30 calves per group were bled.

4. Post mortem examination: The gross lesions observed in the chlamydia positive calves varied. Essentially negative findings, except for catarrhal enteritis and dehydration accompanied by various degrees of serofibrinous synovitis and tendovaginitis occurred. Pulmonary involvement was evident as slight oedema and congestion and sometimes as pneumonitis. A fibrinous pleuritis and pericarditis, meningitis, hepatomegaly, general lymphadenopathy and gastrointestinal involvement varying from mild to severe haemorrhagic, sometimes pseudomembranous, enteritis were less commonly seen. In odd cases, abomasal ulceration associated with petechial haemorrhages, marked congestion and petechial and ecchymotic haemorrhages in the brain and a focal intersitial lymphocytic nephritis were observed. The above lesions were found in varying degrees and varying combinations. However, invariably in positive cases some indication of a fibrinous inflammatory process was found somewhere in the carcase. This most frequently occurred in the joints. The hock joints invariably were affected.

accepted that incorrect pregnancy diagnosis and incorrect recording at the time of pregnancy examination, although unlikely, could have contributed to their numbers.

Examination of the abortion and stillborn losses reveals them to be within apparently acceptable limits. During a chlamydial abortion storm, foetal losses can be extremely high and during an outbreak of EBA have been recorded to range from 25 to 75%. Arthur[1], Rasbeck[21] and Van Dieten[32] give the average incidence of stillbirths as ranging between 5 and 7%. The highest percentage of stillbirths under discussion occurred in the heifer herds and were primarily due to relative foetal oversize in an immature dam. Furthermore comparison of losses with previous years showed no significant deviations.

The conclusion may be drawn that chlamydial organisms had in all probability been present on the farms for some considerable time.

Whereas the abortion losses showed a more or less constant pattern the calf losses and scours incidence showed interesting farm and sectional variations

when comparing various seasons and years. Stress, particularly climatic stress, seemed to play a significant role in the varying pattern seen. The advancing of the calving season on Farm 1 to avoid calving during the latter half of September, October and beginning of November coincided with a dramatic drop in scours incidence and calf loss. Whereas heat stress appeared of primary significance on Farm 1 it is interesting to note that the section most exposed to prevailing wind, namely section 'R' on Farm 2, regularly recorded the highest incidence of pulmonary involvement. According to Storz[25] the epizootology of chlamydial pneumonia in sheep and cattle seems to be closely interdependent with clinically inapparent intestinal chlamydial infection. It is interesting to note that section 'R' consistently had a high scours incidence and that the post mortem of dead calves very often revealed a pneumo-enteric picture. Furthermore Storz states that is it is ecologically significant that active intestinal chlamydial infection does not induce resistance to superinfection with the homologous or other chlamydial strains.

A perfectly balanced host-parasite relationship must be the basis for the clinically quiescent intestinal chlamydial infection seen in ruminants and a shift in favour of the infectious agent may result in some of the clinical manifestations of overt chlamydia-induced diseases of cattle and sheep. In young calves with primary, chlamydia-induced enteritis, dramatic shifts in the bacterial ecology of different intestinal parts have been observed[25].

According to the same author virulence is a most important factor in the pathogenic potential of chlamydial agents and the genesis of some chlamydial induced diseases may depend on the agents degree of virulence than on other factors. The potential clinical manifestations of chlamydial infection is evidently dependent on environmental and/or physiological conditions. Although the intestinal tract appears to be a natural habitat for chlamydial infections in many instances of specific chlamydia-induced diseases of ruminants such as abortion, encephalitis, polyarthritis or pneumonia, it is not known whether intestinal infections also existed. It is of interest, in this connection, that the different diseases of ruminants caused by chlamydial agents never have been observed simultaneously in a given herd of animals or geographic region. Chlamydia-induced abortions and polyarthritis have, however, been observed in the same flocks of sheep but during different seasons.

In the above discussions of chlamydial involvement on Farms 1 and 2 intestinal infection would appear to have played a significant role in the varying clinical and post mortal manifestations seen.

The chlamydial infection appears to have been complicated by *E. coli* as evidenced by the various pathogenic and non-pathogenic serotypes isolated. However, because of the varying pattern found it was concluded that colibacillosis was not of primary significance. No viral pathogens were demonstrated. However, this aspect needs further intensive investigation because of the known association which exists between chlamydia and other pathogens.

The demonstration of chlamydial organisms in the placentas of cows giving birth to strong viable calves and in stillborn dystocia calves from Farms 1 and 2 provides further evidence of quiescent genital chlamydial infection. Further evidence of large scale trans-uterine infection of calves appeared to be the fibrinoid joint lesions seen in young calves. It was on this basis that antibiotic blocking therapy was practised. Various workers have tested chemotherapeutic agents in attempted prevention of chlamydial abortions[9 25]. These drugs were shown to be necessary at sufficient concentration to prevent chlamydaemia thus preventing placental and foetal infection. It is feasible that the antibiotic therapy did help decrease chlamydia concentrations in the new-born calves if infection was in fact present. It is not known to what extent this measure, or the other measures adopted to decrease stress factors, contributed to the significantly lower scour and death rate of the involved calf herd.

The association of chronic lymphocytic nephritis lesions with a chlamydial infection may be pure conjecture. However, as stated above, this condition has been seen in conjunction with typical joint lesions. Furthermore, the odd case of renal focal lymphocytic infiltration was noted in some naturally infected animals [12] as well as in some experimentally infected calves [37].

Hydranenecephaly and arthrogryposis are two congenital abnormalities of new-born calves that have been reported as occurring in outbreak proportions in the same herds as the same time[2]. The isolation of a chlamydial agent from the neural tissue of an hydranencephaly calf may be significant.

According to Storz[25] serological methods are of limited value in the study of intestinal chlamydial infections of ruminants and an interpretation of the above results would therefore appear to be of doubtful significance. It is however, interesting to note that far higher antibody levels were found in all the cow groups than in the calf groups. Thirty three per cent of the first-calf heifers and over 53% of the cows in the three other groups had levels of 1:24 and higher. Taking the diagnostic limit at 1:8 only one positive calf in the no-scour group, only two in the group that had showed typical scours and 17 from the heifer herd were found. Whereas none of the three calves in the first two groups had a level higher than 1:16, ten in the heifer herd did. As stated earlier the calves born to the heifer herd were born in the 6 weeks preceeding the first cow herd calves and were therefore on the average 3 to 4 months older than the calves born to herd C. This age difference may have been of significance in the various calf groups complement-fixing antibody levels despite the youngest calf being 2 months old. Storz[25] found that young calves did not form complement-fixing antibodies following oral inoculation until they were 45 to 51 days of age.

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BOOK REVIEW

BOEKRESENSIE

VERGLEICHENDE DARSTELLUNG DES LYMPHGEFÄSSYSTEMS DER SÄUGETIERE

H. GRAU

Supplement 19 to Zentralblatt fur Veterinärmedizin. Paul Parey, Berlin, 1974. Price R10.

This is a valuable monograph. The author briefly summarizes the available knowledge on the topography of the mammalian lymphatic system and discusses the relevant literature. The lymph centres and vessels of the domesticated animals, a number of laboratory animals and a few primates are described and reference is made to the situation in man. The illustrations are sketchy but helpful. An attempt is made to use a uniform terminology applicable to all mammals and the directives laid down in N.A.V. are followed. In doing so the author is probably under the illusion that those directives are not prescriptive to himself. Nevertheless, he provides a sound basis for comparative anatomical usage. The value of this monograph lies firstly in the fact that the available knowledge on the lymphatic system of laboratory animals is compiled — this information is scattered in the literature and not available in the standard textbooks on veterinary anatomy — and secondly, the compilation of the vast amount of literature on the subject. The publication should find a place in every institute concerned with mammalian anatomy and pathology.

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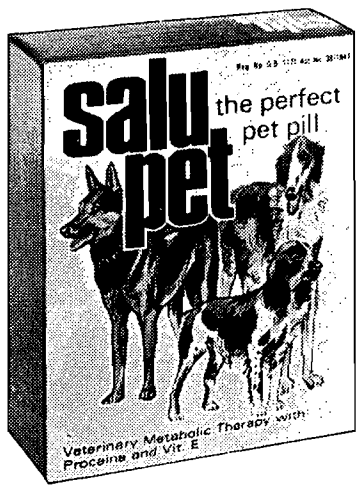

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SOME IMPORTANT PARASITIC AND OTHER DISEASES OF LION, *PANTHERA LEO*, IN THE KRUGER NATIONAL PARK

E. YOUNG

SUMMARY

Lions in the Kruger Park have been found affected by a variety of parasitic and other disease conditions, including trichinosis, filariasis, sarcoptic mange, pentastomiasis, echinococcosis, taeniasis, hepatozoonosis, anthrax, babesiosis and rickets. Six of these may be directly or indirectly transmissible to man or are, at least, closely related to human parasites or disease. Nine of these diseases, or closely related conditions, are also known to infect domestic animal species. The opposite may, of course, also be true — man and his domestic animals may, under certain circumstances, transmit certain diseases to lions, presumably sometimes with fatal results.

INTRODUCTION

It is not the purpose of this paper to provide a complete description of each disease or to revise appropriate literature. It is merely intended to represent a very brief account of the more important diseases of lions in the Kruger Park, with special reference to the known pathogenic effects of these on the lion itself, as well as their suspected significance to man and his domestic animals. Each of these diseases are dealt with very briefly under appropriate headings:

TRICHINOSIS

Trichinella spiralis is a well known and important parasite of man and many animal species. Heavy infestations may lead to death in man but the effects of this parasite on animals seem to be variable.

Trichinosis was diagnosed for the first time in South Africa in 1966, near the Lower Sabie tourist camp in the Kruger Park. The victim was a paralysed lion[10]. A paralysed civet cat *Viverra civetta,* has subsequently also been found infested with *T. spiralis.* In the Kruger Park the spotted hyena, *Crocuta crocuta,* seems to be a very important subclinical host of this parasite - around the Skukuza tourist camp about 85% of them are harbouring *T. spiralis* in encysted form in their musculature[10].

The more recent diagnosis of trichinosis in a warthog, *Phacochoerus aethiopicus*[3], is of considerable practical significance as partly cooked, infested warthog meat may serve as a possible source of human infestation. In East Africa, mortalities in man have already been caused by the ingestion of infested meat from bushpig, *Potamochoerus porcus*[4]. Human trichinosis has, to the best of my knowledge, not yet been recorded in South Africa. Although the distribution of *T. spiralis* in this country is restricted to the Kruger Park, illegal poaching and killing of warthog and wild carnivores can never be absolutely effectively controlled. The prescription of animal organs and products especially those of hyenas, by witch-doctors to their 'patients', within the infested region and elsewhere, may present at least one possible way of transmitting the infestation to man.

FILARIASIS

A post mortem examination on a paraplegic lion revealed an unspecified *Dirofilaria* nematode in its spinal column. No other possible cause of its condition could be found and it was concluded that migratory parasites, such as this one, may sometimes be responsible for this and other relevant nervous symptoms.

Dirofilaria sudanensis is a common parasite of lions in the Kruger Park. Like the above-mentioned specimen they can also attain lengths of about 20 cm and longer. These nematodes can be found anywhere in the subcutis or musculature of the lion but are usually most obvious in the subcutaneous tissues over the thorax behind the shoulder.

SARCOPTIC MANGE

Sarcoptes scabiei represents another parasite with an extremely wide host range. Man and most of his domestic animals are susceptible and may develop skin lesions. In the Kruger Park buffalo, *Syncerus caffer,* blue wildebeest, *Connochaetes taurinus* and impala, *Aepyceros melampus,* represent some of the more common hosts. Springbuck *Antidorcas marsupialis.* and red hartebeest, *Alcelaphus buselaphus,* in the Kalahari are sometimes also severely affected. Severe infestations may result in mortalities. This is particularly true in the case of young lion cubs — in one year one pride of lion in the Kruger Park is known to have lost all of the cubs due to a particularly severe outbreak of sarcoptic mange. This and other forms of mange in wild animals — including notoedric mange in cheetahs — generally respond very well to treatment[9 14 15].

A recent outbreak of mange in sable antelope, *Hippotragus niger,* in the Timbavati Game Reserve, adjoining the Kruger Park, might have originated from infested humans. Labourers on this farm had to receive treatment for sarcoptic dermatitis (J.H. Botha, *pers. comm.*), just prior to the development of a rather severe form of dermatitis in these antelope.

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PENTASTOMIASIS (LINGUATULIASIS)

In the family Linguatilidae, the adult, tongue-shaped parasites are usually found in the nasal and respiratory passages of carnivores, including the domestic dog. In several parts of the Kruger Park up to 100% of the older lions are infested with *Linguatula serrata*. This parasite may be responsible for necrotic rhinitis in affected lions. *L. nuttalli* (A. Fain, *pers. comm.*) has recently also been collected from a lion in the Kruger Park. Eggs of linguatulid parasites are passed out with the nasal secretions or in the faeces of their final hosts to infest herbivorous intermediate hosts. The nymhs of *L. serrata* have been recorded in cattle in South Africa[16]. In the Kruger Park the incidence of infestation is very high in buffalo (60%-70%)[11] and blue wildebeest (70% - 80%)[13], suggesting extremely severe infestation of pastures and drinking places by lions. The final host (*e.g.* lion) acquires the infestation by eating the intermediate host harbouring the immature parasites.

Man can become infested with the immature, as well as the adult forms of *L. serrata*[16]. The necessary precautions should, therefore, be taken when handling possibly infested carnivores, as well as in the preparation of meat and edible organs from possibly infested wild and domestic herbivores.

ECHINOCOCCOSIS

In South Africa the domestic dog acts as the most important source of human infestation. Lions in the Kruger Park are also commonly infested with the adult form of *Echinococcus granulosus felidis* which is remarkably host specific and, like its definitive host, is restricted to the Transvaal. Severe infestations are sometimes associated with diarrhoea, emaciation and debilitation, especially in young and very old lions.

E.g. felidis has not yet been recorded from domestic livestock[8]. Experimental research work in the Kruger Park revealed that the Burchell's zebra represents a suitable intermediate host for these parasites of lions. The incidence of echinococcosis in zebra of the Kruger Park is about 60%.

Although no conclusive evidence of human hydatidosis, due to contact with wild animals, could be traced in the literature, one should always be aware of the seriousness of human *Echinococcus* infestation and take the necessary precautions when handling wild carnivores.

TAENIASIS

Many of the lions in the Kruger Park are infested with tape worms (*Taenia* spp.) including *T. bubesei*. Severely infested individuals may develop enteritis and diarrhoea. Various wild herbivores act as intermediate hosts for the immature parasites (cysticerci) of the *Taenia* spp. of different carnivorous hosts. About 30% of all impala and buffalo and more than 70% of the blue wildebeest in the Kruger Park are infested with cysticercosis[11] [12] [13]. These parasites obviously complete their life cycles when their intermediate herbivorous hosts are captured and devoured by suitable definitive carnivorous hosts.

It is commonly known that man becomes infested with the tape worms *T. saginata* or *T. solium* when ingesting raw or deficiently cooked beef or pork respectively. Stcll[7] in 1947 estimated that about 38,9 million people in the world were infested with *T. saginata* at the time when he made his estimations. It could not yet have been established without doubt whether game meat can also serve as a source of human tape worm infestation. Available evidence suggests that this is highly improbable with regard to most of the common meat producing antelope.

HEPATOZOONOSIS

Unspecified *Hepatozoon* spp. have been recovered from various animal species in the Kruger Park, including the lion, spotted hyena and cheetah, *Acinonyx jubatus*. The organisms are usually found in the walls of capillaries within the myocardium, lungs and skeletal muscles[2]. The significance of this parasite to its wild host species is not at all clear. Hepatozoonosis has, however, already been associated with clinical disease in spotted hyenas and it is suspected that hepatozoonosis may contribute to the seasonal mortalities in hyenas and some other carnivores during late winter and early spring in the Skukuza region of the Kruger Park.

ANTHRAX

Of the carnivora which fed on carcasses of herbivores which had died of anthrax in the Kruger Park, fatalities occurred among lions, cheetahs, civets, leopards (*Panthera pardus*), black backed jackals (*Canis mesomelas*) and genet cats (*Genetta* spp.). Hyenas and wild dogs *Lycaon pictus*, were unaffected [5].

Anthrax is also a well known disease of man and other primates. In the Kruger Park a natural case of anthrax was diagnosed in a vervet monkey, *Cercopithecus aethiops*, while human workers also contracted the disease after the ingestion of infected game meat.

RICKETS

Malnutrition and rickets are among the natural causes of mortality especially in lion cubs. Rickets, as such, is more commonly encountered in young cheetahs, wild dogs and spotted hyenas. Being of relative unimportance as a natural mortality factor in the other species, rickets can, under certain circumstances, exert a significant decimating effect on free living cheetahs. Captive specimens are particularly prone to rickets but these can be effectively treated with mineral-vitamin supplements[9]. It has been proved experimentally that lion cubs will develop rickets when fed on deboned meat alone or when they are withheld from direct sunlight. Advanced cases may develop signs of hypocalcaemic tetany. Such extreme cases have not yet been encountered in nature.

Other Diseases, Parasites and Mortality Factors

Lions in the Kruger Park have been found to be host to not less than five infectious diseases and 18 different species of parasites. Information on most of these infections is rather incomplete.

In addition to the above mentioned disease entities, babesiosis[1] and a suspicious case of sarcosporidiosis (P.A. Basson, *pers. comm.*) have also been diagnosed in lions of the Kruger Park. Ectoparasites also include *Rhipicephalus appendiculatus, R. sanquineus, R. evertsi, R. simus, Amblyomma hebraeum, Haemaphysalis leachi,* and *Hyalomma truncatum. Physaloptera malayensis, Ancylostoma tubaeforme,* a *Pseudophyllidea* sp. and a *Cylicospirura* sp. have also been recovered from lions in this wildlife sanctuary.

Under extreme conditons a very low percentage of all lion cubs in the Kruger Park and elsewhere in Africa reach the age of one year. Mortalities in older lions are also quite high. Much more intensive research is required to establish the relative importance of some of the above-mentioned and other mortality factors. More intensive experimental work is also required to study the pathogenicity, pathogenesis and epizootiological significance of some of the mentioned parasitic conditions which may be transmissible from lions to man, his domestic animals and other wild animal species and *vice versa*. The National Parks Board is already supporting research by the Division of Veterinary Services on the latter group of parasitic diseases and more detailed reports on these zoonoses will be published in the near future.

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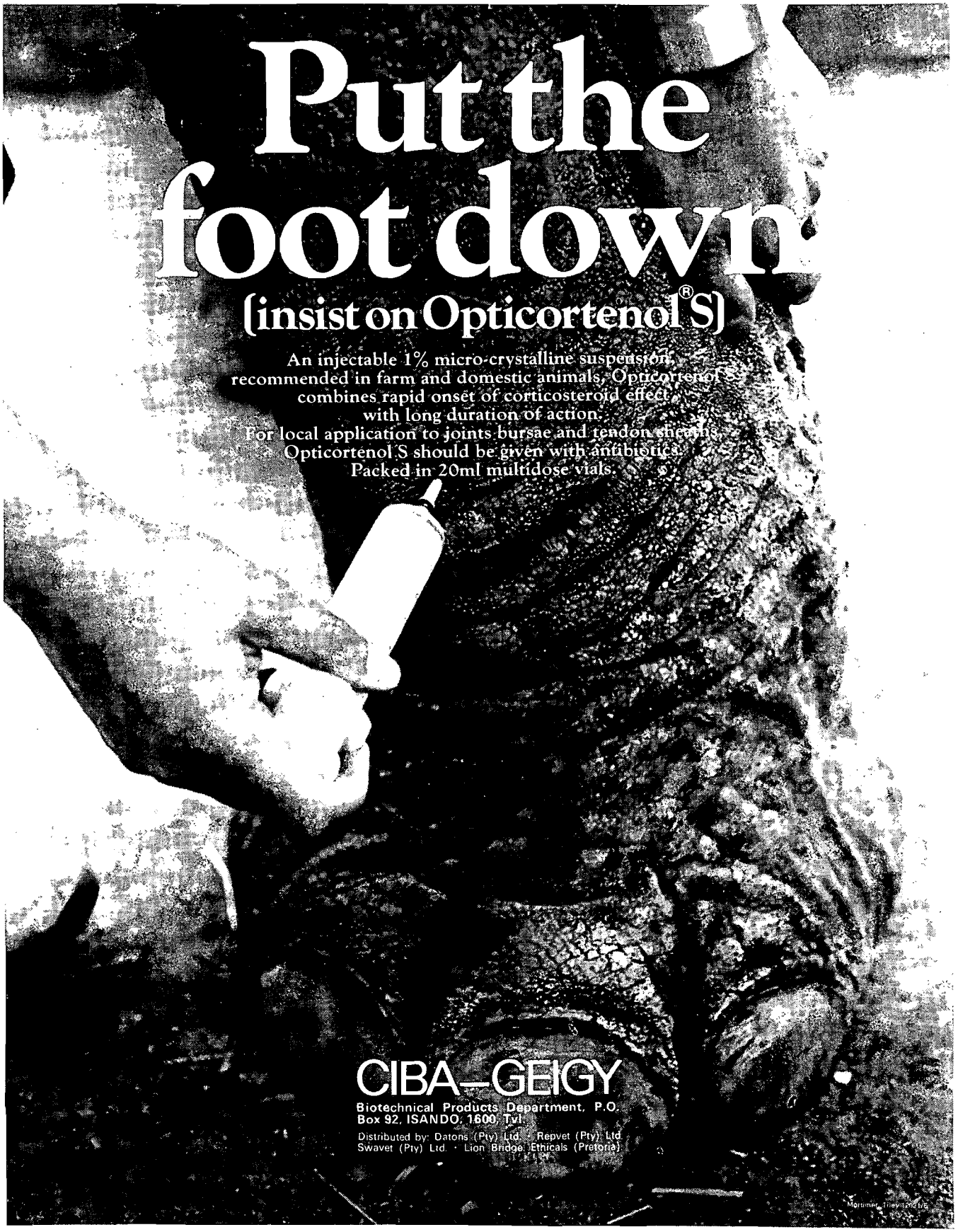

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HAEMOGLOBIN TYPES IN AFRICAN CATTLE

D.R. OSTERHOFF*

SUMMARY

The migration of domesticated animals in Africa is of particular interest to the anthropologist and ethnologist in that it may provide valuable information concerning the migration of human tribes that accompanied these animals.

Haemoglobin C is only found to be present in indigenous breeds of African cattle and can therefore be used as one of the best genetic markers of these animals.

Haemoglobin B has been established in many breeds in Africa and India. It seems that the further away the animals migrated from India, the lower is the frequency of HbB.

Breed comparisons from numerous breeds in Africa are made, including the results of the present investigations on breeds of different countries in Southern Africa.

INTRODUCTION

The migration of different cattle types into the African continent and the relationship between these animals has always been of great interest to human biologists and scientists[4]. The choice of yardsticks in the evaluation of these relationships has been changed from body measurements and photographs to genetical markers such as blood groups and genetical polymorphisms in protein and enzyme types[10].

Since most of the studies have been performed on the haemoglobin types in African cattle[1 8 11 14 16] it was felt that the first survey of cattle breeds and types in Africa could best be performed by studying the haemoglobin types of these animals. The study is by no means complete and it is necessary to collect blood samples from additional breeds and types of cattle to determine not only the haemoglobin types but also study the biochemical polymorphism in the other protein and enzyme types.

Haemoglobin, the oxygen-carrying component of the blood, consists of complex molecules consisting of a protein part, the globin and the effective haeme part. The haeme part comprising only 4% of the haemoglobin molecule contains four iron atoms which are able to bind loosely with four molecules of oxygen. While the haeme part of the haemoglobin is relatively constant, the globin part, a combination of two sets of polypeptide chains and comprising 96% of the haemoglobin molecule varies considerably from species to species and also within a species.

In cattle, Cabannes & Serain[5] were the first to report two electrophoretically distinct components in Algerian cattle. It was pointed out by these workers that the presence of the second haemoglobin was not associated with disease. After the initiation of the starch gel electrophoresis, a third component, called haemoglobin C and a fourth component, haemoglobin D were found[3 6 9 12].

In the investigations before 1964/65 the Allele HbC was not identified because different techniques were used and even in the first starch electropherograms the C band was overlooked. In these investigations, however, the haemoglobin B was always established as correct, while haemoglobin C formed part of haemoglobin A. This could also be confirmed in a study of the Malagasy Zebu[13].

MATERIAL AND METHODS

Blood samples were collected from different types of breeds of animals in Southern Africa: from Rhodesia samples were obtained from the Nkone, Tuli and Mashona, and from Mozambique samples were obtained from the "Landim", a native name for unimproved, indigenous cattle of mixed origin. In Angola cattle were bled in three different regions, Cafa Cunene, Malanje and Quilenques; from the Veterinary Department in South West Africa samples were obtained from Ovambo, Sango and Caprivi cattle. Lesotho, Malawi and Malagasy Zebu cattle could be included, while in South Africa samples were obtained from the Nguni and imported Brahman, the latter for reasons of comparison.

All blood specimens were collected from adult animals to avoid misclassification due to foetal haemoglobin[10]. Starch gel electrophoresis was used throughout, the techniques being described in earlier papers[11 15]. Clearcut separation of the different migration bands was always obtained.

RESULTS

In Table 1 the haemoglobin gene frequencies are presented from the material investigated in this study together with the gene frequencies from earlier investigations[11 12] of blood of cattle of the Afrikaner, Drakensberger, Bonsmara and Ankole breeds.

For the first time haemoglobin D with the slowest migrating molecular structure was found in cattle from Malawi, expressed in heterozygous form as HbA/HbD in two animals and as HbC/HbD in one animal.

DISCUSSION AND CONCLUSIONS

In an attempts to compare the results obtained with those of other authors one has to clarify the problem of correct determination of the haemoglobin types. In

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Table 1: HAEMOGLOBIN GENE FREQUENCIES IN INDIGENOUS CATTLE BREEDS IN SOUTHERN AFRICA.

Breed	No. of samples	Gene frequencies			
		Hb^A	Hb^B	Hb^C	Hb^D
Malagasy Zebu	199	,354	,623	,023	
Brahman	155	,577	,400	,023	
Tuli	199	,580	,399	,021	
Ankole	70	,721	,279	,000*	
Malawi-Zebu (North)	57	,623	,255	,096	,026
Angola-Malanje	134	,682	,235	,083	
Angola – Quilenques	90	,738	,184	,078	
Ovambo	200	,748	,150	,102	
Malawi -Zebu (Central)	55	,700	,140	,160	
Sango	248	,732	,141	,127	
"Landim"	172	,756	,130	,114	
Angola-Cunene	140	,801	,119	,080	
Mashona	124	,866	,117	,017	
Caprivi-Sanga	97	,876	,114	,010	
Nguni	300	,883	,064	,053	
Afrikaner	1 428	,819	,064	,117	
Lesotho-Nguni	100	,910	,060	,030	
Nkone	156	,929	,049	,022	
Drakensberger	27	,833	,037	,130	
Bonsmara	78	,962	,013	,025	

* Investigated before 1965, see text.

the earlier investigations paper electrophoresis or the cellulose acetate technique was used which could not give clear separation of the slow migrating A- and the somewhat faster moving C-band. All results given in Table 1 except those of the Ankole cattle were obtained with the improved starch gel electrophoresis technique[12]. Two examples of these typing difficulties will be given to illustrate the fact that haemoglobin C was typed incorrectly as haemoglcbin A in the earlier investigations.

Different haemoglobin investigations performed on Afrikaner cattle are compared in Table 2.

Table 2: COMPARISON OF SEVERAL STUDIES ON AFRIKANER CATTLE.

No. of samples	Gene frequencies			Author — Year
	Hb^A	Hb^C	Hb^B	
165	,852	,084	,064	Osterhoff & van Heerden 1965[12]
100	,840	,145	,015	Osterhoff & van Heerden 1965[12]
122	,828	,155	,057	Osterhoff & van Heerden 1965[12]
1041	,810	,120	,070	Bouquet et al, 1970[4]
100	,985		,015	Osterhoff & van Heerden 1965[11]
99	,909		,091	Singer & Lehmann, 1963[16]
200	,915		,085	Singer & Lehmann, 1963[16]

The first four groups of animals are regarded as correctly typed, and are included in Table 1, while in the other three groups the frequencies of HbA and HbC were given combined as HbA for the reasons mentioned.

These observations were followed up by a comparison on Malagasy Zebu. In 1968 a study was completed by Petit using the cellulose acetate technique and samples of the same herd were investigated 4 years later with the starch gel technique.

These examples provided evidence for the statement that earlier and present studies can best be compared on the basis of the HbB — frequencies. Table 4 presents the haemoglobin gene frequencies obtained in all studies of African cattle breeds from North to South.

The survey of African cattle is by no means complete, because of all breeds or types of cattle shown in Table 4 very little additional genetic information is available at this stage, except for the South African cattle breeds[10]. In spite of this fact one is inclined after plotting these results on a map of Africa to hypothesise in the following way: Haemoglobin B has been established correctly in the investigations of 39 breeds or types of cattle. Therefore it is believed that

Table 3: COMPARISON OF TWO STUDIES ON MALAGASY ZEBU

No. fo samples	Gene frequencies			Author
	Hb^A	Hb^B	Hb^C	
226	,378	,622		Petit, 1968[13]
199	,354	,623	,023	Present study

this haemoglobin type can be used as a reliable marker in breed comparisons. Haemoglobin B indicates an Asiatic rather than an African ancestry for most of the cattle breeds, considering the high fre-

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Table 4: HAEMOGLOBIN GENE FREQUENCIES IN AFRICAN CATTLE BREEDS.

No.	Breed	Country	Gene frequencies Hb^A	Hb^B	Hb^C	Hb^D	Author
1	Algerian	Algeria	,856	,144			Cabannes & Serain, 1955
2	Zebu Gobra	Senegal	,700	,300			Petit, 1968
3	Zebu Azeuoak	Nigeria	,677	,323			Schmid, 1965
4	N'Dama	Ivory Coast	,975	,025			Petit, 1968
5	White Fulani	Nigeria	,726	,274			Bangham & Blumberg,1958 Lehmann & Ross, 1959
6	Red Bororo	Nigeria	,540	,440	,020		Braend, 1972
7	Zebu Arabe	Chad	,578	,422			Queval et al, 1971
8	Sudan-Zebu	Sudan	,529	,471			Bangham & Blumberg, 1958
9	Mutura	Nigeria	,800			,200	Braend et al, 1965
10	N'Dama	Nigeria	1,000				Braend et al, 1965
11	Gudali	Nigeria	,600	,300	,100		Braend, 1972
12	Zebu Bororo	C.A. Rep.	,617	,383			Petit, 1968
13	Zebu Soudan	C.A. Rep.	,642	,358			Petit, 1968
14	N'Dama	Gabon	1,000				Petit, 1968
15	Ankole	Uganda	,764	,236			Lehman & Rollinson, 1958 Osterhoff & van Heerden, 1965 a
16	Shorth Zebu	Uganda	,678	,322			Lehmann & Rollinson, 1958
17	Barotse	Zambia	,738	,167	,095		Carr, 1964
18	Tonga	Zambia	,417	,417	,166		Carr, 1964
19	Angoni	Malawi	,436	,387	,177		Carr, 1964
20	C. Cunene	Angola	,801	,119	,080		Present study
21	Malanje	Angola	,682	,235	,083		Present study
22	Quilenques	Angola	,738	,183	,078		Present study
23	Malawi-Zebu (N)	Malawi	,623	,255	,096	,026	Present study
24	Malawi-Zebu (C)	Malawi	,700	,140	,160		Present study
25	Mashona	Rhodesia	,866	,117	,017		Present study
26	Tuli	Rhodesia	,580	,399	,021		Present study
27	Nkone	Rhodesia	,929	,049	,022		Present study
28	Caprivi-Sanga	S.W.A.	,876	,114	,010		Present study
29	Ovambo	S.W.A.	,748	,150	,102		Present study
30	Sango	S.W.A.	,732	,141	,127		Present study
31	"Landim"	Mozambique	,756	,130	,114		Present study
32	Rénitélo	Madagascar	,696	,304			Petit, 1968
33	Malag. Zebu	Madagascar	,354	,623	,023		Petit, 1968; Present study
34	Lesotho-Nguni	Lesotho	,910	,060	,030		Present study
35	Afrikaner	S.A.	,819	,064	,177		Present study
36	Brahman	S.A.	,577	,400	,023		Present study
37	Drakensberger	S.A.	,833	,037	,130		Osterhoff & van Heerden 1965 a
38	Nguni	S.A.	,883	,064	,053		Present study
39	Bonsmara	S.A.	,962	,013	,025		Present study

quency of the allele HbB in Indian breeds[9] and also the gene frequencies of the Brahman in South Africa and the Malagasy Zebu and the Rénitélo in Madagascar, both having an apparent close relationship to Indian cattle. With a few exceptions, one may state that the further the *Bos indicus* types migrated away from India to the West and South the lower is the frequency of the allele HbB. There are large "white" areas of the frequency-map of Africa and a study of cattle types of Egypt and cattle in the Horn of Africa would certainly provide more evidence for this hypothesis.

Haemoglobin C must still be studied biochemically, but it certainly is not an abnormal haemoglobin. It could be regarded as a typical "African" haemoglobin, because of its relatively high frequency in the breeds and types of Africa. The significance of haemoglobin D is not known. It is found only in the Muturu breed and in a few animals in Malawi. This haemoglobin variant is also of value in studying polymorphism as well as origin relationships and evolution of cattle breeds.

It is a challenge to the workers in the field of genetic markers and also to international organisations to

complete the survey of the possible genetical markers of the existing breeds and types of cattle in Africa. This survey may result in new theories of cattle relationship and migration and could also assist the colleagues from other faculties in their studies of human relationships.

ACKNOWLEDGEMENTS

The help of the persons and departments involved in the collection of blood samples of different breeds and types of cattle is acknowledged with thanks. Grateful thanks are also due to members of the staff of the Dept. of Zootechnology for their kind assistance in various phases of the investigation.

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BOEKRESENSIE

BOOK REVIEW

FARM ANIMAL BEHAVIOUR

A.F. FRASER

Bailliére Tindall, London, First published 1974. Pp. 196, Figs 55. Price: £2.20.

Hierdie werk deur Andrew F. Fraser, senior lektor in die Departement van Veterinêre Obstetrie aan die Universiteit van Edenburgh, is 'n boek oor etologie (dieregedrag) soos van toepassing op perde, beeste, skape en varke. Die boek is geskrywe om as inleiding te dien vir die vak etologie by die plaasdiere.

Die boek is veral geskik as handboek vir studente in veeartsenykunde en die dierewetenskappe. Die boek behoort ook baie handig te wees vir diegene wat belangstel in plaasdier-etologie.

In die boek word etologie wetenskaplik uiteengesit en beskryf. Die beskrywings is ook pragtig geïllustreer met sketse om dieregedragspatrone sodoende makliker verstaanbaar te maak.

Die boek is beslis 'n konstruktiewe en baie belangrike bydrae tot die vak etologie as 'n toegepaste wetenskap in veeartsenykunde en as 'n vertakking van biologie. Die etologiese navorsing mag tot groot voordeel strek van die plaasdierindustrie en die metodes van produksie.

Die veearts en diereteler kan alleen baat deur kennis te dra van die vakrigting. Die boek kan beslis aanbeveel word, omdat dit maklik leesbaar is en 'n uitstekende inleiding tot die vak etologie gee.

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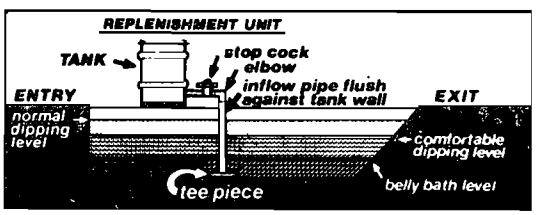

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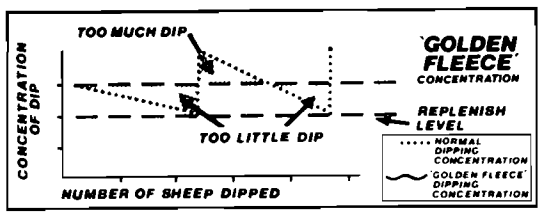

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STANDARD SERUM CHEMICAL AND HAEMATOLOGICAL VALUES IN THE CHACMA BABOON *(PAPIO URSINUS)*

D.G. STEYN*, R.J. HAMILTON-BRUCE, T.J. ZUURMOND AND R. P HARO

SUMMARY

Blood chemical and haematological determinations were carried out on 64 baboons. The blood chemical and haematological values determined were found to be in general agreement with the values for man and other primate species. Some blood constituents however, did show noteworthy differences. Marked sex differences were demonstrated in some blood chemical and haematological parameters.

INTRODUCTION

Little information is available on the blood chemical and haematological values of the chacma baboon. The conditions under which primates are housed, climatic influences, diet and species vary from facility to facility. Normal values for conditioned baboons housed at our primate colony thus needed to be established and were required as a baseline with which other values obtained during experimental procedures could be compared. The values reported by Weber, Brede, Retief, Retief and Melby[32] were determined on 1 400 baboons but included newly captured and conditioned baboons.

MATERIALS AND METHODS

Experimental Animals

All the baboons used in this study had been in the colony for at least 3 months to about 5 years prior to specimen collection. Clinically diseased, pregnant and lactating animals and those which had recently undergone surgical procedures were excluded from the study. The weights of the animals ranged from 8 to 32 kg. The ages of the animals could not be determined due to a lack of appropriate criteria. The youngest animals were, however, probably not less than 3 years. Of the 64 animals included in the study 28 were males and 36 were females.

Experimental Procedures

The animals were housed in single cages. All specimens were collected at 09h00 after overnight starvation and after the intramuscular administration of phencyclidine hydrochloride ("Sernylan" - Parke Davis and Co.) at the rate of 1,5 mg/kg to allow handling of the animal. Blood samples were drawn from the femoral vein about 30 min. after the administration of the anaesthesia.

Blood Chemistry

The blood was collected in ordinary glass tubes and allowed to clot. Thereafter it was centrifuged and the serum used for chemical determinations. For the determination of plasma corticosteroid levels, blood was collected in tubes containing heparin as an anticoagulant, centrifuged and the plasma stored in a deep freeze until a whole series could be examined simultaneously by the method of Bossett & Hinks[5] as modified by Morgenthal (personal communication). The various analytical methods employed in the analyses of the serum and plasma samples are listed in Table 1.

Haematology

The blood was collected in 2 ml vials containing ethylinediaminetetraacetic acid (EDTA) as the anticoagulant. The total leukocyte count, erythrocyte count, haemoglobin content, haematocrit level, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were done on a Model-S Coulter Counter (Coulter Electronics Inc.). The erythrocyte sedimentation rate (ESR) was determined by the Westergren method. White blood cell differential counts were performed microscopically by counting 200 cells on May-Grünwald-Giemsa stained smears. Reticulocyte counts were done microscopically on supravitally stained preparations making use of brilliant cresyl blue stain.

Statistical Analysis

Mean values and standard deviations were calculated for all parameters. A Student's t test was carried out to determine if any statistically significant differences could be demonstrated between the values of male and female baboons. The 95 per cent confidence intervals for the arithmetic means were also calculated[29].

RESULTS

Blood Chemistry

The values for the biochemical serum determinations are presented in Table 2. These values generally fell in the same ranges as those reported for other primates and more specifically for those of *Papio* species[11, 32]. The total plasma protein values were in

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Table 1: ANALYTICAL METHODS EMPLOYED FOR BLOOD CHEMICAL DETERMINATIONS

Determination	Method and Reference	Units
TPP	Weichselbaum [33]	g %
Protein Fractions	Beckman microzone technic Model RIOI cell.	g %
Potassium	IL Flame photometer Model 143	mEq//
Sodium	IL Flame photometer Model 143	mEq//
Chloride	Cotlove, Trautham & Bowman [8]	mEq//
CO_2 Content	Natelson microgasometer Model 650	mEq//
Urea	Fawcett & Scott [12]	mg %
Cholesterol	Watson [31]	mg %
Calcium	Baron & Bell [4]	mEq//
In.Phos.	Goldenberg & Fernandez [15]	mEq//
Glucose	Werner, Rey & Wielinger [34]	mg %
SAP	Rick & Hausamen [26]	mU/ml
SGPT	Schmidt & Schmidt [28]	mU/ml
SGOT	Schmidt & Schmidt [28]	mU/ml
LDH	Amelung [2]	mU/ml
TCS	Bassett & Hinks [5]	mcg %

TPP = total plasma proteins; In. Phos. = inorganic phosphorus; SAP = serum alkaline phosphatase; SGPT= serum glutamic pyruvic transaminase; SGOT = serum glutamnic oxaloacetic transaminase; LDH = lactic dehydrogenase; TCS = total plasma corticosterioids.

agreement with the value of 6,4 g per cent reported by Weber *et al*[32] for *P. ursinus* and corresponded with the values reported for other *Papio* species[11] [13]. The albumin: globulin ratio (A:G) of 1,03 was also in agreement with the values of four groups of baboons[11]. value of 47,56 mg per cent obtained in this group of baboons was the same as the value reported by Weber *et al*[32]. If this value is compared with the value for other *Papio* species, *e.g.*, *P. cynocephalus* as tabulated in the literature[24], however, it seems to be a

Table 2: BLOOD CHEMISTRY VALUES FOR THE CHACMA BABOON UNDER STANDARD CONDITIONS.

Determination	Units	N	Mean	S.D.	Range
TPP	g %	64	6,43	0,74	4,4–87
Albumen	g %	39	3,43	0,59	1,49–4,34
Alpha–1 globulin	g %	39	0,16	0,06	0,02–0,37
Alpha–2 globulin	g %	39	0,16	0,22	0,32–1,09
Beta globulin	g %	39	1,09	0,24	0,59–1,62
Gamma globulin	g %	39	1,38	0,36	0,87–2,57
Potassium	mEq//	64	3,75	0,43	3,10–4,80
Sodium	mEq//	64	144	4,65	135–156
Chloride	mEq//	64	104	3,95	90–110
CO_2 content	mEq//	63	28	5,27	9,8–40,1
Urea	mg %	63	48	10,93	19–80
Cholesterol	mg %	63	127	47,80	46–408
Calcium	mEq//	45	5,16	0,52	4,3–6,2
Inorg. Phosph.	mM//	45	1,63	0,60	0,3–3,1
Blood glucose	mg %	37	96,46	27,03	43–160
SAP	mU/ml	64	555	398	145–1548
SGPT	mU/ml	64	14	6,75	5–36
SGOT	mU/ml	64	16	6,16	6–35
LDH	mU/ml	64	266	101	133–759
Corticosteroids	mcg %	35	22,6	10,59	4–46

N = number of samples; SD = standard deviation, TPP = total plasma protein; Inorg. Phosph. = inorganic phosphorus; SAP = serum alkaline phosphatase; SGPT = serum glutamic pyruvic transaminase; SGOT = serum glutamic oxaloacetic transaminase; LDH = lactic dehydrogenase.

The mean serum potassium concentration was somewhat higher than the value of 3,3 mEq/ℓ previously reported for *P. ursinus*[32]. The sodium, chloride and carbon dioxide values were similar to previously reported values[11] [32]. The mean blood urea characteristic of the chacma baboon to have a higher value than other primates.

The mean cholesterol value was slightly higher than the value of 100 mg per cent reported by Weber *et al*[32] but lower than the values reported for baboons by

Gillman, Gilbert and Savage [14], and McCraw and Sim. [20] De la Pena and Goldzieher [10] demonstrated differences related to geographical distribution.

Serum calcium and inorganic phosphorus were in general agreement with previously reported values although both values were at the upper limit of the normal values as reported for baboons[10 11 32]. The blood glucose levels were slightly higher than the 88 mg per cent reported for chacma baboons[32], more or less the same as those reported for *Papio* species[10 11 14 20] but lower than the non-fasting values listed by Burns, Ferguson and Hampton[6]. The baboon values compared closely with the values of 60 to 100 mg per cent reported to be normal for man[9]. The serum alkaline phosphatase (SAP) values were much higher than the recorded values for humans confirming previous results[11 32]. The values for serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT) were lower than those previously reported for chacma baboons [32] and *Papio species* [7]. The values, however, corresponded to the values reported for man [9]. The values for lactic dehydrogenase (LDH) were lower than the values recorded for baboons[10 11 32]. It was also lower than the value reported for man [9] but it was more or less in the same range as the values reported for other primates particularly *Macaca mulatta* [23].

The value for the concentration of plasma adrenocorticosteriods reported for this group of baboons represents the total plasma adrenocorticoid value. The steroids present in the baboon *(P.hamadryas)* are mainly hydrocortisone, cortisone, corticosterone and 17 alpha-hydroxy-progesterone. Hydrocortisone occurs in the highest concentration [16]. No reference for corticosteroid values for *P. ursinus* could be found in the literature. The value determined in the animals in this study was lower than the value reported for *M. mulatta* as tabulated in the literature[23].

Haematology

The data pertaining to the haematological findings are summarized in Table 3.

The mean total leukocyte count with a mean value of 7 718 was considerably lower than the values reported by some authors[10 22 32] but corresponded with the values reported by others[7 13 19]. The leukocyte count was also considerably lower than the values for 10 other primate species as tabulated by Hall[18]. Even unimportant injuries like scratches and minor bites on tails and limbs are rapidly reflected by an increased leukocyte count and fluctuations of the leukocyte count seem to occur much more rapidly in the baboon than in man[22].

The differential white blood cell count was in agreement with previous reports[10 13]. The ratio between lymphocytes and neutrophils was, however, the reverse of that found by Hall[18] in 10 species of non-human primates. There was a marked variation in the individual animals with lymphocyte counts ranging from 21 to 83 per cent.

The mean erythrocyte count was higher than the 4,79± 0,35 obtained by Burnes *et all*[7] and the value of 4,45 ± 0,50 reported by De la Pena and Goldzieher[10] but generally lower than the values for most of the species listed by Hall[18].

The mean haemaglobin value and the haematocrit were slightly higher than the values of 13,5± 2,3 and 42,2± 5,6 respectively reported by Weber *et all.*[32] and significantly higher than the 11,8 ± 1,16 and 35,8,± 8,7 reported by De la Pena and Goldzieher[10]. The reason for these relatively high values is not clear as these animals are housed in an area almost at sea level. High level, therefore, could not have played any role.

The mean erythrocyte sedimentation rate (ESR) was lower than the value of 6,2± 5,3 mm/h obtained by Weber *et all.* [32] but was comparable to the value found in another study for 10 baboons[25]. The range in these normal or conditioned baboons was remarkably narrow if the wide range during disease is considered.

The red blood cell indices were in general agreement with reported values[7 13]. In the main, the values also corresponded with the values found in man[9].

The mean reticulocyte count was found to be 1,08± 0,68 which was higher than the values previously reported[13 32]. The platelet count was in agreement with the value of 360 000 reported by Foy *et all.*[13].

TABLE 3: HAEMATOLOGICAL VALUES IN THE CHACMA BABOON UNDER STANDARD CONDITIONS

Determination	Units	N	Mean	S.D.	Range
Leukocyte Count	$X10^3/mm^3$	64	7,718	2,630	3,30–15,70
Lymphocyte Count	per cent	44	44,50	13,20	13–75
Neutrophil Count	per cent	44	51,05	13,40	21–83
Monocyte Count	per cent	44	2,15	1,36	0–9
Eosinophil Count	per cent	44	2,00	1,80	0–9
Basophil Count	per cent	44	0,30	0,67	0–3
Erythrocyte Count	$X10^6/mm^3$	64	5,26	0,78	4,22–6,53
Haemoglobin	g per cent	64	14,11	1,67	11,3 –21,4
Haematocrit	per cent	64	42,62	4,33	32–51
ESR	mm/h	62	2,35	2,53	1–12
MCV	$/u^3$	64	80	3,10	72–89
MCH	/u/ug	64	27	2,10	22–34
MCHC	per cent	64	33	1,70	28–36
Reticulocytes	per cent	34	1,08	0,70	1–2,9
Platelets	$X10^3/mm^3$	36	312	108	100–660

N = Number of samples; SD = standard deviation; ESR = erythrocyte sedimentation rate; MCB = mean corpuscular volume; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration.

Differences between Males and Females

Table 4 summarizes the differences found between males and females in some parameters. Significant differences ($P < 0,05$) were found in the plasma albumin and beta globulin concentrations between males and females with the albumin fraction being higher in males than in females and the beta globulin fraction lower in males than in females. Similar findings were reported by De la Pena *et al.*[11] who also found lower albumin and A:G ratios in female baboons. The A:G ratio for males of the group in the present study was 1,16 as compared to 0,97 in females. Although the difference was marked, it was not statistically significant.

Serum sodium ($P < 0,05$), serum carbon dioxide content ($P < 0,05$) blood urea ($P < 0,01$) and SAP levels ($P < 0,01$) were higher in males than in females whereas serum cholesterol ($P < 0,05$) and blood sugar ($P < 0,01$) were lower in males than in females.

The males had higher red blood cell counts ($P < 0,05$), haemoglobin concentrations ($P < 0,01$) and haematocrit levels ($P < 0,01$) than females whereas a higher platelet ($P < 0,05$) and reticulocyte count ($P < 0,05$) occurred in females. Blood loss due to menstruation in the female baboon may be responsible for the differences observed between the sexes.

by some investigators, may be a characteristic of the baboon. Similar values to those found in this study have been recorded previously[17 11 32]. It has been stated that the rise in total protein at puberty in human females is not accompanied by an increase in the albumin fraction[35]. Both oestrogens and progesterone may cause an inhibition of albumin synthesis. If the same should apply to the baboon, it could explain the lower albumin values found in the females because all of the females included in the study had passed the stage of puberty.

The low serum potassium value was referred to earlier. If the range of potassium values is examined one finds that it varies from 3,1 to 4,8 mEq/ℓ. Muscular trauma, necrosis, haemolysis, pH status, aldosterone, etc. may affect the potassium level. Excitement in some squirrel monkeys caused a critical rise in serum potassium[18]. This reaction has not been observed in the baboon. High levels of potassium are encountered, however, in some animals during the advanced stages of dysentery.

The sodium value was found to be similar or slightly higher than the accepted human value. Altshuler, Stowell and Lowe[1] reported similar findings and thought that this may be the result of different levels of water intake by the simian when compared to man.

Table 4: SIGNIFICANCE OF DIFFERENCE IN MEAN VALUES FOR MALE AND FEMALE CHACMA BABOONS UNDER STANDARD CONDITIONS

Determination	N	Male	N	Female Mean	Significance Level
Albumen	20	3,60 ± 0,49	19	3,25 ± 0,65	P<0,05
Beta globulin	20	1,02 ± 0,25	19	1,16 ± 0,21	P<0,05
Sodium	28	145 ± 4,97	36	143 ± 4,15	P<0,05
CO_2 Content	28	29,1 ± 4,23	35	26,6 ± 5,80	P<0,05
Urea	28	52 ± 10,77	35	44 ± 9,80	P<0,01
Cholesterol	28	110 ± 27,81	35	140 ± 55,93	P<0,05
Blood sugar	17	83,5 ± 17,57	18	108,7 ± 29,09	P<0,01
SAP	28	782 ± 439	36	379 ± 253	P<0,01
Erythrocytes	28	5,155 ± 0,50	36	5,056 ± 0,90	P<0,05
Haemoglobin	28	14,86 ± 1,76	36	13,53 ± 1,36	P<0,01
Haematocrit	28	44 ± 3,89	36	41 ± 4,28	P<0,01
Platelets	20	271 ± 91,65	22	350 ± 109,3	P<0,05

N = Number of samples; ± standard deviation: SAP = serum alkaline phosphatase.

DISCUSSION

In discussing the results it may be wise to follow the example of De La Pena *et al*[11] by emphasizing the fact that these values represent the findings in a particular set of baboons under particular dietary and environmental conditions and that they, therefore, cannot be applied, without suitable qualification, to other sets of baboons. The data obtained are in general agreement with previous reported data and the mean values are within or very nearly within the human range. Certain differences between the values recorded in the literature and those found in this study as well as differences demonstrated between male and female values warrant further discussion.

The rather low total plasma protein value, when compared to values reported for other primate species

They also reported slight but consistently higher sodium levels in male *M. mulatta* than in females. In the present study the value in males was not merely slightly higher but significantly higher than the female values. Altshuler *et al*[1] speculated that the difference may be a reflection of the weak sodium retention properties of the androgenic hormones.

All baboons in this experiment received a controlled diet with a protein content of about 20 per cent which may explain the high blood urea values recorded. The higher urea level found in the males, a phenomenon also demonstrated in other species, is probably a reflection of the protein anabolic effect of testosterone[1].

Cholesterol is normally carried on or by the beta globulin proteins and the low beta globulin found in males may be a reflection of the lower cholesterol

values recorded for males. Males show changes in the beta globulin fraction when subjected to emotional stress[17] Beader and Werthessen[3] reported that over 1 000 seperate lipid determinations showed that the average total cholesterol level for newly imported baboons and a group inbred for a number of generations was 114 mg per cent. A higher mean value for male baboons than for female baboons was recorded in free-living animals within 24 h of being captured[30]. This is contradictory to the findings in this experimental group of conditioned animals. The change in diet of the baboons in captivity may partly explain the differences in levels but why this should affect one sex more than the other and then oppose the findings in man is not at all clear.

The serum levels of calcium and inorganic phosphorus did not reveal any sex related differences although these values were rather high compared to the values in man. The serum concentration of inorganic phosphorus appears to be intimately related to carbohydrate metabolism. During increased carbohydrate utilization the level tends to decrease and during fasting an increase is usually observed[9]. Haemolysis will contribute to serum inorganic phosphorus whereas starvation prior to blood collection will eliminate any effect of carbohydrate metabolism that may cause a depression of serum phosphate concentration[9].

The blood glucose levels are in general agreement with levels reported by other investigators. Overnight fasting is apparently not adequate to prevent adrenaline mediated glycogenolysis in the liver[18]. The exercise and excitement of a previous day's capture may cause marked increases in fasting blood glucose levels, particularly in normal adult males. The high blood glucose levels in the females could have been brought about by higher elevations of circulating catecholamines due to excitement and nervousness in the more subordinate females. Starvation, recent feeding, excitement and the effect of anaesthesia are factors to be considered when interpreting blood sugar levels as such disturbances may have a marked effect on blood glucose levels.

The high SAP values found in the baboons are in agreement with other published reports. Not only baboons, but also other nonhuman primates exhibit these high values, which are much higher than the values which are considered as normal for man[1]. Sex related differences in SGPT and SGOT or LDH levels could not be determined in baboons.

The plasma corticosteroid value determined for the baboons falls more or less between the accepted value for man and those of other primates. The circadian rhythm, immobilization and various other stress factors may alter the values. In monkeys a nocturnal rise with a maximum at 6 a.m. has been demonstrated. The 9 a.m. values were about 60 percent of the 6 a.m. level and the fall continued until 9 p.m. when the nocturnal rise commenced once again[21].

Various other factors, which are difficult to assess, undoubtedly have an effect on the level of plasma corticoids. The stress effect of social grouping are manifest primarily in the subordinate numbers of a group and are intensified by increasing the group density[27]. Social and physical environmental changes, fear and trauma associated with handling and management procedures may all add to the difficulties of interpreting results. This may also explain the variations encountered when sampling a group of animals under apparently similar conditions.

The results of the haematological investigations were in excellent agreement with previous reports. The values for the total white blood cell count obtained during this study, however, were lower than other reported values. Benhar and Samuel[7] reporting on 12 baboons recorded an even lower value of infections due to disease and wounds or even minor scratches must of course not be overlooked in the interpretation of elevated values.

The most striking findings in the haematological picture were the significant differences between male and female animals which were found in the red blood cell count, haemoglobin concentration and haematocrit levels. These findings were in agreement with numerous reports on similar differences demonstrated in some species of nonhuman primates as well as the difference normally accepted for the sexes in man. The red cell indices all fell at the lower limit of normality for man or in some cases even slightly lower.

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EXPERIMENTAL *PHOMOPSIS LEPTOSTROMIFORMIS* MYCOTOXICOSIS OF PIGS

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SUMMARY

The susceptibility of the domestic pig to intoxication by the causative mycotoxin of lupinosis was established experimentally. The symptomatology and pathology of the disease produced by the administration of toxic cultures of the fungus, *Phomopsis leptostromiformis* (Kühn) Bubák ex Lind to pigs are described. The toxin induced severe loss of weight and, in many cases, posterior paresis or paralysis. The principal gross lesions were generalised icterus, orange-red discoloration of the liver, nephrosis and, in some, enterorrhagia. Microscopically there was severe necrosis of hepatocytes and kidney tubular epithelium as well as myocardial degeneration. In the more chronic cases hepatocytes became anaplastic and arranged in acini. The production of toxin by *P. leptostromiformis* on yellow maize is reported and a method for production of toxic material is described.

INTRODUCTION

It has now been well established that lupinosis is a mycotoxicosis caused by the fungus *Phomopsis leptostromiformis* (Kühn) Bubák ex Lind (= *Phomopsis rossiana* (Sacc.) Sacc. et D. Sacc.[7 10 18 19 20]). Its morphology and synonomy have been described by Van Warmelo & Marasas[17]. The mycotoxin responsible for the disease is also produced on substrates other than lupin material[20].

Lupinosis has been described in various species namely sheep[1 4 5 6 18 19], cattle[12], horse[8] and three species of experimental laboratory animals[3 4 7 10 13 14]. Only one brief report exists in the literature on a possible field outbreak of lupinosis in swine[11]. In this instance pigs fed a ration containing ground, bitter lupin seeds suddenly became ill and showed signs of inappetence, depression, recumbency, increase in body temperature, slight icterus of the sclera, constipation, vomiting and lack of milk production.

Because there appears to be a tendency to make more use of lupins in pig rations due to the increase in price of soybeans[9] it was decided to investigate the susceptibility of pigs to the mycotoxin produced by the fungus, *P. leptostromiformis*.

MATERIALS AND METHODS

In our laboratory over the past 4 years we have used for experimental purposes the isolate of *P. leptostromiformis* described as No. B16 (= PRE 44350). It was isolated from pods of *Lupinus albus* L. cult, Pflugs Gela, which were obtained from the Hermon district, Cape Province in October 1969[17 18 19]. Subcultures of this isolate have been deposited in the American Type Culture Collection (ATCC 22849), Centraalbureau voor Schimmulcultures (CBS 754.70) and the Commonwealth Mycological Institute (IMI 146035). Stock cultures were maintained on slants of 1,5% malt extract agar in McCartney bottles at 4°C. As we have determined that pure cultures of the fungus grown on autoclaved maize (*Zea mays* L.) kernels induced lesions in sheep, rabbits, guinea pigs and mice identical to those caused by cultures on *L. albus* seeds (Marasas, Kellerman, Anderson and Van Rensburg 1971, unpublished data), and since maize was more easily obtained we adopted it as a standard medium for production of toxic material. In this experiment the procedure was as follows: Spore suspensions of *P. leptostromiformis* for use as inoculum were prepared in sterile water from 21 day-old sporulating cultures grown on 1,5% malt extract agar[17 18].

Yellow maize kernels in distilled water were autoclaved for 1 h on each of 2 consecutive days at 121°C and 103 k Pa in 1,0 *l* glass fruit jars (200 g kernels in 150 ml distilled water/jar). The autoclaved kernels in each jar were inoculated with 2,5 mℓ of spore suspension. The jars were shaken to distribute the spores evenly and incubated in the dark at 25 to 28°C for 4 weeks. Thereafter the contents of the jars were minced in a meat mincer, air-dried at room temperature in flat metal pans, ground to a fine powder in a mill and stored in a refrigerator at 4°C until used.

DOSING OF FUNGAL CULTURES TO EXPERIMENTAL PIGS

PILOT EXPERIMENT

A pilot experiment using one pig was done in 1971. A Large White gilt about 4 months of age received *Phomopsis* culture plus maize medium mixed with her normal pig growing ration at 5 g/kg body weight for 2 consecutive days. This resulted in anorexia and apathy for several days after which the animal recovered. When she had fully recovered her appetite, toxic material was again mixed into her feed. This was repeated for approximately 8 weeks by which time a total of approximately 950 g of toxic culture and medium had been consumed. She died and was autopsied on the 58th day of the trial. A litter mate kept as a control was fed only the pig growing ration.

EXPERIMENT I

Six Landrace X Large White pigs, 2 months old and representing both sexes were purchased from a

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breeder and fed *ad lib.* on a commercial balanced ration known as " pig growth meal*". They were allowed 2 weeks to adapt to their new surroundings and feed. Pig No. 1 was starved for 24 hours and then given toxic material (fungus plus substrate) at the rate of 20 g/kg body weight mixed with a sufficient quantity of its ration for it to consume in a few minutes. The same procedure was followed with Pig No. 2, but it received 10 g/kg body weight of toxic material. Both pigs consumed the full dosage. Pig No. 2 was again offered 10 g/kg 4 days later, but only a few mouthfulls were eaten. Pigs No. 3 and 4 were each given a single dose of 20 g/kg body weight of the toxic material in a similar way while Nos. 5 and 6 only received the normal ration and served as controls. The animals were weighed every week. Pigs surviving the experimental period of 2 weeks were slaughtered by stunning and exsanguination. All pigs were autopsied and material collected from them for histopathological examination.

EXPERIMENT II

Because the toxin causes complete anorexia in pigs for several days following its administration repeated administration of toxic material at short intervals by the method described above is impossible. The dosing of pigs with large amounts of any material is also virtually impossible. Therefore intragastric rubber fistulas** were installed in eight pigs according to the method described by Michael & Buck[12]. These animals were of the same mixed breed and of both sexes as those in Experiment I and at the time of purchase weighed approximately 35 kg (see Table 1). The surgery was performed under Trilene*** anaesthesia after premedication with Stresnil.****

The pigs were allowed 1 week to adapt to the fistulas. Toxic material was then administered to the experimental animals and autoclaved minced and dried yellow maize kernels to the controls through the fistulas directly into the stomach as follows: The required quantity was weighed and mixed with tap water (the same as was available in their drinking troughs) into a semifluid mass and then pumped through the fistulas by means of a stomach pump. The pigs did not object to this. Dosage rates were as follows: Pigs F1 and F2 received 10g toxic material/kg body weight daily on 5 consecutive days of the week until death supervened. Feed and water were withheld for 16 h prior to administering the material. Pigs F3 and F4 were given 2,5 g toxic material/kg body weight on 3 days of the week, *i.e.* Mondays, Wednesdays and Fridays. Toxic material was administered at the rate of 1,25 g/kg body weight to Pig F5 once a week only and to F6 fortnightly for the first month and thereafter once a week. The control pigs F7 and F8 received 5 g/kg body weight of the yellow maize kernels on Mondays and Fridays during the experiment. The pigs were examined daily and weighed at weekly intervals.

Pigs surviving the experimental period of 8 weeks and those very sick or *in extremis* were stunned and exsanguinated. All animals were autopsied and specimens from various organs were taken and fixed in 10% buffered formalin. Blocks prepared from these were embedded in paraffin wax. Sections were then cut and stained with haematoxylin and eosin (H & E) in a routine manner for microscopical examination.

RESULTS

Some of the experimental data and results are summarized in Table 1.

PILOT EXPERIMENT

CLINICAL AND GROSS PATHOLOGY

The experimental animal was at the time of death in a very poor physical condition and her weight was

* Epol Feeds (Pretoria) (Pty.) Ltd.
** Hudson Vulcanising Company.
*** I.C.I. South Africa (Pharmaceuticals) Ltd.
**** Ethnor Laboratories (Pty.) Ltd.

Table 1: SUMMARY OF EXPERIMENTAL DATA

Experiment	Pig		Weight in kg			Duration of experiment in days and manner of death	Total amount of toxic material received in g	Number of doses
	No.	Sex	Commencing	Death	Gain or loss			
Pilot		f	28	25	−3	58D	950	8
I	1	f	26	24,5	−1,5	7E	520	1
	2	f	26	22,5	−3,5	13E	260+x	2
	3	m	30	–	–	3K	600	1
	4	m	34,5	33	−1,5	5K	690	1
II	F1	m	36	32	−4	12E	3 550	10
	F2	f	39	38	−1	7D	1 950	5
	F3	f	40	28	−12	19E	740	9
	F4	f	41	30	−11	15D	605	6
	F5	f	36	16	−20	42D	200	7
	F6	m	41	36	−5	46E	296	6
	F7	m	41	47	+7	7K	0	0
	F8	f	41	80	+39	56K	0	0

f = female m = castrated male K = killed E = killed in extremis or very ill D = died x = estimated 80g eaten at 2nd feeding.

less than half that of the control animal (*i.e.* 25 kg compared with 69 kg). A generalised icterus was present and the skin was congested and cyanotic. The liver was of normal size, a yellow-brown colour and of increased consistency. The bile was a curry yellow, turbid and floccular fluid. Subcutaneous and intermuscular oedema was conspicuous – especially in the ventral neck region. The large intestine contained hard, dry faecal balls coated with mucous and blood. Hydropericardium, pulmonary oedema and sub-epi-, and endocardial haemorrhages were present.

HISTOPATHOLOGY

The lesions in this animal were of a more chronic nature than any of the subsequent cases. The liver presented the most severe changes. The basic lobular architecture was well preserved but within the lobules, however, hardly any hepatocytes were present. There was no orderly cord-like arrangement of parenchymal cells. The majority of hepatocytes had been replaced by small anaplastic cells showing a tendency to acinar arrangement. Many of these cells contained small lipid vacuoles. What few hepatocytes were left contained numerous small fat droplets in their cytoplasm and were frequently multinuclear. Yellow pigment globules were present in some of them. The nuclei varied in size and shape, some were very small and pycnotic, others were large and bizarre in that they had a multiplicity of shapes while a few cells contained larger than normal round nuclei. Cells containing the latter occurred most frequently at the periphery of the lobule. The centres of the lobules were congested and haemorrhagic. There was some intralobular round cell and fibroblast infiltration and proliferation.

Several myocardial fibres contained small clear vacuoles while in others the nuclei were enlarged and some proliferation of sarcolemma nuclei had occurred. In the left papillary muscle there was a rarifaction of the myocardium with homogenous eosinophilic clumping of sarcoplasm in these areas. Some fibres were calcified (Fig. 3).

EXPERIMENT I

CLINICAL AND GROSS PATHOLOGY

Pig 1 weighed 26 kg on the day the experiment commenced and 24,5 kg when it was killed *in extremis* on the 7th day. From the third day it was anorectic, very lethargic and showed a very slight yellowish tinge to the sclera.

When necropsied the most interesting finding was a slightly smaller than normal orange-brown liver with a definite increase in consistency. The bile was of a normal quantity but mucoid and dark-yellow. The intima of the large blood vessels was a light yellow colour and the kidney cortex pale brown and streaky in appearance on cut surface indicating mild degenerative changes.

Pig 2 had the same initial weight and was killed 13 days later for necropsy at which time it had lost 3½ kg body weight. The clinical signs exhibited were identical to those of Pig 1.

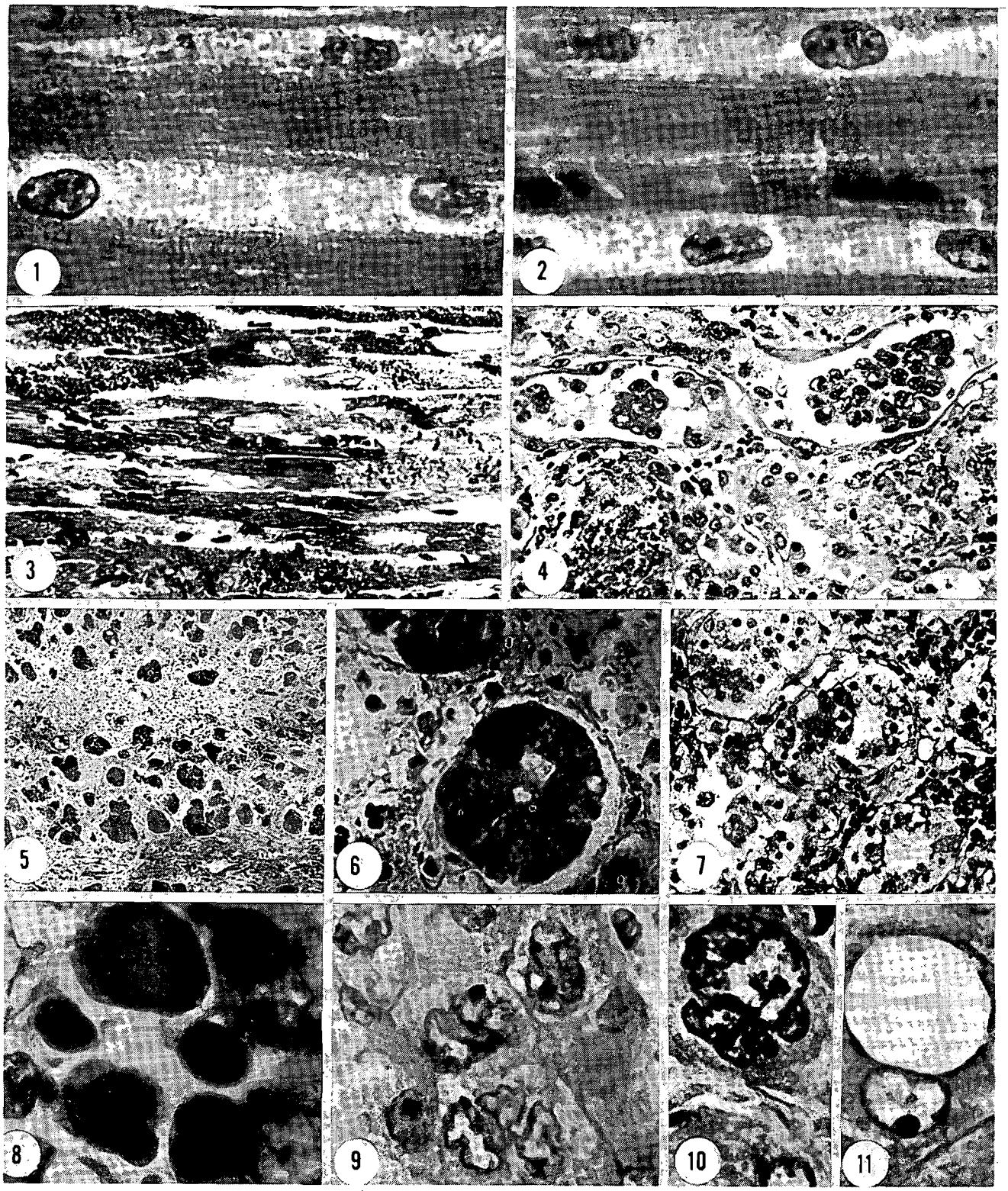
Macroscopic lesions were a slight icterus, an enlarged orange-yellow liver which was firmer than normal in consistency, but not as firm as in the case of the former pig. The bile was a light green (resembling unripe grapes) and of jelly-like consistency. The kidneys were enlarged and a light biscuit-brown colour.

Pig 3 was killed for necropsy on the third day post toxin administration. Macroscopically the liver was a slightly paler brown than normal with bile resembling that of the former pig.

Pig 4 weighed 34½ kg before feeding of toxic material and was killed on Day 5 after showing anorexia for 2 days and having lost 1½ kg of body weight. Macroscopically there was a conspicuous icterus best observed in the intima of the large blood vessels. The lymph and joint fluid were a picric acid yellow. The liver had a firmer consistency and the lobulation was more distinct than normal with the lobules having a brown central portion and greyish periphery. The bile was a clear dark yellow colour with an orange granular deposit. The kidneys were a light fawn. Despite the anorexia clinically the stomach was well filled with food on examination.

HISTOPATHOLOGY

The histopathological changes of the livers of Pigs 1 and 2 were very similar — the difference being mainly a matter of degree. The lobular architecture was not disturbed although the parenchyma within the lobules was disrupted. Focal areas of leukocytic infiltration occurred in the portal areas as well as in the lobules especially in Pig 2. Fatty changes of the hepatocytes were very mild and bile duct proliferation within the lobule was present but inconspicuous. The liver of Pig 1 especially showed an increase of collagen and fibroplasia in the portal areas. These were accompanied by some bile duct epithelial cell hyperplasia. The normal cord-like arrangement of hepatocytes was disturbed, the sinusoids being compressed by encroaching hepatocytes in many instances. There was a marked variation in the degree in which hepatocytes stained with H & E. The cytoplasm of some was very granular and light pink, in others it was intensely and homogenously eosinophilic or it contained basophilic blotches. The nuclei presented even a greater variation. Many cells were binucleate or multi-nucleate with up to six nuclei per cell. The nuclei differed markedly in size, even in the same hepatocyte. Most nuclei were round with a distinct basophilic nuclear membrane, vesicular nucleoplasm and one or more distinct nucleoli. Some had large, bizarre shapes and gave the impression of "budding" as described by Petterson & Coackly[15] while others contained distinct pink pseudo-inclusions which resulted from cytoplasmic invagination. Some nuclei on the other hand were pyknotic and undergoing karyorrhexis. A moderate number of hepatocytes contained round, discrete or multiple, small eosinophilic globules in the cytoplasm while in others there was a large eosinophilic mass lying in a vacuole — in some cases containing pyknotic nuclear remnants. In general the centrilobular hepatocytes were smaller than the more peripherally situated ones. Among these central cells were some which contained conglomerates of a nongranular brown pigment



Figs. 1&2 Myocardial degeneration in Pig 3. H&E X 1200

Fig. 3 Myocardial degeneration and calcification in the pig used in the pilot experiment. H&E X 250

Fig. 4 Kidney of Pig F4 showing necrosis and desquamation of tubular epithelium with cast formation. H&E X 250

Fig. 5 Liver of Pig F6 showing grouping of hepatocytes into acinar structures. H&E X 80

Fig. 6 Higher magnification of Fig. 5. H&E X 250

Fig. 7 Liver of the pig used in the pilot experiment showing acinar arrangement of hepatocytes. H&E X 250

Fig. 8 Large eosinophilic globules in hepatocytic cytoplasm of Pig F3. H&E X 1200

Fig. 9 Crenated nuclei with nuclear division in Pig F5. H&E X 1200

Fig. 10 Large "budding" nucleus from the kidney of Pig F4. H&E X 1200

Fig. 11 Binucleate hepatocyte with one large vesicular nucleus in Pig F3. H&E X 1200

resembling bile in bile canaliculi in the cytoplasm. This pigment was negative for stainable iron. Its nature could not be determined.

The kidney of Pig 1 revealed a large percentage of cells with mitotic figures in the tubular epithelium.

The histopathological changes in the liver of Pig 3 were similar to those of the foregoing, but were not quite as extensive. There was no increase in connective tissue, and the nuclear changes were mostly necrotic in nature, namely pyknosis and karyorrhexis — the latter frequently resembling mitotic figures. In the kidney several epithelial cells in the convoluted tubules exhibited mitoses. The cytoplasm of the majority of these cells was swollen and had a granular appearance. Under high magnification small clear intracytoplasmic clefts could be determined in many of them. This was interpreted as being a sign of early hydropic degeneration. In the myocardium were numerous focal areas involving groups of muscle fibres where small eosinophilic droplets had formed around and in the vicinity of the nuclei (Figs. 1 & 2).

In the liver of Pig 4 small aggregations of monocytic cells and a few neutrophiles were seen in some lobules. The centrilobular cells were atrophic. A degree of leukostasis also occurred in the central zone. The most conspicuous change occurred in the peripheral areas. Numerous hepatocytes had very pyknotic nuclei and a cytoplasm which was condensed and eosinophilic around the nucleus but clearer at the periphery of the cell. Some, however, were frankly karyorrhectic. The nuclei of some liver cells were enlarged and bizzare in shape and many cells were binucleate or even multinucleate. The nuclei in these cells were mostly of normal size while in some a very clear margin and large distinct nucleolus was the only abnormality seen. Several hepatocytes contained distinct single, golden-brown pigment globules in their cytoplasm while in others rounded-off masses of eosinophilic cytoplasm were present, some containing nuclear debris. Fatty change was not observed but there was a slight increase in fibrous connective tissue between lobules, as well as in bile duct epithelial cells and bile ducts in some portal areas. The latter was not conspicuous.

The kidney in this case was severely affected: the majority of epithelial cells in the convoluted tubules showing granular eosinophilic cytoplasm with pyknotic or karyorrhectic nuclei. A few cells contained, as in the liver, rounded masses of necrotic cytoplasmic and nuclear debris in their cytoplasm. In many areas necrotic cells had desquamated and been replaced by regenerating epithelial cells.

The two pigs kept as controls were normal in all respects.

EXPERIMENT II

CLINICAL OBSERVATIONS

Of the two pigs receiving 10 g/kg of toxic material five times a week, Pig F1 showed a greater tolerance. Anorexia was first noticed in Pig F2 from the 3rd day and was complete by Day 5. From the 4th day scleral icterus was apparent. By the afternoon of the 6th day it was found to be very weak and staggered around in its pen, being partially inco-ordinated. Respiration was laboured and it made weak moaning sounds. It died that night. Its weight dropped from 39 to 38 kg during this time. Pig F1 was still feeding a little by the 8th day and was reasonably lively. By the 18th day it was very icteric, listless, anorectic and showed polydipsia. Weight loss amounted to 3 kg from Day 1 to 12 when it was killed *in extremis*.

Pigs F3 and F4 weighed 40 and 41 kg respectively at the commencement of toxin administration. One week later both showed severe loss of weight and weighed 34 and 37 kg respectively. Anorexia was more pronounced in Pig F4 than Pig F3 at this stage, and by the 10th day it remained prone most of the time and was inappetent. Its voice was much weaker than normal. By the 11th day a very slight icterus was detectable in the sclera. It became gradually weaker, drank but did not eat and died during the night of the 13th day when it weighed 30 kg, *i.e.*, a loss of 11 kg over a 13 day period.

Pig F3 showed severe polydipsia from the 8th day while anorexia increased. On the 14th day it weighed 28 kg, *i.e.* a loss of 11 kg over the 2 week period which was identical to that of Pig F4. By the 16th day anorexia was complete, it only drank water and was jaundiced. On the 17th day it was *in extremis*, very icteric, and walked with swaying, partially incoordinated hindquarters. The ears and snout were intensely congested and cold to touch. It was killed for necropsy at this stage.

Pigs F5 and F6 weighed 36 and 41 kg respectively at the commencement of toxin administration. Pig F5 became less interested in feeding from the 5th day. On the 8th day it weighed 28 kg and appeared very weak and gaunt but no sign of icterus was present. It lingered on like this eating almost nothing and drinking very little water. The faeces became dry and hard. After 2 weeks it weighed only 23½ kg and by the 5th week, 18 kg. It eventually became dehydrated, was unable to get up unaided and showed weakness especially of the hindquarters and finally died after 6 weeks weighing only 16 kg. Pig F6 responded severely to the toxin and became anorectic on Day 5. It had lost 8 kg of weight during the first week and was very weak but not icteric. On Day 8 anorexia was complete, it had poor control over its hindlimbs and assumed a dog-sitting position. Toxin administration was suspended for 1 week and on Day 14 it appeared clinically normal and regained much of its weight, *i.e.*, it weighed 35 kg. At this stage toxin was again administered. The pig seemed in good health the following week and in fact gained more weight until it weighed almost 41 kg 4 weeks after the first administration. From then toxin was administered at weekly intervals. In the 6th week anorexia again set in. The hindquarters swayed when walking but the habitus remained good. Body weight dropped by 2 kg. Its condition deteriorated rapidly in the 7th week, the hindquarters became very inco-ordinated and eventually almost completely paralysed. The skin was very hyperaemic and tachycardia was present (its pulse rate was 164 per minute). At this stage it was killed for necropsy.

The control Pigs F7 and F8 did very well and gained weight from 41 to 47 kg and 41 to 45 kg respectively in the 1st week. Unfortunately Pig F7 suffered a blowfly strike in the surgical wound during the second week which led to peritonitis and it had to be discharged from the experiment. Pig 8 weighed 80 kg when it was slaughtered during the 7th week of the experiment. The autoclaved maize kernels did not have any

detrimental effect as far as could be ascertained. Details of variations in body weight of some of the pigs in Experiment II are given in Fig. 12.

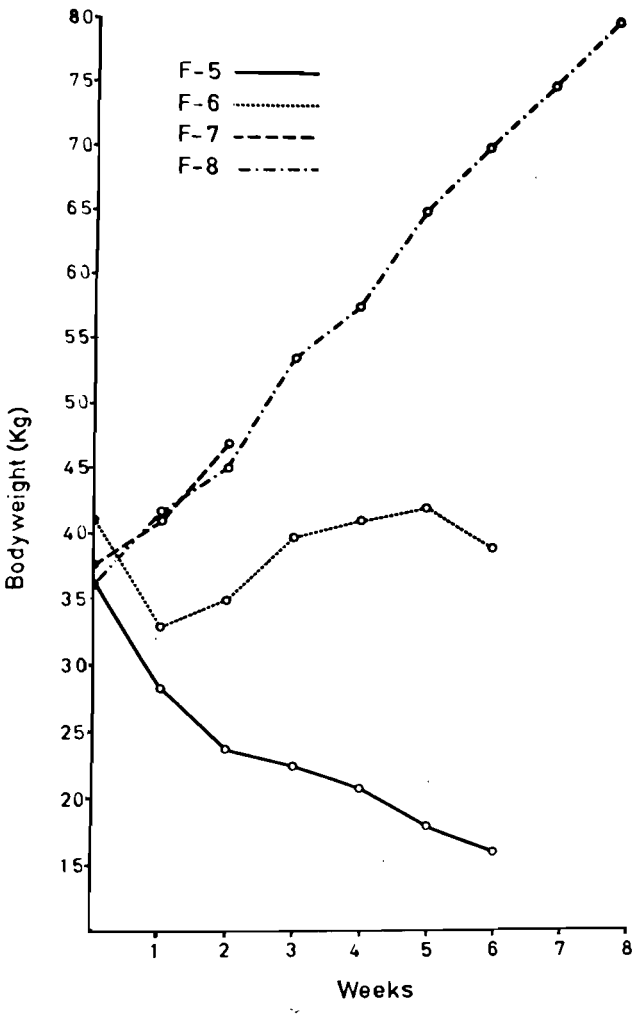


Fig. 12: Graphic presentation of changes in body weight encountered in pigs surviving more than 2 weeks in Experiment II.

GROSS PATHOLOGY

Pig F1 showed severe generalised icterus and had an orange-brown, atrophied liver which was increased in consistency. The bile was very viscous. There was a nephrosis, the kidneys being swollen and a very light brown colour. The urine was brownish yellow and contained a considerable quantity of yellow floccular precipitate. The small and large intestinal mucosa was diffusely hyperaemic. The carcass of Pig F2 was cyanotic, congested and icteric. The liver was not atrophic and the bile was yellow-green and more gel than fluid in nature with floccules in it. Its volume was not increased. The intestinal contents contained a small amount of free blood mixed with the contents. A moderate hydropericardium (± 25 ml) was present.

Of Pigs F3 and F4 the former lived ionger and showed a more severe terminal icterus which was especially conspicuous in the intima of the blood vessels and in the sclera and fascia layers which were ochre in colour. The skin was yellow, but the subcutaneous fat was more a dirty pink due to congestion and revealed little of the icterus. The liver was also ochre in colour, and was slightly atrophic with a slight increase in consistency as opposed to a normal-sized brown liver with distinct lobulation in Pig F4. The bile in both pigs was a very thick slimy material. Both pigs were constipated and had extensive suggilations in the intestinal wall while severe enterorrhagia was present only in F4. In both there was a mild nephrosis but the urine was normal in appearance.

The lesions in Pigs F5 and F6 differed considerably. Pig F5 was dehydrated, emaciated, icteric and showed haemoconcentration. Although the liver was slightly atrophic its consistency was not firmer than normal. Its colour was orange-brown. The bile resembled that of Pigs F2 and F4. Numerous focal ecchymoses occurred in the intestinal wall with much free blood in the lumen of the gut. The urine was brown (but no floccules were present in it.) Subendocardial ecchymoses were present. The carcass of Pig 6 was slightly icteric and had a bled-out anaemic appearance. There was a severe haemoperitoneum with many haemorrhages in the omentum and mesentery and subperitoneally on the diaphragm. Numerous haemorrhages of 0,5 — 1cm in diameter were disseminated throughout both lungs. No free blood was present in the intestine. The blood in the heart and elsewhere was unclotted at the time of necropsy despite an interim between death and autopsy of approximately 10 hours. The liver was a light orange-pink and the consistency was not increased. The bile was a yellow-orange, floccular viscous material. The urine contained a floccular sediment.

Pig 8 was normal in all respects except for the presence of the rubber fistula in the stomach.

HISTOPATHOLOGY

Generally speaking the histopathological changes encountered in the fistulated pigs were similar to those seen in Experiment I. It seemed therefore that the effect did not differ significantly whether toxin was administered as a single dose or repeatedly. In Pig F1 the larger bile ducts were filled with a pink proteinaceous mucoid like material. The kidney changes in Pigs F1 and F2 were of a similar nature but were much more severe than those in any other pig and furthermore were more advanced than the hepatic lesions in F2. The nuclei of some convoluted tubular epithelial cells contained pseudo-inclusions similar to those mentioned above in the liver while the cytoplasm was condensed into an eosinophilic globule in others. Some nuclei were enlarged. A few cells were binucleate. The changes therefore were similar to those observed in hepatocytes. The descending and ascending loops of Henle contained protein rich pink casts. The epithelium of the collecting tubules was hypertrophied and proliferative in areas — several cells having more than one nucleus. In some the nuclei were pyknotic while those of others were in mitosis. Several tubular epithelial cells had a foamy cytoplasm proximal to the nucleus. Necrotic cellular debris occurred in the lumen of several ducts.

In the myocardium of F1 were numerous focal areas of degeneration in which muscle fibres were more eosinophilic than normal and cross striations indistinct.

The lesions in Pigs F3 and F4 followed a similar pattern to the abovementioned in that the kidney of F4 was much more severely affected than the liver. In the liver of Pig F3 parenchymal cells containing

eosinophilic globules were more numerous than in any of the other pigs. The nuclei of several hepatocytes consisted merely of a rim of chromatin surrounding a clear space and contained a marginally situated nucleolus. The perinuclear cytoplasm in most cells was intensely eosinophilic and granular while at the periphery it had a more basophilic and foamy appearance. The major changes in the liver of Pig F4 included bi- and multinucleism, variation in nuclear size, and the presence of intranuclear pseudo-inclusions. Most nuclei exhibited irregularly folded membranes. The kidneys were more severely affected than the liver and showed degenerative and necrotic changes of the tubular epithelium with desquamation and the formation of cellular casts in the tubules resembling those of Pigs F1 and F2. Some desquamated epithelial cells, however, were not necrotic and occurred in clusters in the widened lumens of affected tubules. Mitotic figures were present in the cortex and medulla and it appeared as if most of the desquamated cells were replaced by flatter epithelial cells in a process of regeneration. Some cells contained single large or several smaller nuclei. Eosinophilic droplets occurred in the lumens of many tubules.

The myocardium of Pig F3 had focal areas of rarifaction and of more eosinophilic staining shrunken fibres.

In Pigs F5 and F6 the former showed lesions more of the acute type. In the liver the nuclear abberations were very conspicuous, some nuclei being large and irregularly shaped. The majority, however, had a crenated appearance and several contained large pseudo-inclusions. In the kidneys bilirubin casts were present in the lumens of several tubules and focal areas of slight fibrosis associated with a mild round cell infiltration occurred in the cortex. In Pig F6 the liver was severely affected and the lesions more or less resembled those of the pig examined in the pilot trial. Most hepatocytes had disappeared and only a few islands of these cells were left in the lobules. They were surrounded by loose connective tissue. These hepatocytes tended to assume an acinar arrangement (Figs. 5&6). Some contained fat droplets or yellow-brown pigment globules. Several were binucleate while others had large vesicular or large irregular shaped nuclei. Unfortunately *post mortem* changes were fairly advanced and obscured the renal pathology.

The mucosa of the gall bladder was focally infiltrated by round cells in Pigs F1 and F3. There was a severe congestion with focal microscopic haemorrhages in the brain and spinal cord of Pigs F2, F4, F5 and F6. Large focal haemorrhages occurred in the lungs of Pig F6.

DISCUSSION

These experiments have conclusively demonstrated that pigs are susceptible to intoxication by the mycotoxin produced by *P. leptostromiformis* although due to the anorexia produced by the toxin it is improbable that many acute deaths will occur under natural circumstances following ingestion of sublethal amounts. The growth stunting effect of the toxin is significant, however, and its presence in pig rations could lead to serious economical losses. It is therefore essential that lupins used for pig feed should be uncontaminated.

The results obtained in these experiments indicate that the mycotoxin affects primarily the liver, kidneys and myocardium of pigs. In animals receiving relatively large amounts of toxic material the hepatic changes were primarily degenerative and necrotic in nature together with severe nuclear abnormalities. An interesting observation was the rapidity of the development of hepatic fibroplasia following mycotoxin ingestion. In Pig F1 there was a definite fibrosis present within 7 days. It is also interesting to note that in pigs the toxin did not induce nearly as severe a degree of fatty change or bile duct proliferation in the livers as it does in sheep[1 4 5 6 18 19] cattle[1 2] and mice[3 4 7 10 13 14]. Liver reactions similar to those in other species, however, were the presence of pseudo-inclusions in the nuclei, binucleate cells, large bizarre nuclei and eosinophilic globules and pigment in the cytoplasm of hepatocytes. Those pigs exposed to smaller doses exhibited lesions of a more chronic nature manifested by the replacement of hepatocytes with anaplastic cells which tended to be arranged in acini. The renal changes were mainly necrosis of epithelium particularly of the proximal and distal convoluted tubules. The renal damage produced by the toxin is more severe in pigs than in any of the other animal species mentioned.

Only one reference has been encountered concerning the myocardial damage caused by the mycotoxin of *P. leptostromiformis* in sheep[19]. It has, however, been the experience of one of us (I.B.J.V.R.) that in the majority of experimental cases of intoxication with this mycotoxin in sheep, rabbits, guinea pigs and mice, severe myocardial lesions have been present. In this series of the disease in pigs six of the 11 animals used showed focal areas of myocardial hyalin degeneration and/or necrosis.

The liver lesions, particularly those of the animal in the pilot experiment in which the primitive appearance and acinar arrangement of hepatocytes as well as the nuclear changes of these cells were prominent, compel one to consider the possibility of this mycotoxin being carcinogenic. Pigs have recently been proved susceptible to the hepatocarcinogenic activity of aflatoxin [16].

No explanation can be offered for the finding that the weight loss induced by the toxin is not positively correlated to the dosage. Pigs F3 and F4 which received the "medium" dosage showed by far the most severe and rapid loss of weight.

No lesions were detected in the central nervous systems which could have accounted for the posterior paresis which was a constant and rather severe clinical sign.

The preliminary results obtained in these experiments do not indicate any difference between gilts and castrated males in susceptibility to the mycotoxin.

ACKNOWLEDGEMENTS

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CASE REPORT GEVALVERSLAG

SCORPION STINGS IN DOGS

D.J. THORNTON*

During the last 8 years eight cases of scorpion stings in dogs have been encountered in the Graaff-Reinet district. In all but one of these the owner had witnessed the incident, and the majority of them have occurred in times of drought in farm dogs, hunting dogs or dogs living in the outskirts of the town.

The most frequent history is that the dog is seen playing with a scorpion and then it suddenly receives a sting either on a paw of a front leg or a lip. The animals which I have examined within an hour of such an episode have shown no evidence of swelling or abrasion of the skin at the site of the sting although almost immediately after the accident has occurred they do manifest signs of localized pain, such as rubbing a leg against the muzzle in the case of a sting on the lip, or licking the affected area. Within 1 to 2 hours of receiving a sting on a paw, the animal shows lameness of the affected limb which soon progresses in severity to the "carrying leg" type. Shortly after this generalized signs are noticed: there is decided evidence of agony with continual crying and the animal can no longer stand and goes down to lie in a position of lateral recumbency showing "paddling" movements of its limbs and dyspnoea. In addition, at this stage, many of the animals have shown fits lasting 2 to 3 minutes and occurring at relatively regular intervals during which consciousness appears to be lost and involuntary defaecation, urination and vomition occur.

The first case I encountered was a Fox Terrier weighing about 8 kg. When first seen it was showing generalized signs which, despite treatment consisting of the intravenous administration of 5 ml Scorpion Antivenom (produced by the South African Institute for Medical Research), progressed until death supervened 4 to 6 hours after being stung.

Subsequent cases were treated as follows:

Ten ml of Scorpion Antivenom were injected parenterally as soon as possible, 5 ml (i.e. the contents of one vial) being administered intravenously while the remainder was given subcutaneously irrespective of the body mass of the dog (none of which have happened to have been very small). In addition, 1 ml Vecortenol (Ciba, containing 25 mg glucocorticosteroid/ml) was administered intramuscularly, 0,5 ml adrenalin (1:1 000 B.D.H.) subcutaneously, and 2 ml pethidine hydrochloride (Pethidine, Burroughs Wellcome 50 mg/ml) and 0,5 ml acetylpromazine (Boots, 2 mg/ml) intravenously. At times it has been necessary for me to obtain the Scorpion Antivenom from the local provincial hospital. All animals treated in this manner recovered completely within 6 to 12 hours.

The second case I encountered was a 10 year old Dachshund weighing about 14 kg. This animal enjoyed romping in the veld and was prone to dig up stones with its paws and teeth. The owner who farmed 64 km from Graaff-Reinet witnessed it being stung on the lip by a scorpion. Within an hour the dog exhibited typical signs and fits and was frothing at the mouth. I was consulted telephonically and in order to expedite specific treatment the owner and I met each other approximately half way between the farm and town. After treating the dog as described above, I returned to my consulting rooms with it in the car in order to keep it under observation. Six hours later the owner fetched the still partially sedated dog which made an uneventful further recovery.

In none of the eight animals did any evidence of local injury develop at the site of the sting at any stage. In cases where the actual deed of a scorpion stinging a dog is not witnessed, a diagnosis must therefore be made on the evidence presented of a localized pain particularly if a paw or a lip is involved as well as on the more generalized clinical signs described above.

ACKNOWLEDGEMENTS

I thank Prof. R.C. Tustin for assistance in preparation of the manuscript and Miss. L. Colyn for typing it.

* Private Practitioner, P.O. Box 219, Graaff-Reinet, 6280.

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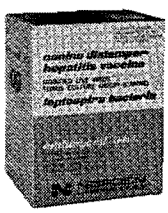

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TO THE EDITOR

AAN DIE REDAKSIE

EQUINE ELECTROCARDIOGRAPHY

Sir,

It was with great interest that I read the article entitled:"Some aspects of equine electrocardiography " by Johanna M. Kruger and W.L. Jenkins, which appeared in the Journal of South African Veterinary Association, vol. 45, P. 139 of 1974.

In this paper, under the heading, Sinus Arrhythmia, the authors mentioned that "if exercise does not abolish this form of arrhythmia, it is considered pathological in origin."

In my own investigations on equine cardiac rhythm using radiotelemetry which were carried out at the School of Veterinary Medicine, Bristol University in England, I encountered 46 horses demonstrating sinus arrhythmia at rest and/or after exercise.*

Interestingly enough, sinus arrhythmia, mostly in a transient form lasting on an average not more than 1½ minutes, was detected in 32 cases at the end of exercise. None of these horses showed any sign of heart disease or lack of stamina.

In 10 other horses, sinus arrhythmia was observed both at rest and after the end of exercise and these animals again showed no signs of cardiac disability.

In my opinion, post exercise sinus arrhythmia in the horse is due to readjustment of the autonomic nervous system and has no clinical significance.

Yours faithfully,
A. REZAKHANI
Department of Clinical Studies
School of Veterinary Medicine
Pahlavi University
Shiraz
IRAN.

* Reference: Rezakhani A. 1974 Studies on cardiac rhythm in the horse Ph.D. Thesis: University of Bristol.

TO THE EDITOR

AAN DIE REDAKSIE

VENIPUNCTURE OF CATTLE

Sir,

Dr. R. Every in the September 1973 issue of your journal recommended use of a jugular vein compressor for jugular venipuncture in the bovine animal.

Workers in this country have found the easiest, quickest way of collecting samples of bovine blood is to stab the ventral caudal vein, in the midline on the ventral aspect of the tail, with a fine scalpel blade. The handle end of the blade can be wrapped in the blade packet to provide a convenient, cheap handle, with which the blade can be safely held between thumb and forefinger. The fingers of the same hand are used to grasp a bottle in which the blood is collected. The other hand is used to manipulate the tail.

We have bled in excess of 150 animals in an hour on many occasions using this method. It is practical in all sizes of cattle. Blood samples collected in this manner are quite suitable for serological studies, as in brucellosis testing. No ill side effects have been observed in any cattle to date. We have bled many thousands of cattle this way: some as many as 18 times.

Yours faithfully,
C. MAYBERRY,
Department of Agriculture
Esperance, 6450,
Western Australia.

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Caution: The lid switch should not be used to start machine as its current switching capacity is not meant to be used instead of the timer.

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This useful head spins up to 12 micro tubes with an approximate G force 10,000 Centrifuges small quantities of blood or other liquids. To fit this head merely remove the two screws holding the standard head. Replace the screws and slip the micro head over the spindle. Will also fit existing centrifuges.

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This special Rotor has been produced to hold glass tubes of approximately 200 ul capacity. If further sampling is to be made of serum and/or cells after centrifugation it is possible to run the haematocrit in one slot and this larger tube in the adjacent immediate slot. The Macrotor can be purchased as an additional head for any new or existing centrifuges or as an alternative to the standard head in a new centrifuge. A Special reader for haematocrits taken in this larger tube is available on request.

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BOOK REVIEW BOEKRESENSIE

FISH AND SHELLFISH HYGIENE

Technical Report Series 550. Report of an expert committee, World Health Organisation, Geneva, 1974. Pp. 62, Tabs 2, Publ. Price: Sw. fr. 6.

This welcome addition to the wellknown Technical Report Series is published in collaboration with FAO. It deals concisely and expertly with a most interesting subject, providing up to date and practical information furnished by acknowledged experts from all over the world.

The Report is divided into the following sections: Environmental Factors, Principle Human Diseases, Principle Diseases of Aquatic Food Animals, Biotoxins of Marine Fish and Shellfish, Epidemiological Investigations, The Safe Handling of Fish and Shellfish, Special Considerations with reference to Molluscs, Use of Chemicals, *etc.* in the cultivation of Fish & Shellfish, Special Problems in Warm Climates and Developing Countries, Public Health Problems in International Trade, Fish and Shellfish Hygiene Inspection Services, Requirements for Training and Education, and finally Recommendations. The Report ends with two tables summarising the characteristics of Fish and Shellfish-borne Diseases in Man and a most useful Selected Bibliography.

The Report is highly recommended.

L.W. v.d. H.

BOOK REVIEW BOEKRESENSIE

LEHRBUCH DER ANATOMIE DER HAUSTIERE

BAND V. ANATOMIE DER HAUSVÖGEL

A. SCHUMMER

Paul Parey, Berlin, 1973. Pp. 215, Figs 215, Price: R26,00.

Traditionally avian anatomy is reserved for the back pages in textbooks of comparative veterinary anatomy where it is treated as an afterthought. Authoritative texts dealing with the subject from the veterinary point of view are practically non-existent. Those intended for students' use are outdated, vague and unreliable; the illustrations are poor and they all seem to have an inexhaustible source of reproductions of the protruded drake's penis for the edification of the readers. During the past few years textbooks in this style appeared on the Eastern German horizon and recently the book under review was published in the West. When our colleagues on that side of the *Oder-Neisser Linie* get this one in hand they will probably kick it aside and snort: *"Im Westen nichts Neues"* or perhaps, "That's for the birds". And in this manner of mood the reviewer started paging through the book well aware of the fact, however, that the author is held in high repute and that it is an eagerly awaited volume in a superb series of text books. He was given added courage when it was noticed that among the references occurred the name of a certain P.J. du Toit who even indulged in avian anatomy during his heyday.

On par with the sister volumes the book essentially displays the same properties which label them as text-books of quality, namely: clear and accurate diagrams, meticulous labelling, precise and unambiguous prose, references to the standard sources and a convenient index. The organ systems are described systematically with reference to the functional aspects and the adaptations peculiar to the species. Each chapter includes a little histology, but this is a flirtation rather than a serious affair. Excellent illustrations depict the abdominal topography. The coloured plates of the air sacs are beautifully produced and give the reader a clear concept of their topography and extent. The illustrations on the genital system, expecially the female tract, are disappointing. Very few students will be able to appreciate the oviduct of the laying hen and its lining from these illustrations. References cited are not necessarily covered in the text.

The German style is admirable, being concise and yet achieving a readability which is unusual in such works. Technically well produced, the book can be recommended unreservedly to students and veterinarians who have a good reading knowledge of German.

J.M.W. LE R.

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The new maximum 90 ordinary shares and 45 supplementary shares provide monthly sick pay benefits up to R1 255 and in the event of permanent incapacity an annual income of approximately R10 000.

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' A variable premium rate is now introduced and rises from $12\frac{1}{2}$ cents per R1 000 per month payable to age 30, to 15 cents from ages 30 to 40, 25 cents from 40 to 50 and 60 cents from 50 to 65.

With the increase in ordinary shares the amount of life cover available under the scheme rises to R90 000.

These marked improvements in the Group Life Scheme are the result of the very favourable experience ever since it was launched 16 years ago and come at a time when higher protection for the family becomes increasingly necessary.

For further information write to the General Manager, P.O. Box 6268, Johannesburg 2000.

VERHOOGDE SEKURITEIT VIR DIE PROFESSIES

Belangrike verhogings in voordele om te voorsien in die professies se behoefte vir verhoogde sekuriteit, is onlangs deur die Professionele Voorsieningsvereniging aangekondig.

Stygende inkomstes volgende op inflasie vereis verhoogde beskerming van verdienvermoë, en om hierdie behoefte te bevredig het die Vereniging die aantal aandele waarvoor 'n lid kan inteken, verhoog.

Die nuwe maksimum 90 gewone aandele en 45 aanvullende aandele verskaf maandelikse siektevoordele tot R1 255, en in die geval van blywende ongeskiktheid, 'n jaarlikse inkomste van ongeveer R10 000.

Ingeval van siekte of ongeluk is die professionele man, afhanklik van sy vermoë om te praktiseer, veral vatbaar. Sy eerste sorg moet dus wees vir die beskerming van sy inkomste, waarvan die handhawing van sy lewensstandaard, voldoende voorsiening vir sy aftrede en die allerbelangrike beskerming vir sy gesin in die geval van sy dood afhang.

Aansienlik verhoogde beskerming vir sy gesin teen aanmerklik verminderde premietariewe word verskaf deur die Vereniging se gewysigde Groeplewensversekeringskema. Die lewensversekering deur bestaande lede onder die skema gehou, word $2\frac{1}{2}$ maal verhoog vanaf 1 Augustus 1975 sonder bewys van versekerbaarheid, en alle lede onder ouderdom 50 sal op hierdie verhoogde beskerming in werklikheid minder betaal as wat hulle vir hul huidige dekking betaal.

'n Wisselende premietarief is nou ingestel en styg vanaf $12\frac{1}{2}$ sent per R1 000 per maand betaalbaar tot ouderdom 30, tot 15 sent vanaf ouderdom 30 tot 40, 25 sent van 40 tot 50, en 60 sent vanaf 50 tot 65.

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Hierdie aanmerklike verbeterings in die Groeplewensversekeringskema is die gevolg van die heel gunstige ondervinding sedert sy totstandkoming 16 jaar gelede, en kom op 'n tyd wanneer hoër beskerming vir die gesin al hoe meer nodig word.

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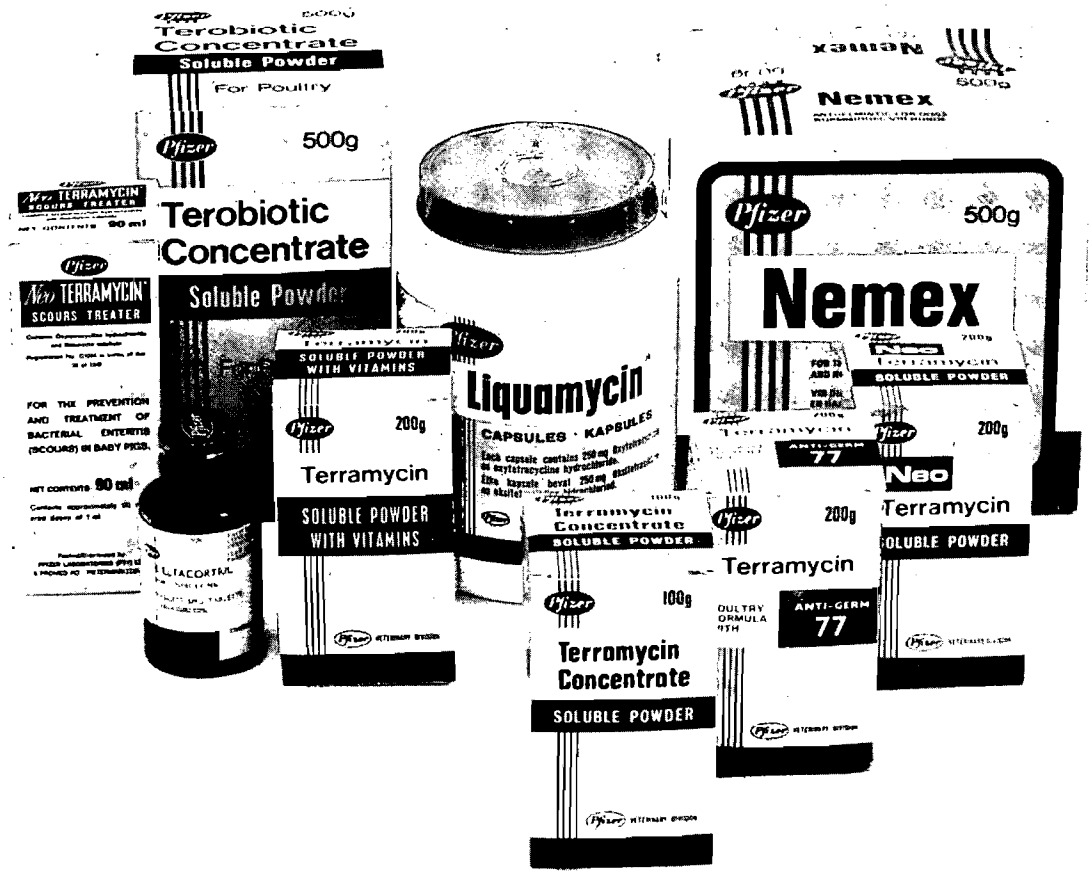

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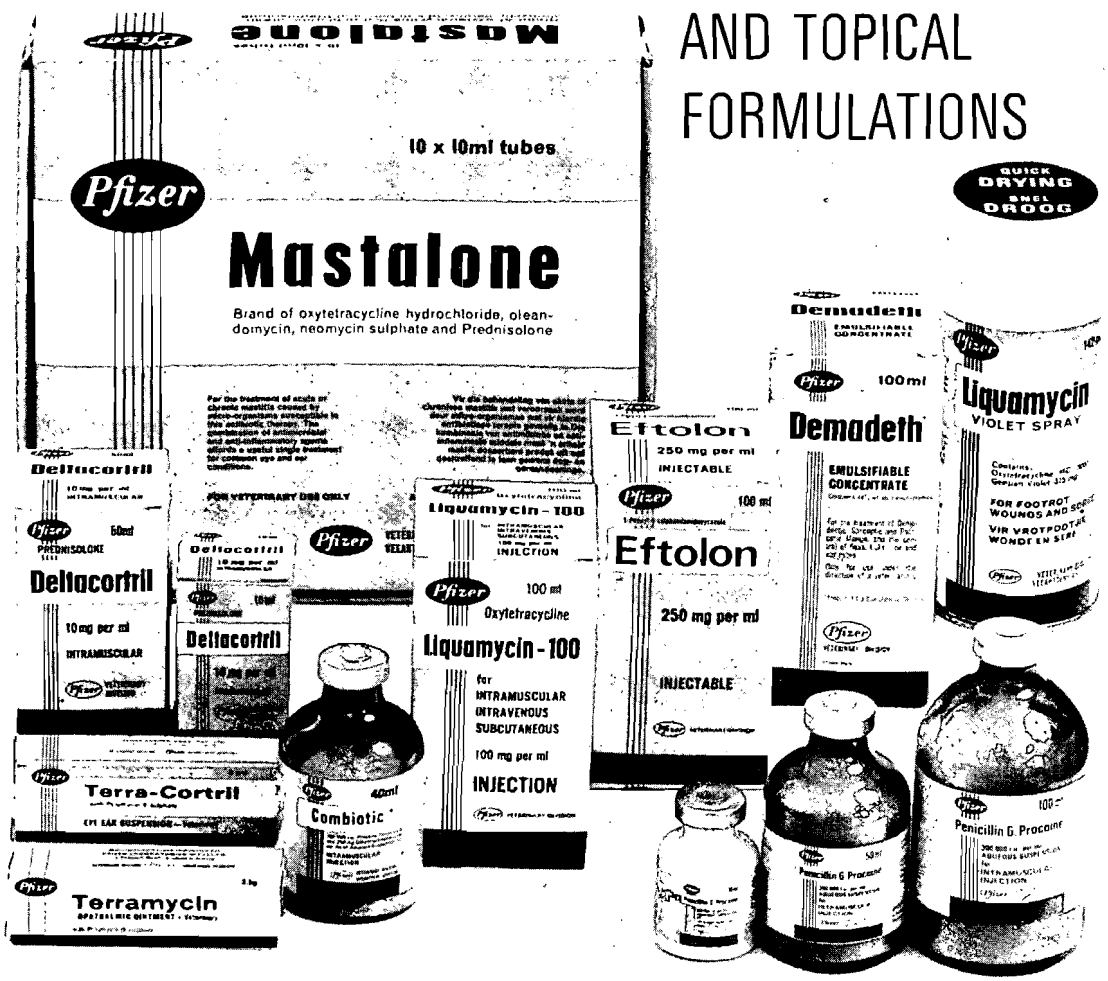

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IN MEMORIAM

PETRUS LAFRAS UYS
4.3.1919 - 27.4.1975

Petrus Lafras Uys is op die 4de Maart 1919 op die plaas Sybrandskraal, distrik Pretoria gebore; as kleinkind van wyle Kommandant Petrus Lafras Uys, het hy behoort aan een van die vooraanstaande Voortrekkerfamilies in Transvaal.

Gedurende sy jeug het hy hom bevind op die familieplase in die Transvaalse bosveld, waar hy te doen gehad het met die verskillende veesiektes wat in die omgewing voorgekom het, diè vroeë ondervindings het sy belangstelling geprikkel ten einde hom te bekwaam as veearts.

Hy het sy matrikulasie-eksamen aan die Hoërskool Bronkhorstspruit afgelê en die B.V.Sc. graad verwerf aan die Universiteit van Pretoria in 1943.

Vanaf 1944 was hy Staatsveearts in Dundee, waar hy met mej. Rina Fourie getroud is. In 1946 het hy tot die privaatpraktyk te Potchefstroom toegetree. In 1949 is hy aangestel as Staatsveearts te Potgietersrus, waar hy onder andere verantwoordelik was vir die eerste vasstelling dat "*Pavetta harborii*" verantwoordelik was vir 'n grootskaalse uitbraak van gousiekte.

In 1954 word hy aangestel as Veearts van die Stadsraad van Pretoria waar hy twee jaar later bevorder is tot Senior Veearts, welke pos hy tot sy afsterwe beklee het.

Dwarsdeur sy loopbaan in Volksgesondheidsdienste het hy besonder belang gestel in dié gespesialiseerde rigting en was hy onder die eerste groep veeartse wat die Diploma Veterinêre Volksgesondheid verwerf het, toe dié na-graadse kursus vir die eerste keer in 1963 ingestel is.

Hy was 'n ywerige en pligsgetroue lid van die Witwatersrand en Pretoria Publieke Raadgewende Gesondheidskomitee waarvan hy in 1962 die voorsitter was. Hy het op verskeie van die liggaam se subkomitees gedien, insluitende die Verordeninge-subkomitee en die Melk-subkomitee, synde die sameroeper van laasgenoemde. Hy het die groep verteenwoordig op verskeie van die Suid-Afrikaanse Buro vir Standaarde se Komitees, wie se taak dit was om standaarde vas te lê vir toebehore wat betrekking het op die melknywerheid en ook gedien op die Standaard Melk Verordeninge-komitee, saamgeroep deur die Transvaalse Provinsiale Owerheid. Hy was 'n ywerige byeboer en kenner vir baie jare, tot oorgevoeligheid vir bysteke hom genoop het om hierdie voorliefte te staak. Innig lief om te hengel en 'n seun van die natuur wie se kennis van die Transvaalse bosveld aansteeklik was en 'n plesier vir enige persoon wat die voorreg gehad het om dit met hom te kon deel.

Hy was 'n besielde en getroue Kerkman van die Gereformeerde Kerk, wat hom na aan die hart gelê het en waaraan hy met liefde baie van sy tyd opgeoffer het en ywerig gedien het. Hy sal lank onthou word vir sy barmhartigheidsdade en die helpende hand wat hy daadwerklik uitgesteek het aan sovele.

Aan Rina, sy vrou, kinders – Petrus, Haneka en Louise, wil ons hiermee ons innige simpatie en medelye betuig.

IN MEMORIAM

G.A. KRONSBEIN
6.4.1913 — 16.8.1974

Gert August Kronsbein is op 6 April 1913 in Aus, Suidwes-Afrika gebore. Op eenjarige leeftyd het die gesin Duitsland toe vertrek met verlof. Kort na hulle aankoms breek die Eerste Wêreldoorlog uit. Terwyl sy vader gekommandeer is, het hy met sy moeder en broer by sy grootouers op die eeue-oue familieplaas van sy moeder gewoon. Hier het hy die plaaslewe en diere leer ken en liefkry.

In 1921 het die Kronsbein-gesin teruggekeer Suidwes toe waar sy vader in Grootfontein en Windhoek onderwys gegee het en waar hy ook skool gegaan het. Sy liefde vir en belangstelling in diere is gedurende vakansietye op sy ouers se plaas voortgesit sodat hy na die Abitur-eksamen besluit het om veearts te word.

In 1932 - 1933 het hy in Pretoria en Onderstepoort gestudeer. Deur middel van 'n beurs is hy Duitsland toe waar hy van 1934 - 1936 te Leipzig sy studies voort sit en in 1936 in die Staatseksamen slaag. Na praktiese werk in slagpale keer hy in 1937 terug Suidwes toe. Weens 'n tekort aan staatsveeartsposte in Suidwes bestuur hy in 1938 sy vader se plaas.

Na deelname aan 'n longsiekteveldtog aan die Okavango en die bestryding van Runderpes in Tansania is hy gedurende die oorlogsjare geïnterneer.

In 1946 is hy met mej. Elfriede Philipsen in die huwelik bevestig. Vanaf 1948 - 1957 was hy staatsveearts in Calvinia. Hier is drie van sy vyf kinders gebore.

Na deelname aan die Bek-en-Klouseerveldtog aan die Molopo van 1957 - 1958 en die dood van sy vader, het hy gevra om Suidwes toe verplaas te word waar hy van 1958 - 1962 op Gobabis gestasioneer was en aan die Bek-en-Klouseerveldtog deelgeneem het.

Die heengaan van sy sewejarige seun Heiner in 'n ongeluk kort na die aankoms in Gobabis was vir hom 'n harde slag.

Vanaf 1962 tot sy dood op 16 Augustus 1974 het Gert as Staatsveearts, Windhoek en as lektor vir Veeartsenykunde in Neudamm gewerk.

Hy word oorleef deur sy eggenote en vier kinders.

Die skrywer hiervan het vir Gert leer ken as student op Onderstepoort en was bevoorreg om jare lank saam met hom te werk in Suidwes-Afrika. Al sy kollegas het hom geken as 'n korrekte en pligsgetroue beampte en 'n ware vriend, en hy het hulle hoogste agting geniet.

Met al die verantwoordelikheid wat op hom gerus het was sy toewyding en energie 'n inspirasie vir almal om hom, en die geleentheid om hierdie eerbewys aan hom te betoon word waardeer.

TREFFERBLAD

FEATURE PAGE

TRAUMATIESE *DERMATITIS CRUSTOSA* VAN DIE BEESUIER

Met die ondersoek van 'n Fries melkkudde is drie mastitis negatiewe diere gevind met uitgebreide letsels aan die vel van die uier. Al drie diere was hoë produseerders, een in die 7de en 2 in hul 8ste laktasie. Die uiers was groot en deur die agterbene saamgedruk. In een geval was die aanhegting aan die buikwand goed en in die ander gevalle was die aanhegting los. Almal het ondertoe gestrek tot onder die tarsaalgewrigte. Die groot uiers het die koeie gestrem as hulle loop en veral die los aangehegte uiers het heelwat geswaai.

Die vel letsels is geïllustreer in die Figuur. Geen parasiete of virusse is gevind in skraapselondersoek nie. 'n Groot verskeidenheid bakterieë waaronder ook mastitogene soos *Escherichia coli, Bacillus* spp., *Corynebacterium* spp. en *Staphylococcus aureus,* het voorgekom. Herhaalde plaaslike antiseptiese behandeling het egter geen verbetering gebring nie.

Daar was klein areas van sereuse eksudaat en rowe, tussen in maar veral perifereel versprei tot areas van hiperkeratose, oppervlakkige ulserasies, hiperemie en bloeding. Die patologiese toestand was algeheel oppervlakkig en beperk tot areas van droë huid, opvallend sonder hare en met veelvuldige klein krakies en barsies. Die huid was verder elasties en vryelik beweeglik oor die orgaan en net effens verdik. Die huid aan die bene wat met die aangetaste areas van die uier kontak gemaak het, het geen soortgelyke letsels getoon nie, en was met kort stywe hare bedek.

Gedurende laktasie was die veltoestand beperk tot die laterale en kaudale dele waar die groot, diep en laaghangende uiers die meeste blootgestel was aan wrywing deur die binnekante van agterbene. Met opdroog van die koei het die letsels herstel behalwe vir areas van hiperkeratose aan die uier. Gedurende die edemateuse perinatale fase van uierontwikkeling het die toestand weer teruggekeer.

Hierdie patologiese toestand word beskryf as *dermatitis crustosa* van suiwer traumatiese oorsprong wat blykbaar veroorsaak word deur die aanhoudende en herhaalde wrywing van die binnekante van die bene teen die uier.

Insending: W.H. Giesecke, Navorsingsinstituut vir Veeartsenykunde, Onderstepoort 0110, en L.P. Theron, Posbus 103. Olifantsfontein 1665.

TRAUMATIC *DERMATITIS CRUSTOSA* OF THE BOVINE UDDER

During an investigation in a Friesland dairy herd three mastitis negative cows showed extensive lesions of the udder skin. In every instance the cows were high producers in their 7th (1 cow) and 8th (2 cows) lactations respectively, with udders that were voluminous and tightly compressed between the posterior extremities. One was attached firmly, the other two loosely to the abdominal wall; all extended appreciably below the tarsal joint. Due to their bulkiness the udders encumbered the cows when walking and the loose attachment particularly caused pendulous swaying.

The skin lesions are depicted above. Neither parasites nor viruses were revealed by examination of material scraped from the affected parts. Bacteriological culture yielded a wide variety of bacteria including mastitogens such as *Escherichia coli, Bacillus* spp., *Corynebacterium* spp. and *Staphylococcus aureus.* Repeated local antiseptic treatment was unsuccessful.

There were small areas of serous exudation and scab formation interspersed amongst, but particularly at the periphery of areas of focal hyperkeratosis, shallow ulcerations, hyperaemia and haemorrhage. The entire pathological condition was superficial and limited to areas of very dry chapped udder skin displaying multiple minute cracks and a marked loss of hair. The skin was otherwise elastic, freely moveable on the gland surface and only slightly thickened. The legs did not show any comparable lesions in the areas of contact with the udder but were covered by very short stiff hair.

During lactation the skin condition was confined to those lateral and caudal portions of the udder most exposed to rubbing of the voluminous, deep and rather pendulous udders against the inner side of the extremities. During the dry period the lesions healed except for some hyperkeratosis of the shrunken non-lactating udders. During the edematous peri-natal phase of udder development the condition returned.

The pathological condition represents a *dermatitis crustosa* of purely traumatic origin apparently associated with continuous recurrent rubbing of the inner aspects of the hindlegs against the udder.

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