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NOSEMATOSIS: REPORT OF A CANINE CASE IN THE REPUBLIC OF SOUTH AFRICA

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SUMMARY

The first case of nosematosis of dogs in the Republic of South Africa is reported. The most significant clinical features of the disease were blindness, ataxia and convulsions. The pathological changes included meningo-encephalitis with microgranulomas and glial nodules, associated with extensive thrombosis and haemorrhage; interstitial nephritis and focal granulomatous hepatitis. The spores of an organism identical to *Nosema cuniculi* were demonstrated in some of the lesions in the brain, kidneys, liver and spleen.

INTRODUCTION

A previously undescribed infectious disease which caused motor paralysis in rabbits was reported and the causal organism illustrated by Wright and Craighead in 1922 [1]. The protozoan organism was named *Encephalitozoon cuniculi* by Levaditi *et al* the following year [2]. The granulomatous lesion which is considered typical for the infection in the brain of the rabbit was illustrated in an earlier (1917) publication by Bull [3]. The world literature contains many subsequent accounts of the disease in rabbits, mice and other rodents. It was reported first in South Africa among rabbits and mice by Malherbe and Munday in 1958 [4]. The identical organism was recovered from the cerebrospinal fluid and urine of a Japanese boy having a febrile disease accompanied by cerebral symptoms [5]. The evidence presented is considered as conclusive of the infection of a human with encephalitozoa [6].

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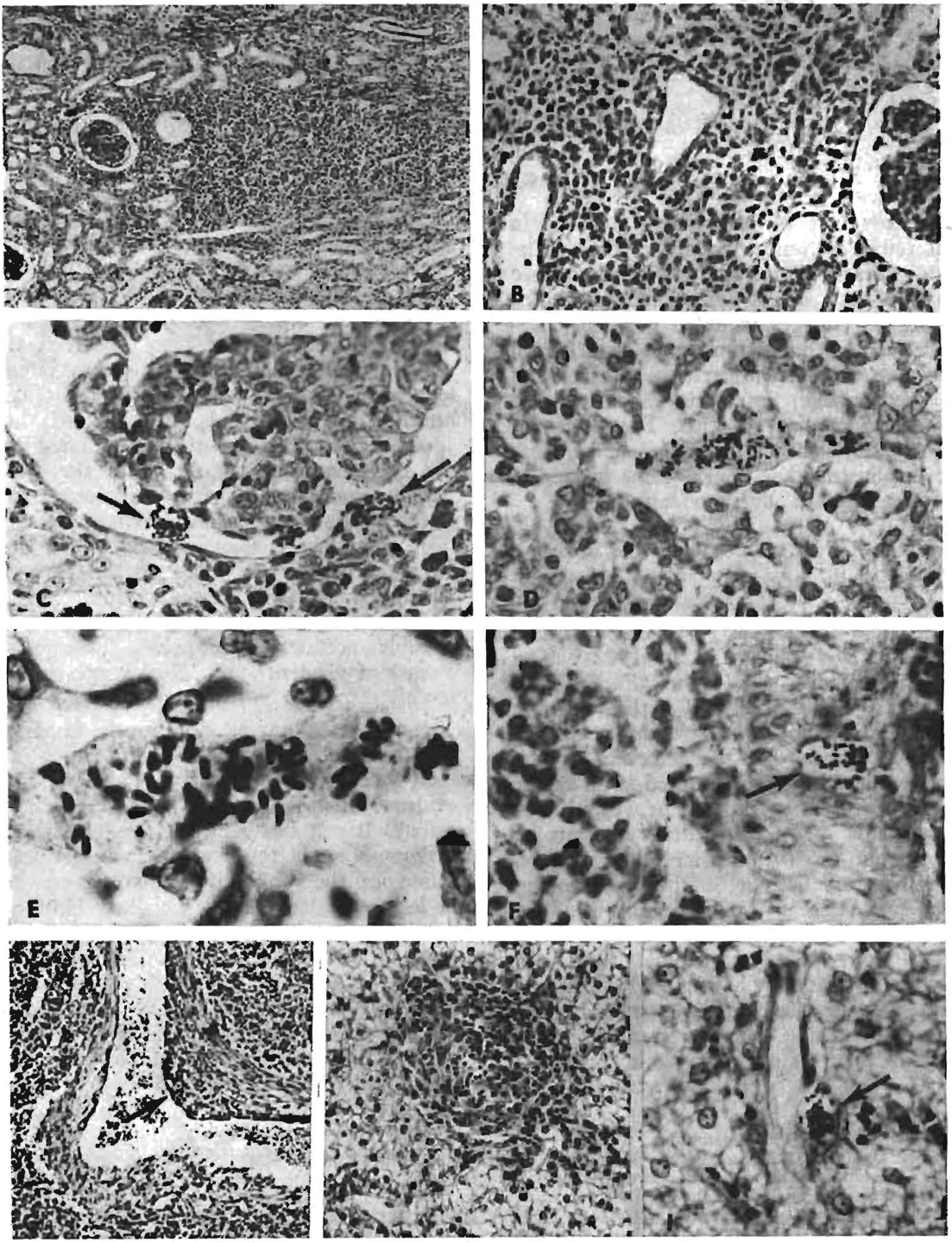
After studying the developmental cycle and the morphology of the organism electron-microscopically, Lainson *et al*[6] confirmed the microsporidial nature of "*Encephalitozoon*". They demonstrated that the Gram-positive forms of the organism seen in the tissue of the host were spores. They observed that under suitable conditions the spores extrude long polar filaments with infective sporoplasm attached to their ends. Because of these specific characteristics, they considered that the organism clearly belongs to the *Nosematidae* and proposed that the correct name of the one found in various animals, including man, should be *Nosema cuniculi*. They drew attention to the probability that nosematosis affecting man, with its source from rodents, is a zoonosis causing subpatent infections and possibly accounting for some undiagnosed disease of virulent type. They suggested that in this respect it parallels toxoplasmosis.

Innes *et al*[7] drew attention to the important fact that the disease could be present in mice used for experimental purposes and presented evidence of its occurrence in a "disease free" colony. It can be deduced from the lengthy bibliographies of this and other recent publications on the subject that it is very common in laboratory animals, especially rabbits and mice. Conversely, there are relatively few reports of the disease in dogs. The most convincing cases in dogs were reported by Plowright[8] and Plowright and Yeoman[9]. In his first report Plowright[8] described nephritis and meningo-encephalitis in three Foxhounds from a litter of six. Because of the history that pups of the bitch's previous litter were similarly affected clinically, the infection was thought possibly to have been acquired *in utero*.

Plowright[8] drew attention to previous reports by Kantorowics and Lewy[10] and Peters and Yamagiwa[11] on the occurrence of organ-



isms morphologically identical to *Encephalitozoon cuniculi* in sections of dog brains. Some of the descriptions and illustrations in these two earlier publications may be suspicious for *E. cuniculi*, but others are more suggestive of *Toxoplasma gondii*.

Material

Four puppies from a litter of seven showed convulsions and keratitis, became blind at the age of four weeks and had to be destroyed. Brain, liver, kidney and spleen from only one of these cases were collected in 10% formalin for histopathological examination. These tissues were embedded in paraffin wax, sectioned at 3 microns thickness and stained with haematoxylin and eosin (H. and E.), Giemsa, Gram's stain[12] and by the method of Wright and Craighead[1].

Results

Macroscopic

The examination of the fixed specimens of brain revealed marked congestion, haemorrhages and thrombosis of some of the vessels in the meninges and haemorrhagic foci and small areas of encephalomalacia in the brain substance. Other than splenomegaly with prominent Malpighian corpuscles, the remainder of the tissues did not show recognisable changes.

FIGURE 1

A. Nephritis involving both cortex and medulla. H. & E. X 75.6.

B. Inflammatory focus in the kidney with some plasma cells, lymphocytes and epithelioid cells. H. & E. X 192.

C. Renal corpuscle showing the presence of organisms in the parietal layer of Bowman's capsule and within Bowman's space (arrows). Gram. X 480.

D. Granulomatous reaction surrounding groups of intraepithelial organisms within the medulla of the kidney. Gram X 480.

E. The same groups of organisms shown in D. Gram X 1200.

F. A group of organisms apparently within the media of a trabecular artery in the spleen (arrow) Gram X 614.4.

G. Splenic vein with a focal round cell reaction in the intima and adjacent trabecular tissue (arrow). H. & E. X 100.8.

H. Typical medium-sized microgranuloma in the liver. H. & E. X 192.

I. Central vein of a liver lobule with a group of intraendothelial organisms (arrow). Gram X 480.

Microscopic

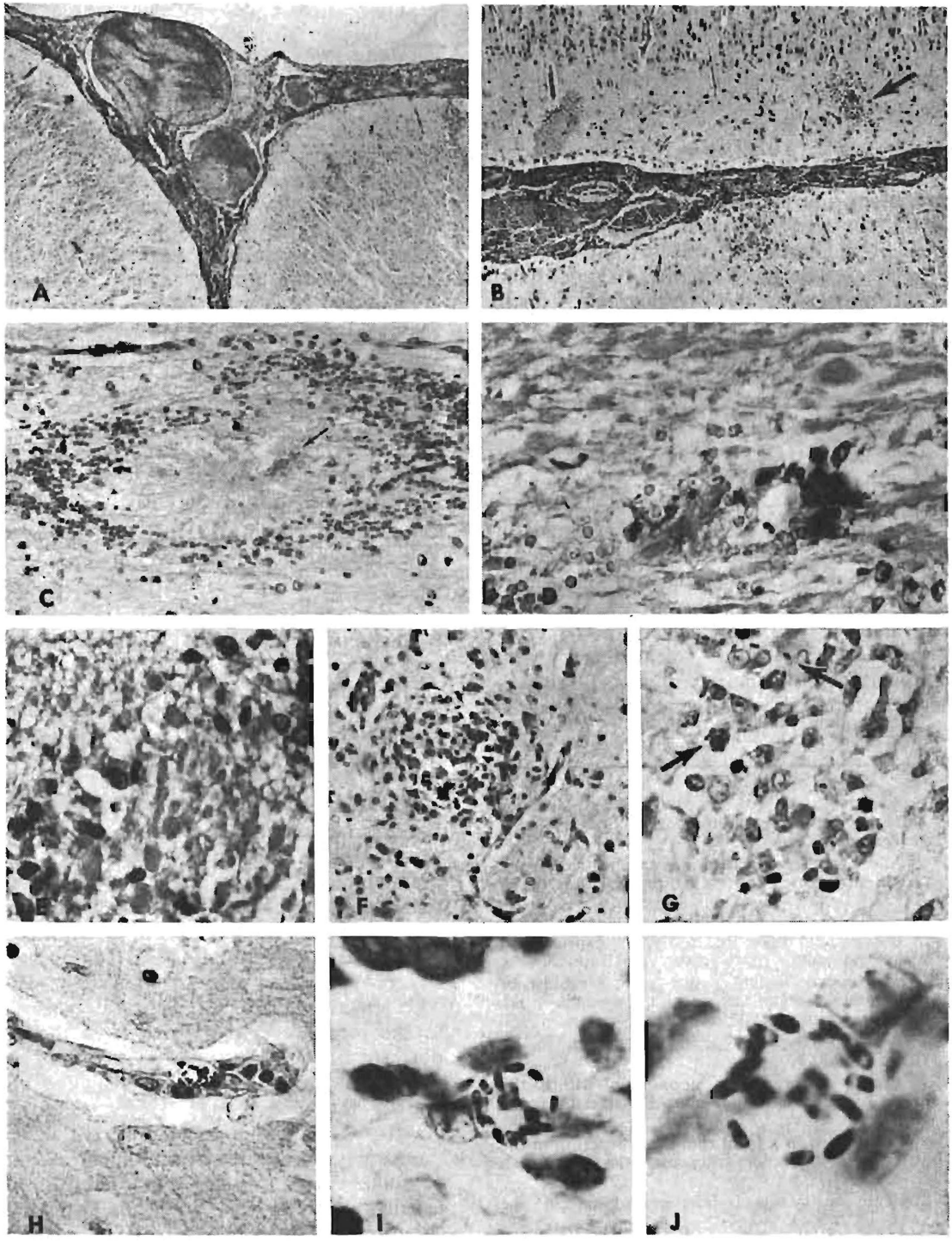
Kidney

There was interstitial nephritis extending from the cortex corticis into the medulla (Fig 1 A, B). The inflammatory cells were of mononuclear type with those of the lymphocytic series predominating. Plasma cells were numerous. The infiltrate, present mainly between tubules, was sometimes in close contact with the parietal layer of Bowman's capsule. In the medulla, particularly the outer zone, there were a number of areas where the tubules had been replaced largely by granulomatous reactions. With either the Gram or Giemsa stain numerous free, short, straight or very slightly curved, blunt-ended, cigar-shaped organisms (1μ by 2.3μ) were readily demonstrated in these granulomatous areas (Fig. 1 D, E). Similar organisms in clusters of ten or more were observed in the tubular epithelium, in the parietal layer of Bowman's capsule and within Bowman's space without any apparent host reaction (Fig. 1 C).

Brain

Sections of cerebrum including the hippocampus, cerebral peduncles, thalamus, cerebellum and medulla oblongata were examined. Lesions were present in all of the brain sections; the cerebral hemispheres being the most severely affected.

In sections of the cerebrum there were significant lesions in the leptomeninx and the vessels. There was an increased thickness of the leptomeninx due to the presence of numerous leucocytes of small and large mononuclear types, congested blood vessels and haemorrhage (Fig. 2 A, B). Many of the larger veins contained thrombi (Fig. 2 A). The affected portions of vessels were usually in the more superficial portion of the sulci, but some vessels were so involved that thrombosis extended over a portion of either one or the other adjacent gyri. Groups of Gram-positive organisms similar to those in the kidney were present in the endothelium of some of the vessels containing thrombi, usually, however, at a distance from the thrombi. Thrombi were sometimes present in veins in the depths of the sulci. Haemorrhages



in the meninges of the sulci as well as focal haemorrhage in the brain substance were common.

In the white matter of the cerebrum there were many necrotic capillaries with small fibrinoid thrombi (Fig. 2 D), many of which were surrounded by a zone of degeneration and necrosis varying from 30-120 microns in diameter and encircled by an outer layer of haemorrhage (Fig. 2 C). With H. and E. stain these affected zones of white matter were eosinophilic and either coarsely granular, roughly club-shaped or rather amorphous; some of these apparently represented swollen axis cylinders. Others were very similar, but had a radiating linear appearance and were probably of glial origin. Focal haemorrhage and foci of necrosis of similar nature without one accompanying the other were present, often near, but sometimes distant from visible bloodvessels. On a series of twenty sections from one block the organisms were not demonstrated within these foci. Much of the white matter showed focal accumulations of mildly eosinophilic, proteinaceous, oedematous substance, rarefaction and vacuolation which led to encephalomalacia in some areas. In one of these areas blood had accumulated to form a small haematoma. A few distinct foci of swollen axis cylinders were present (Fig. 2 E).

Focal areas of gliosis with some accompanying infiltration of lymphocytes or plasma cells were present in both gray and white matter. More circumscribed foci of cells of endothelioid or epithelioid nature were present also. Numerous individual organisms could be demonstrated in a few of these microgranulomas as well as in the areas of gliosis, either free or in macrophages (Fig. 2 G, I). Such areas of gliosis and the microgranulomas were often associated with small bloodvessels (Fig. 2 F, B). In a number of small blood vessels, colonies or groups of the Gram-positive organisms, without any detectable surrounding cyst wall, were present in the absence of a recognisable host response (Fig. 2 H).

The walls of some smaller vessels appeared more cellular than normal and there was an occasional cell in the Virchow-Robin space, but perivascular cuffing was not a prominent feature of the disease in this dog. A few neutrophiles were present in the white matter as well, probably in response to the necrosis.

In sections of brain other than the cerebrum, there were focal areas of haemorrhage, gliosis and microgranulomas.

Spleen

The Malpighian corpuscles were large and densely populated with small leucocytes. In the media of one of the trabecular arteries there were oval-shaped vacuoles containing numerous Gram-positive, cigar-shaped organisms (Fig. 1 F). They could be seen with the H. and E. stain as basophilic objects clustered together in a mass. There was no cellular response to such groups. The intima of several trabecular veins was hypercellular and contained small round cells which were also present in the adjacent connective tissue of the trabeculae (Fig. 1 G). No organisms were demonstrated in these lessions.

Liver

There was a very mild increase in the number of leucocytes around the branches of some hepatic and portal veins. The pro-

FIGURE 2

A. Marked thrombosis and haemorrhage in the leptomeninx. H. & E. X 30.

B. Haemorrhagic leptomeningitis seen within a sulcus with two adjacent glial nodules in the molecular layer (arrow). H. & E. X 75.6.

C. Cerebral white matter showing a necrotic fibrinoid capillary (arrow) surrounded by zones of degeneration and haemorrhage. H. & E. X 192.

D. Necrotic capillary containing a fibrinoid thrombus and surrounded by haemorrhage and swollen axis cylinders. H. & E. X 480.

E. Swollen axis cylinders in cross section. H. & E. X 480.

F. Microgranuloma in the brain in close proximity to a capillary. H. & E. X 192.

G. Small focus of reaction in the cerebrum containing scattered organisms (arrows). Gram X 480.

H. Small bloodvessel in the cerebral cortex with a group intraendothelial organisms unaccompanied by any host response. Gram X 480.

I. Another group of organisms with a mild cell reaction. Gram X 1200.

J. The same group of organisms showing the distinct polar vacuoles and other typical features of the organism. Gram X 1920.

minent lesions, however, were the multiple microgranulomas throughout the section (Fig. 1 H). There were no obvious restrictions to, nor preferences for any part of the liver lobule. Though the granulomas were somewhat circumscribed, they had no fibrous capsules. Their diameter varied between 100-150 microns. A group of causal organisms without any significant host reaction was demonstrated in one of the vessels (Fig. 1 I). None were noticeable in the microgranulomas. However, it must be pointed out that serial sections were not made.

No inclusions suggestive of canine distemper or infectious hepatitis were found in any of the sections.

Discussion

The findings in the present case were very similar to the first two cases reported by Plowright[8], the necropsy of his third case having been made at a much later stage. Practically without exception the significant findings in his first two cases were observed in the available tissues of the present case. Both the host response and the morphology of the organisms were identical. There were, however, additional prominent features not present in his cases. These specifically were the more marked thrombosis, encephalomalacia, swollen axis cylinders, the focal perivascular necrosis in the white matter and the presence of organisms additionally in the spleen and liver. Neuronal changes in the present case were of questionable significance. Organisms were also not demonstrated within any of them.

As in Plowright's cases, there was no isolation, no biological or other means of identification of the organism, but the combination of nephritic and meningo-encephalitic lesions supported by the demonstration in the affected tissues of Gram-positive organisms, morphologically identical to *Nosema cuniculi*, would seem to justify the diagnosis of a case of canine nosematosis (encephalitozoonosis). By its morphological characteristics and affinity for certain stains the causal organism can be differentiated from *Toxoplasma gondii*, the only other protozoon of the dog with which it is likely to be confused. The short, cigar-shaped spore with its rounded ends, polar vacuoles and affinity for Gram stain are typical of *Nosema* sp. (Fig. 2 I, J). Furthermore, no recognisable cyst walls were present around the groups or colonies of intraepithelial or intraendothelial organisms.

Because of the difficulty one experiences in finding the organism in H. and E. stained material, cases are likely to be missed unless Gram or Giemsa stains are applied.

Whether this disease in dogs is more common than previously realised remains to be determined. In all problematic cases of nephritis and encephalitis of dogs, especially puppies, nosematosis should be given consideration. Since the organisms are voided in urine[1], selective staining of urine sediments and intraperitoneal injection of mice[8] could aid in the clinical diagnosis.

Acknowledgements

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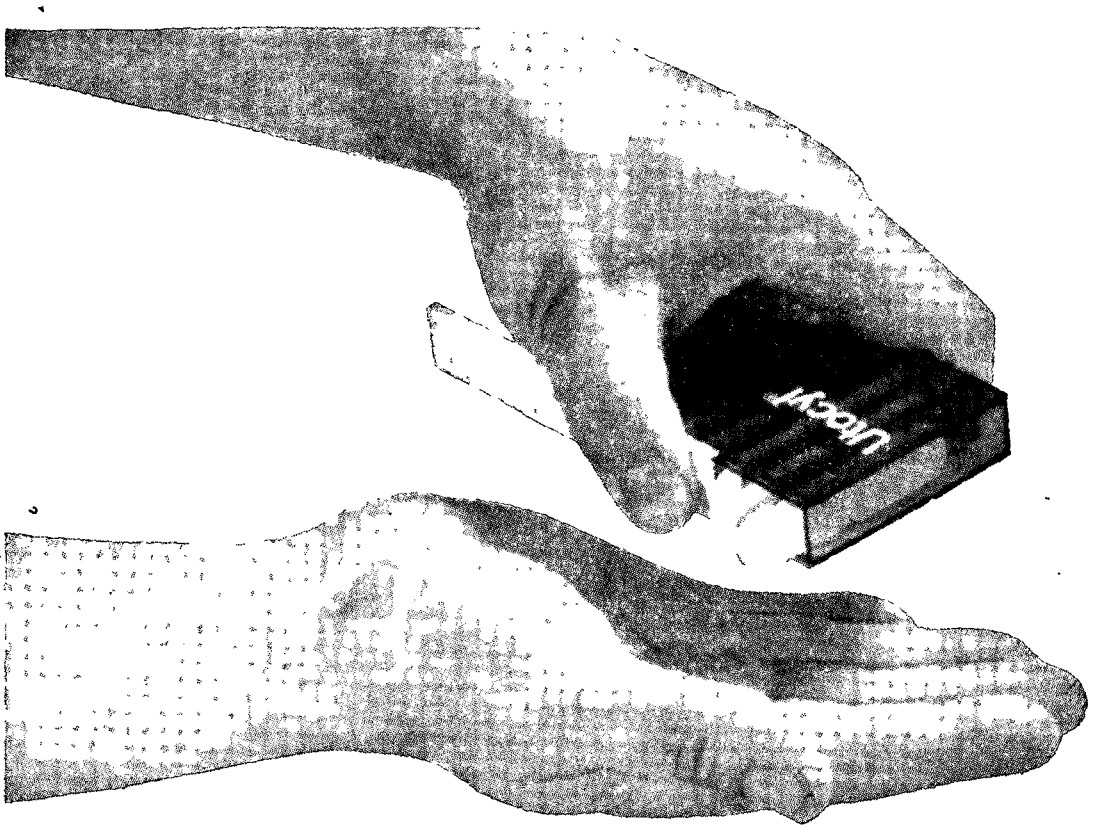

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CORYNEBACTERIUM PYOGENES: THE PROBLEM OF VACCINATION AGAINST INFECTION

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SUMMARY

An aerated liver meat broth was found to be an excellent medium for the cultivation of *Corynebacterium pyogenes* and potent high titre haemolytic toxin could be produced in Levy milk medium.

Immunity tests showed that mice cannot be satisfactorily protected against infection by recognised methods of immunization. A possible explanation is that immunity to *C. pyogenes* may be associated with a hypersensitivity reaction.

INTRODUCTION

Despite the importance of *C. pyogenes* as an animal pathogen[1], relatively little work has been done on producing immunity to it.

Lovell's[2] earlier work, primarily aimed at toxin production, was followed by experiments on antitoxin production in cattle using alum-precipitated toxoid. He found that the haemolysin and the mouse lethal toxin were identical and that toxin production in culture was maximal after 48 hours' incubation, a finding subsequently confirmed by Matthews and Derbyshire[3]. Lovell[4] obtained a good antibody response in sheep but the results in cattle were disappointing and he suggested that cattle were not easily immunized. Weitz[5] was however able to produce a good antitoxin response in cows.

In 1947, Weitz and Langridge[6] compared the protective value of toxoid, formalin-killed cells and a mixture of the two against mastitis in ewes. These workers were, however, able to show an increase in resistance only when a low intramammary challenge dose was used. The results were such that it was impossible to determine whether toxoid or cells were responsible for the degree of immunity obtained.

Extensive field trials with *C. pyogenes* toxoid in controlling bovine summer mastitis in England were most disappointing. Neither Weitz[7] nor Lovell and his co-workers[8] could show any decrease in the incidence of summer mastitis in thousands of cows over a period of four years.

Parvanta[9] and Derbyshire and Matthews[10] studied the immunization of mice against *C. pyogenes* infection. They compared the value of a number of different vaccines and, although they could demonstrate a slight increase in resistance to infection, were unable to produce a solid immunity.

The apparent inability to produce a satisfactory *C. pyogenes* vacine has a number of possible explanations.

The effect of strain differences, especially as they concern field trials, must be considered. Although *C. pyogenes* strains vary considerably as far as pathogenicity and toxin production are concerned,[2 3] Ryff and Browne[11] have conclusively shown that there is no serological difference among the strains.

In the earlier work especially, the vaccines used usually consisted of toxoid alone. Weitz[7] suggested that the poor results obtained might have been due to the low antigenicity of the toxoids used, or because the toxin was not the protective antigen.

The results obtained with killed bacterial cells were based on the ability of such a vaccine to protect mice against acute septicaemic infection, but in nature *C. pyogenes* causes chronic purulent infections and the re-

sults obtained in mice are not necessarily applicable in the field.

This study was therefore undertaken to examine methods suitable for producing potent toxin and to select a medium which would support a dense growth of cells.

The immunity experiments in mice were designed to determine whether toxoid or cells would give the best protection against purulent infections.

Materials And Methods

Media

The media, used in this study, were prepared according to the described techniques. [Bovine nutrient broth[12], Robinson's broth[13], Wright's broth[13], horse meat broth[14], Todd Hewitt broth[15], Lovell broth[4], Levy milk[16], liver meat broth[17], modified].

The latter medium was prepared by boiling 1.8 kg minced horse liver and 3.6 kg minced horse meat in 6 L. distilled water for one hour. The supernatant fluid was decanted and the pH adjusted to 8.0. Peptone (Bacteriological Oxoid) 1g/1; KH_2PO_4, 1.74 g/1 and $Na_2HPO_4.12H_2O$, 2.82 g/1 were added to the fluid which was boiled for a further 30 minutes. The pH was then adjusted to 7.8, the broth filtered through butter muslin and paper pulp and distributed in 500 ml quantities in 1 l Erlenmeyer or 4 L. Roux flasks. The medium was sterilized at 120°C for 45 minutes.

The production flasks containing the different broths were inoculated with the fluid from a tube of a 48-hour meat particle broth culture. The culture media were aerated through glass spargers with air or with 30% CO_2 in air. The large Roux flasks were either laid down flat for static cultures or agitated in a shaking machine. Eight flasks of each medium were used and the experiments were repeated three times.

The cell yield was estimated after 48 hours' incubation at 37°C. Cells, obtained from 5 ml of culture by centrifugation, were resuspended in 5 ml of saline, washed twice and the density compared with Brown's standard opacity tubes.

Haemolysin was titrated by the method described by Lovell[2].

Vaccine preparation

Toxin was produced from strain 14425 in small static Roux flasks containing 200 ml medium. After 48 hours' incubation the clear whey was decanted and filtered through paper pulp and Ford F.C.B. pads. The filtrate was then centrifuged at 3,000 g for 30 minutes to remove nearly all the cells and the haemolytic titre of the supernatant fluid found. As a rule the minimal haemolytic dose (m.h.d.) of toxin chosen for preparing toxoid lay between 0.001 ml and 0.002 ml. The filtrate, containing enough formalin to give a 0.5% concentration, was non-toxic and non-haemolytic after 48 hours' incubation at 37°C.

Cellular vaccine was prepared from agitated 48-hour liver meat cultures of strain 23402. The density usually corresponded to Brown's opacity tubes No. 8 or 9. The cells were killed by addition of formalin (final concentration 0.5%) to the cultures.

Combined vaccines were prepared by suspending the desired number of cells in toxoid.

Immunity experiments

Pathogenicity tests in mice were done by intraperitoneal injection of different numbers of organisms obtained from 48-hour blood tryptose agar cultures under 30% CO_2. Deaths were recorded daily and the survivors killed and autopsied six days later and examined for abscessation and purulent peritonitis.

In every experiment groups of fifty mice were used to test each vaccine. Each animal was given two injections, each of 2 ml, with an interval of three weeks between injections. The route varied according to the requirements of the various experiments.

The immunized mice were challenged ten days after the second injection by subcutaneous or intramuscular injection of 0.5 ml of a suspension of strain 23402 of an opacity corresponding to Brown's tube No. 6. Controls were challenged simultaneously. Deaths were recorded daily and the survivors were killed and autopsied after six days and examined for subcutaneous or intraperitoneal abscesses.

Experimental Results

Twenty strains which had been isolated from abscesses in cattle were examined in order to compare their toxigenicity. After repeated tests, strain 14425 proved to be the most consistent toxin producer and was selected for further experiments.

Preliminary experiments to find the influence of a number of variables on growth and toxin production were done with Lovell broth[4] without meat particles in Erlenmeyer flasks. Increasing or decreasing the incubation time of 48 hours and temperature of 37°C only decreased toxin production. Adjusting the pH and additional buffering of the medium also had no advantageous effect but the addition of horse serum (final concentration, 2.0%) did promote toxin production.

It was further established that although CO_2 had a slight stimulating effect on growth it was detrimental to toxin production. Toxin production in aerated flasks was decidedly better, but still rather low; the m.h.d. ranged from 0.25 ml to a maximum of 0.03 ml. It was also shown that the pH of the flasks through which 30% CO_2 and those through which air was bubbled dropped to the same extent. The detrimental effect of CO_2 on toxin production was therefore not due to excess acid formation. However, when the medium was aerated by shaking it in Roux flasks, a even better cell yield was obtained but without toxin production.

It thus became clear that conditions suitable for bacterial growth were not suitable for toxin production and that it would be necessary to separate the two processes.

The following media were compared for their ability to support growth: bovine nutrient broth, Robinson's broth, Wright's broth, horse meat broth, Todd Hewitt broth, Lovell broth and liver meat broth. They were all tested without meat particles in Roux shake flasks and it was clear that liver meat broth was considerably superior to any of the others. A cell density corresponding to Brown's opacity tube No. 9 was regularly obtained.

These broths were also tested with meat particles in static Erlenmeyer flasks for ability to promote toxin production and also compared with Levy milk. Toxin production in all the media containing meat particles was fair and the m.h.d. varied from 0.25 ml to 0.016 ml. Levy milk was however much superior and an average m.h.d. of 0.007 ml was obtained. Even better results were obtained with Levy milk in static Roux flasks laid flat. In large flasks with 500 ml medium the average m.h.d. was 0.007 ml and in small flasks with 200 ml medium it was 0.0025 ml.

Pathogenicity tests with a large number of strains showed that there were two pathogenic varieties. One, e.g. strain 11425, which was very toxigenic, caused acute death within 48 hours while the mice that survived seldom showed any purulent lesions; the other, e.g. strain 23402, produced acute deaths only when a very large number of organisms was injected but purulent lesions were common in the survivors. This difference is clearly shown in table 1.

Table 1.—Pathogenicity of two strains of *C. Pyogenes* for mice

Density Brown tube No.*	Strain					
	14425			23402		
	Acute death	Purulent infections	Negative	Acute death	Purulent infections	Negative
4	1/10	0/10	9/10	0/10	3/10	7/10
5	0/10	1/10	9/10	0/10	4/10	6/10
6	4/10	0/10	6/10	0/10	8/10	2/10
7	6/10	2/10	2/10	2/10	8/10	0/10

* Mice received bacterial suspension, of the Brown tube opacities noted, by the intraperitoneal route.

Strain 23402 was selected as challenge strain in the immunity experiments and was also used to prepare cellular vaccine.

The results of three experiments designed to determine whether toxoid or cells are mainly responsible for immunity, are given in table 2.

TABLE 2.—COMPARISON OF PROTECTIVE VALUE OF TOXOID AND KILLED CELL VACCINE

Vaccine	Infection rate %			Average % protection minus controls
	Experiment			
	1	2	3	
Toxoid	57.5	69.0	72.5	8.7
Bacterin	66.25	41.7	80.0	12.3
Toxoid plus Bacterin	56.2	45.84	77.37	15.2
None (*Controls*)	70.0	62.0	93.0	0

Mice were immunized subcutaneously and challenged intraperitoneally

Only a very mild increase in resistance was found using toxoid and killed cell vaccine. Even with a combination of the two, the demonstrable immunity was extremely poor and insignificant. An experiment was therefore conducted to find if the route of immunization would influence the development of immunity. As can be seen from table 3, demonstrable immunity could only be found when the mice had been immunized by the subcutaneous or intraperitoneal routes and challenged intraperitoneally.

TABLE 3.—COMPARISON OF PERCENTAGE INFECTION AND IMMUNITY AFTER IMMUNIZING AND CHALLENGING BY DIFFERENT ROUTES

Immunizing Route	Challenge route			
	Subcutaneous		Intraperitoneal	
	Infection rate %	% protection *minus control*	Infection rate %	% protection minus controls
Subcutaneous	78.3	—10.3	59.8	+13.2
Intramuscular	80.0	—12.0	82.5	— 9.5
Intraperitoneal	84.4	—16.4	56.3	+16.7
Controls	68	0.0	73	0.0

When the mice were challenged subcutaneously quite a different picture was found. Not only were more mice infected in the immunized than control groups but the subcutaneous abscesses were also more extensive.

CONCLUSIONS

Cultural conditions suitable for producing a dense growth of *C. pyogenes* are detrimental to toxin production. Toxin production is inhibited by carbon dioxide and excessive aeration but is stimulated by serum. Levy milk in static Roux flasks laid flat was found to be the most satisfactory medium for producing high-titre toxin.

A liver meat broth without meat particles is an excellent fluid medium for the production of dense cultures of *C. pyogenes*. The yield is greatly improved by aeration.

The immunity produced in mice is very poor and, in fact, certain groups show an increased susceptibility to challenge infection after immunization. This may be a typical Koch's phenomenon and suggests that im-

munity to *C. pyogenes* may be closely associated with a hypersensitivity reaction.

These results, substantiating those obtained by other workers, show that immunity to *C. pyogenes* cannot be obtained by conventional methods and, for this reason, a new approach must be sought.

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FATAL ENTEROBACTERIAL SEPTICAEMIA IN LAMBS

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SUMMARY

Fatal septicaemia affecting lambs from birth to eight weeks old is described and the symptomatology, incidence and economic importance briefly discussed.

Following the initial diagnosis in 1963, colisepticaemia has been reported from various sheep-raising areas in the Republic of South Africa and the available evidence indicates that the incidence of this highly fatal disease is increasing.

Colisepticaemia is caused by a large variety of *E. coli* serotypes with 078:B80 by far the most prevalent. It is characterized by severe nervous symptoms, meningitis with accumulation of excessive amounts of cerebro-spinal fluid, ascites, hydropericardium, endo- and epicardial haemorrhages, tumour hepatis without any marked gastro-intestinal disturbances.

Salmonellosis on the other hand is of little importance. *Salmonellae* were isolated as the only possible pathogens from specimens of two lambs representing an incidence of 4.3% out of a total of 46 lambs investigated. E. coli caused 87% and combined E. coli-salmonella infection was responsible for 8.7% of cases.

INTRODUCTION

Howarth[1] described a fatal septicaemic condition in adult sheep with abortions a very common phenomenon. The diagnosis of colisepticaemia subsequently made, was based on bacteriological examinations carried out on the genital tract of affected ewes as well as on foetal organs which yielded pure cultures of *Escherichia coli.*

Subsequently, fatal colisepticaemia in lambs was described in Australia[2,3,4], Britain[5] and in New Zealand[6]. The symptomatology described by these authors varied considerably especially as regards the age incidence.

Roberts[2] reported a fatal syndrome in lambs of four weeks of age, which resembled "white scours" very closely except that diarrhoea was not a constant diagnostic feature. These lambs showed nervous involvement manifested by arched backs and continuous paddling movements carried out with extended legs. At post-mortem, marked purulent meningo-encephalitis was a diagnostic lesion. *Escherichia coli* was isolated, in pure culture, from all the organs including the meningeal exudates. A year later the same author[3] encountered fatal infections in lambs suffering from arthritic lesions from which *E. coli* serotype 078 was isolated, also in pure culture.

Charles[4] confirmed the high incidence of fatal colisepticaemia in Australia affecting lambs 6 weeks to 8 weeks of age. Death usually followed 48 hours to 96 hours after the appearance of the first symptoms which included circular movements, monoplegia, lateral recumbency and paddling movements. Here, also, no evidence of diarrhoea was found. Pure cultures of *E. coli* were obtained from the brain tissues which were characterised by accumulation of excessive amounts of cloudy cerebrospinal fluid.

Sterlicki and Shaw[5] on the other hand described clinical cases of fatal colisepticaemia in lambs one to 3 days old. All lambs were born alive but a large percentage refused to suck and went down 24 hours later, struggling vigorously. At post-mortem, no gastro-intestinal lesions were found. One lamb showed extensive petechial, sub-endocardial and sub-epicardial haemorrhages, fibrinopurulent arthritis affecting all joints and con-

gested meninges accompanied by excessive amounts of cerebrospinal fluid. The second lamb, too weak to stand, showed a laterally flexed neck and weak paddling movements. *E. coli* 078 was isolated in pure culture from blood, heart, liver, joints and brain of the first lamb and also from the brain of the second.

S. enteritidis var. typhimurium has been described as the cause of severe losses in lambs in Australia and New Zealand[7] and in Cyprus[8]. The high incidence of *S. typhimurium* amongst sheep in New Zealand has again lately been emphasized[9].

S. enteritidis var. dublin on the other hand has not been described as the cause of fatal infections in lambs although mortality in young goats 1 to 8 weeks old[10] and abortion in ewes[11] has been associated with this serotype. In kids the disease resembled calf paratyphoid very closely viz. dullness, anorexia and diarrhoea[10].

During 1964/1965 mortalities varying from negligible up to 60 per cent had been reported from various parts of the Republic including the Orange Free State, Natal, Eastern Transvaal, the Karoo and the north eastern Cape areas. Specimens representing 42 of these logical examinations. Two acute outbreaks outbreaks were forwarded for routine bacterio affecting lambs within 12 to 24 hours of birth and 6 weeks to 8 weeks old respectively, were also investigated. From two additional farms diseased lambs, 3 to 6 weeks old, were submitted for clinical observations and detailed investigations.

Routine Bacteriological Examinations

Material and Methods.

Specimens consisting of brain, liver, spleen, lung and intestinal contents were forwarded in sterile containers either in 50 per cent glycerine in Selenite F enrichment medium or on ice. Specimens from lambs brought in for post-mortem were collected under sterile conditions and immediately subjected to detailed bacteriological examinations.

On arrival all specimens on ice, in glycerine or those freshly collected, were homogenized, a loopful directly plated on MacConkey agar while the remaining portions were suspended in Selenite F. After 24 hour incubation at 37°C a loopful of the enriched suspensions was streaked out on MacConkey agar and again incubated. Various representative morphological colonial growths, isolated from the MacConkey agar plates, were identified biochemically and serologically. Portions of lungs from lambs with pneumonic lesions were also examined for the presence of *Pasteurella* and *Corynebacterium.*

Pathogenicity Tests

All *E. coli* isolates were subjected to the rabbit gut segment test for detection of pathogenicity as described by De, Battacharya and Sarkar[12] and Taylor, Maltby and Payne[13]. Pathogenicity was determined by acute deaths as well as by macroscopical pathological changes in the gut segments. *E. coli* isolates were designated as belonging to pathogenic serogroups when the gut segments were extended contained muco-haemorrhagic exudate and showed acute inflammation of the mucous membrane.

Results

A minimum of 10 colonial isolates from a specimen from each lamb was examined, the results of which are recorded in Table 1.

Table 1.—*E. coli* and *Salmonella* isolated from diseased lambs representing 46 outbreaks of fatal septicaemia.

	Pure Culture	*Mixed Infections*	*Total (%)*
E. coli.....	36	7	93.4
Salmonella.....	2	4	6.6

1. *Colisepticaemia.*

Pathogenic *E. coli* were isolated from the specimens of 43 (93.4 per cent) lambs; from 36 (78.2 per cent) in pure culture and from 7 (15.2 per cent) in association with *Salmonellae, Pasteurella* as well as with members of the non-pathogenic *Enterobacter*-subgroup.

E. coli serotypes: The results of slide agglutination tests carried out on *E. coli* isolated from 29 lambs in O and K diagnostic sera using heat-killed and live cultures, respectively, are shown in table 2.

TABLE 2.—*E. coli* SEROGROUPS

No. of isolates serologically Identified[a]	E. coli serotypes						
	078:B80	0128:B12	026:B6	0101:K?	09:K?	035:K?	Unidentified[c]
29	13	3	2	2	1	1	7

a. Only 29 of *E. coli* isolated from 40 lambs were typed.
b. Preliminary identified as 0128:B12. Cross reactions occurred also with 0127 antiserum.
c. Non specific reactions in the available O and K diagnostic antisera.

Of the 22 *E. coli* isolates finally typed, 13 (59.1 per cent) belonged to serogroup 078:B80, 3 to 0128:B12, 2 to each of serogroups 026 and 0101, one to each of 09 and 035 while 7 remained serologically unidentified. These gave either non-specific reactions or did not agglutinate at all in any of the available diagnostic sera.

Clinical Investigations:

The high mortality rates, on two farms, effecting up to 50 per cent of lambs, were thoroughly investigated and representative specimens were subjected to detailed bacteriological examinations.

Case One:

This self-contained flock of about 1,000 sheep had been regularly vaccinated against bluetongue, enterotoxaemia and lamb dysentery. No mortality had occurred during the 1962 and 1963 lambing seasons. As was the custom, all pregnant ewes were brought in during April, 1964, from good quality veld grazing and put on green oats supplemented with a good quality hay. All ewes had free access to a bonemeal-salt lick as well as to a mixture consisting of mineral protein 16 per cent, phosphate 7 per cent, calcium 0.08 per cent with total fibre content not exeeding 15 per cent.

Lambing commenced on 20th April, 1964, and the first mortality occurred 7 days later when 8 (2 per cent) lambs born the previous day were found dead on the morning of the 27th April. The subsequent accumulated mortality figures which are shown in table 3 increased from 9% during May to 14% in June and 15% in July with a total loss of 39.5 per cent of the total lambcrop.

TABLE 3.—MORTALITY INCIDENCE OF LAMBS 12 HOURS TO 24 HOURS AFTER BIRTH. FIRST LAMB BORN ON 20.7.1964

	Cumulative mortality figures amongst 441 lambs born during April to July 1964				Total
	Period 27.4 to 30.4	1.5 to 31.5	1.6 to 30.6	1.7 to 31.7	
Total.....	8	98	53	15	174
Percentage.....	2	9	13.5	15	39.5

The mortality rate during the 1965 season was even higher; one lamb out of 14 born up to 30th April died, followed by 38 out of 216 born during the first 10 days of May. The mortality rate increased with the season resulting in a total loss of over 50 per cent.

Symptoms:

All lambs were born alive but a large percentage was too weak to suck or did not attempt to do so. Mortalities usually occurred during the night, 90 per cent within 12 to 24 hours after birth.

The few sick lambs seen alive developed immediately prior to death, severe nervous disorders which included ataxia, paresis, lateral recumbency, lateral or dorsal flexion of the neck, irregular paddling movements, continuous muscular twitching, groaning and frequent attempts at straining.

Post mortem examinations carried out on 20 lambs during the two seasons revealed:

1. Very mild gastro-intestinal lesions; the abomasums of all the lambs which died within 12 hours were empty, while some of the lambs 2 to 3 days old showed a slight enteritis with marked congestion of the mesenteric blood vessels of all lambs examined.
2. Marked *tumour hepatis* with acute stasis and degenerative changes.
3. Hydropericardium accompanied by petechial haemorrhages, subendo- and epicardially.
4. Acute oedema and emphysema of the lungs some of which showed subpleural haemorrhages especially on the dorsal aspects of the anterior lobes.
5. Acute congestion of the meninges accompanied by accumulation of excessive volumes of cerebro-spinal fluid.

Histo-pathological examinations carried out of brain, liver, heart and spleen revealed: Acute congestion of all the organs, sub-endocardial haemorrhages, massive concentration of gram-negative bacteria in the small blood vessels and surrounding tissues of the brain and liver.

Bacteriological:

E. coli, preliminary identified as serotype 0128:B12 was isolated from the liver, brain, spleen, heart and intestinal tract of all 7 lambs examined; from 5 lambs in pure culture and from the specimens of the remaining two in association with members of the *Enterobacter*-group.

Case Two:

A high mortality rate affecting lambs 6 weeks to 8 weeks of age was reported on a farm on the high veld of the Eastern Transvaal. From the specimens of 10 lambs submitted over a period of 30 days, *E. coli* 078:B80 was isolated in pure culture or in conjunction with *Pasteurella haemolytica* where pneumonia occurred as a complication.

As in the first case all deaths occurred during the night. Early in the season enteritis was a constant feature, which disappeared later on. All the lambs showed a mucopurulent discharge from the nostrils and some developed a cough. At post-mortem the most characteristic lesions were: Mucopurulent haemorrhagic sinusitis, acute congestion of meninges, tumour hepatis, hydrothorax, hydropericard, ascites with marked congestion of the mesenteric blood vessels. *Oestrus ovis* larvae were found in the sinuses of only one lamb which was presented in a state of complete paralysis. Two lambs showed in addition to the lesions described, extensive fibrinous pleuropneumonia.

Pathogenic *E. coli* 078:B80 was recovered from the organs including the nasal exudates of all 6 lambs while *Pasteurella haemolytica* was an additional isolate from the lung lesions of both lambs affected with pneumonia.

Other cases:

Subsequently, four additional outbreaks of fatal septicaemia have been reported, only 3 of which were confirmed bacteriologically. The salient feature of the unconfirmed outbreak, clinically diagnosed as *Clostridium welchi* infection, was abortion while a large percentage of the lambs born alive, died shortly afterwards. Specimens submitted for confirmation of the clinical diagnosis, yielded negative results but the characteristic nervous symptoms shown by some of the lambs shortly before death suggested colisepticaemia.

Specimens submitted from the other three outbreaks, yielded pure cultures of *E. coli* in two instances, while from the third *E. coli* and *Salmonella* were isolated. In the first of these outbreaks, mortality occurred at birth or within a few hours. *E. coli* was isolated from the brain, liver and faeces of two lambs submitted. These *E. coli* isolates apparently belong to the same pathogenic serogroup as identical non-specific agglutination reactions were obtained in the available OK-diagnostic antisera but no reactions at all when heat-killed cultures were tested in the corresponding O antisera. As in the case of unidentified *E. coli* isolated elsewhere, final

typing of this isolate depends on detailed serological tests to be carried out with a complete range of diagnostic antisera which are at present being produced.

Pathogenic *E. coli* 078:B80 as well as *S. enteritidis* var. *dublin* were isolated from specimens of the second outbreak where mortality occurred within one hour after appearance of the first symptoms. The symptoms shown in these lambs, which were affected at the age of 4 weeks, included swaying movements ataxia, torticollis and lateral recumbency, with only a few showing diarrhoea.

E. coli 078:B80 was also isolated, in pure culture, from the third outbreak where purulent meningitis appeared as a constant post-mortem lesion in the affected 4 to 6 week old lambs.

Experimental:

Two groups of 4 lambs each were exposed to *E. coli* 078:B80 and 0128:B12 isolated from natural outbreaks of colisepticaemia affecting lambs 4 weeks to 8 weeks and 12 hours to 24 hours old, respectively. Each serotype was administered intranasally into 4 lambs, 2 of which were 24 hours old and 2, 4 weeks of age. Each lamb received 1.0 ml of a 24-hour broth culture, carefully administered in 0.5 ml quantities into the posterior nasal cavities. Two lambs in each group were used as negative controls while a further two received 1.0 ml of a non-pathogenic *E. coli* culture.

Results:

The effect of age on susceptibility to *E. coli* serotypes is evident from the results shown in table 4.

TABLE 4.—EXPERIMENTAL TRANSMISSION OF COLISEPTICAEMIA. RESULTS OF EXPOSING LAMBS AT DIFFERENT AGES TO *E. coli* 078:B80 AND 0128:B12, INTRANASALLY

	Lambs 24 hours old			Lambs 4 weeks old		
	078:B80	0128:B12	Controls*	078:B80	0128:B12	Controls*
No. exposed	2	2	4	2	2	4
Morbidity (%)	50	50	0	100	0	0
Mortality (%)	50	50	0	50	0	0
Transmission (%)	50	50	0	100	0	0

*Half (2) was exposed to non-pathogenic *E. coli* and the other half (2) was unexposed.

E. coli 078:B80: Both lambs exposed at the age of 4 weeks developed nervous symptoms 36 hours later but only one case ended fatally. The symptoms observed were dullness ataxia and paraplegia. At post-mortem the most striking lesions were: Ascites (± 100 ml.), marked congestion of the mesenteric and meningeal bloodvessels, excessive accumulation of cerebrospinal fluid, acute haemorrhagic sinusitis without any gastro-intestinal lesions. *E. coli* 078:B80 was isolated in pure culture from all the organs including the brain and sinus exudate.

Of the 2 lambs exposed at the age of 24 hours, only one died of acute septicaemia, the other lamb was not affected at all. The post-mortem lesions revealed were acute sinusitis with slight congestion of the meninges. Repeated efforts to recover *E. coli* from the different organs were unsuccessful.

0128:B12: This *E. coli* serotype was not pathogenic for 4-week old lambs at all, while only one of the two lambs, infected at the age of 24 hours, died within 48 hours of exposure, after revealing typical clinical symptoms i.e. lateral recumbency, opisthotonus, continuous paddling movements and irregular muscular twitching. The post-mortem was characterized by absence of gastro-intestinal lesions, slight congestion of the meninges with marked haemorrhagic sinusitis, where 0128:B12 occurred as the only isolate.

2. *Salmonellosis.*

It is obvious from the results reported in table 1 that Salmonellosis is infrequently as-

sociated with fatal infections in young lambs in South Africa.

Different *Salmonella* serotypes were isolated from 6 (15 per cent) lambs only, as follows:

from 2 lambs in pure culture;

from 3 lambs in association with pathogenic *E. coli;*

from 1 lamb in association with members of the non-pathogenic *Enterobacter*-group.

Salmonella serotypes. The *Salmonella* serotypes isolated and their association with other enteric bacteria are listed in table 5.

Although all *Salmonellae* must be considered as potential pathogens, in only 3 of the 6 lambs could Salmonellosis be ascribed as the cause of death with certainty. These were, one lamb from which *Salmonella* and *Enterobacter* were isolated and two lambs in which *Salmonellae* occured as the only possible pathogens.

In the remaining 3 lambs death must be attributed to mixed *Salmonella* and *E. coli* infections to colisepticaemia as such.

TABLE 5.—SALMONELLA SEROTYPES ISOLATED FROM DISEASED LAMBS

	No. of lambs from which the following *salmonella* serotypes were isolated			
	S. typhimurium	S. dublin	S. thompson[1]	Salmonella not identifiable*
Pure culture	0	2	1	0
Salmonella and *E. coli*	2	0	0	1
Salmonella and *Enterobacter*	1	0	0	0

[1]Occurred in conjunction with var. *dublin.*
*Not identified on account of its mucoid nature.

Symptoms and Post-mortem lesions.

The following two outbreaks were investigated:

Case one: Four lambs, 6 weeks to 8 weeks old, were submitted for clinical observation and diagnosis. They originated from a flock in which severe losses amongst lambs of the same age were being experienced. All were in a poor condition and appeared to be suffering from malnutrition. Besides emaciation no diagnostic post-mortem lesions were found.

The diagnosis of colisepticaemia, made tentatively, was subsequently confirmed bacteriologically. But in addition to *E. coli* 035 and 0101, *Salmonella typhimurium* was also isolated from all 4 lambs. *E. coli* and *Salmonella* belonging to the same serogroups were also isolated from specimens subsequently submitted from the same outbreak.

Case two: Fresh carcases representive of mortality in lambs 3 to 5 weeks old were brought in for detailed investigation. Although the history was vague, it appeared that mortality usually occurred at night without any noticeable symptoms being evident the previous evening.

In contrast to the previous case, all lambs were in excellent condition but similarly, no diagnostic pathological lesions were found at post-mortem. Specimens, collected under sterile conditions from two lambs only, were submitted for detailed bacteriological and virological examination.

S. enteritidis var dublin was isolated in pure culture from the brain, liver and spleen of one of these lambs while *S. enteritidis var thompson* was an additional isolate from the second.

DISCUSSION

As far as the author is aware the present work represents the first successful diagnosis of fatal Colisepticaemia in lambs in the Republic of South Africa.

From the results of routine bacteriological examinations carried out on pathological material, it is evident that colisepticaemia is widely distributed in this country. Specimens from which *Escherichia coli*, belonging to different pathogenic serogroups were isolated have been received from the Western Cape, Karoo, N.E. Cape, Orange Free State, Eastern and S.W. Transvaal as well as from Natal.

A comparison between the number of specimens, representing specific outbreaks, submitted during the last 3 years indicates a gradual increase in the incidence of fatal colisepticaemia amongst lambs in South Africa. Specimens representing 4 outbreaks, 2 of which were amongst goats (kids) were received during 1963 in comparison to 12 during 1964 and 26 during the first six months of 1965. The number of reported cases where up to 50 per cent of the lamb crop was affected also increased from one in 1963, to two in 1964 and 4 in 1965.

Although the majority of the specimens submitted, represented sporadic outbreaks affecting a small percentage of lambs in a flock only, in the two major outbreaks investigated losses varied from 20 per cent to 50 per cent. On another farm in the Orange Free State where colisepticaemia was diagnosed for the first time in 1963 and since then confirmed annually, losses of more than 500 lambs were reported during the 1963 and 1964 lambing seasons.

From the results obtained during this investigation the following two pertinent conclusions are evident. Firstly, a large variety of *E. coli* belonging to different pathogenic serogroups are capable of causing fatal infections; more than 7 heterologous *E. coli* serotypes have been isolated from specimens representing 29 outbreaks. Although a large variety of serotypes have been incriminated it is significant that *E. coli* 078:B80 was the serotype predominantly associated with these outbreaks; 59.1 per cent of the 29 incidents reported was caused by this serotype. Secondly it would appear that the incidence of specific *E. coli* serotypes responsible for fatal infections is largely determined by the age of the lambs affected; *E. coli* 078:B80 was isolated from natural cases of fatal septicaemia affecting lambs 4 weeks and older but never from lambs under the age of one week.

This finding is in agreement with reports from Australia[2 3 4] where fatal infections caused by 078:B80 was diagnosed in lambs 6 weeks to 8 weeks old.

In contrast to this, Sterlecki and Shaw[5] described an outbreak of fatal septicaemia in lambs 2 to 5 days old, from which *E. coli* 078:B80 was recovered in pure culture. The symptomatology described by these authors was very similar to those observed in two of the acute outbreaks mentioned here where up to 90 per cent of mortality occurred within 12 hours to 24 hours of birth. There remains no doubt that *E. coli* other than 078:B80 was responsible for the high mortality rates described here which affected up to 50 per cent of the lambing crops. Whereas *E. coli*, preliminary identified as serotype 0128:B12 was responsible for acute losses in lambs 12 hours to 24 hours old, the other serotype not yet typed, caused mortality in lambs at birth or shortly afterwards.

Supportive evidence as to the differences in age susceptibility to fatal infections by specific *E. coli* serotypes is to be found in the results of the experimental transmission trials. Attempts to reproduce colisepticaemia by exposing 4 week old lambs to 0128:B12 were unsuccessful while only 50 per cent of lambs exposed at the age of 24 hours to 078:B80, contracted the disease, but all the lambs exposed at the age of 4 weeks to 078:B80 developed diagnostic symptoms, 50 per cent of which ended fatally. Similarly, only one of the two lambs infected with 0128:B12 within 24 hours of birth died showing post-mortem lesions typical of cases from which the exposure strain was recovered.

In general, the symptoms and lesions observed were very similar to those described by Charles[4], Roberts[2] and Sterlecki and Shaw[5], viz. severe nervous involvement, general congestion especially of the meninges, excessive accumulation of cerebrospinal fluid, hydropericard, *tumour hepatis*, endo- and epicardial haemorrhages without distinct gastro-intestinal disturbances. Whereas Charles[4]

and Sterlecki and Shaw[5] described nervous symptoms lasting for 24 hours to 96 hours prior to death, clinical symptoms were hardly ever noticed in these outbreaks; the majority of lambs died at birth or shortly afterwards or during the night without revealing any symptoms the evening before.

As described by Sterlecki and Shaw[5], a large percentage of lambs did not attempt to suck. On closer investigation it became evident that these lambs were too weak to suck on account of existing infection at the time of birth. This finding strongly suggests prenatal infection from a grossly infected uterus, a condition previously described[1] where uterine infections followed by abortions was a diagnostic feature of fatal colisepticaemia amongst adult sheep. But in not one of the outbreaks confirmed bacteriologically were abortions reported, neither did any of the ewes suffer any noticeable ill-effects.

Lesions found in 6 to 8 week old lambs, not previously described, were: acute muco-haemorrhagic sinusitis, severe ascites, marked congestion of the mesenteric blood vessels and fibrino-pleuropneumonia caused by *Pasteurella haemolytica.*

Although *Oestrus ovis* larvae were found in the nasal cavities of one 6 to 8 weeks old lamb slaughtered in extremis, isolation of *E. coli* 078:B80 from the nasal exudate of all the lambs and the absence of gastro-intestinal disturbances strongly suggests or nasal route of infection. This contention was subsequently supported by the successful intranasal transmission of fatal *E. coli* infections where the development of haemorrhagic sinusitis was a constant lesion.

The occurrence of ascites in natural cases of fatal colisepticaemia was, at the time, considered an indication of heavy worm infestations especially as some of the lambs were in rather poor condition, but the accumulation of excessive amounts of abdominal fluid in experimentally produced cases, with the absence of this lesion in the controls, labelled ascites as one of the diagnostic features of colisepticaemia caused by *E. coli* 078:B80. Marked congestion of all the mesenteric blood vessels was also an additional lesion found in outbreaks caused by both the *E. coli* serotypes 078:B80 and 0128:B12.

Fibrinous pleuro-pneumonia, on the other hand, was an inconstant lesion present in about 20 per cent of 6 to 8 week old lambs fatally infected with *E. coli* 078:B80. This finding, together with the fact that pathogenic *E. coli* 078:B80 was isolated from all the lambs including those with lung lesions, led to the suggestion that *Pasteurella*-pneumonia is an inconstant secondary complication rather than a primary disease condition.

In contrast to a recent report from New Zealand[14], Salmonellosis in lambs is of little importance in South Africa. Although *Salmonellae* have been isolated from 15 per cent of diseased lambs, in only 4.3 per cent could the cause of death, which appeared to be sporadic, be attributed to Salmonellosis.

S. enteritidis var. typhimurium, the serotype responsible for high mortality in sheep in New Zealand, has never been isolated as the only possible pathogen from the 6.5 per cent of lambs in which it occurred during this investigation. In the majority of these outbreaks pathogenic *E. coli* has also been found which together with symptoms observed, have led to the suggestion that *var. typhimurium* was a secondary invader rather than the primary cause of death.

S. enteritidis var. dublin on the other hand was the only *Salmonella* serotype isolated in pure culture from two lambs representing one acute outbreak. In neither of these lambs was diarrhoea or enteritis a diagnostic feature, a condition which has been described in Salmonellosis in kids.

In one outbreak where diarrhoea did occur in association with *S. dublin* infection, the diagnosis of Salmonellosis was to a large extent obscured by the presence of *E. coli* belonging to the pathogenic serogroup 078:B80.

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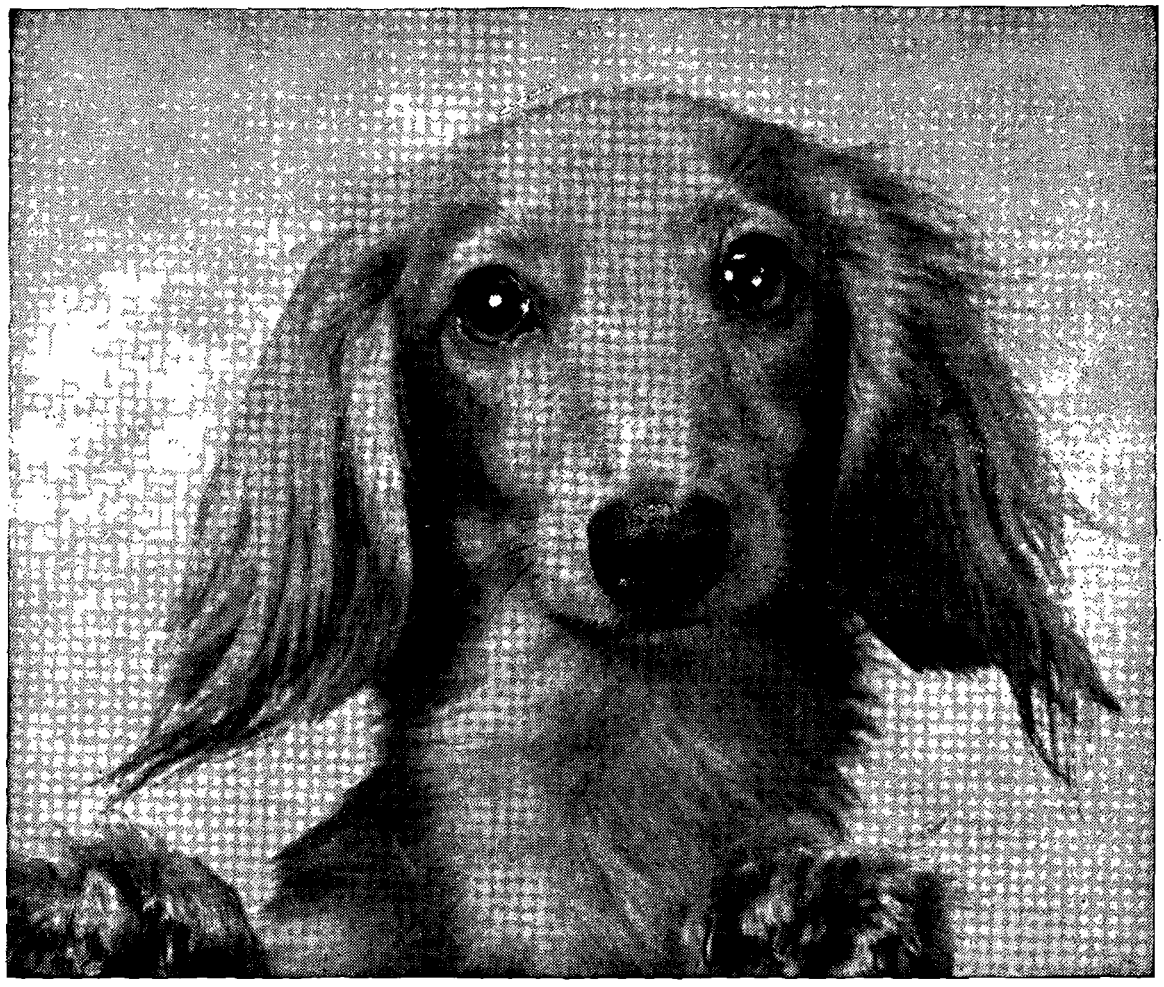
CORRECTION

The Reliability of the California Mastitis Test as a field test for Leucocytes in Milk — G. Crewe. J. S.Afr. Vet. Med. Assoc. 36(4) 1965, p.503:

The *commercial C.M.T. solution used was Rufus Mastitis Testing Solution not "Mastest" (T.M.) solution.

(Editor).



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A LARVAL ANTHELMINTIC TEST

R. K. REINECKE

Veterinary Research Institute, Onderstepoort

SUMMARY

(1) A procedure of infesting sheep, to test anthelmintic efficacy against all stages of larval development, is described. This was successful with *Oesophagostomum columbianum, Haemonchus contortus* and *Trichostrongylus colubriformis*, but could only be used against a single stage of development of *Gaigeria pachyscelis*.

(2) A Day O control gives proportions between the various larval stages on the day of treatment.

(3) Anthelmintic efficacy was assessed by comparing the worm burdens in treated sheep with those in the controls killed on the same day, i.e. 8 days after treatment.

(4) The value of the Day O control in establishing the larval stages refractory to treatment is discussed.

(5) I.C.I. 50,627 at 15 mg/K was 89.0% to 100.0% effective against all stages of development of *O. columbianum, H. contortus, T. colubriformis* and against fifth stage *G. pachyscelis*.

(6) At 10 mg/K it retained a 89.0% to 100% efficacy against fifth stage and adult worms of all species, but its efficacy dropped to only 57.2% and 74.4% against fourth stage larvae of *H. contortus* and *O. columbianum* respectively.

INTRODUCTION

Modern anthelmintics effective against all stages of development of parasitic nematodes, have made new methods of assessing the efficacy of these compounds essential. While Banks and Michael[1] and Gibson[2] developed methods to test anthelmintic efficacy against specific stages of the parasite life-cycle, this laboratory has attempted to include all stages of larval development in anthelmintic tests[3 4 5].

This paper describes further refinements of this test, hereafter referred to as the "larval anthelmintic test" using a new, highly potent anthelmintic, dl -2, 3, 5, 6, -tetrahydro-6-phenyl-imidazo (2,1-b) thiazole hydrochloride to establish the validity of this method of assay. The compound will be referred to hereafter as I.C.I.50,627.

MATERIALS AND METHODS

1. Ten weaned Dorper (Dorset Horn X Black Head Persian) lambs, reared and maintained under worm-free conditions, were used. These lambs were infested orally with infective larvae of *Oesophagostomum columbianum, Haemonchus contortus*[4] and *Trichostrongylus colubriformis*[5] and percutaneously with *Gaigeria pachyscelis* larvae.

2. The experimental design is summarized in Table 1.

3. Procedures *post-mortem* were modified from those described[5] as follows:

(a) Before sieving, worms were killed by the addition of approximately 5 ml concentrated iodine solution per litre of ingesta or digested gut.

(b) After removal of the ingesta, the intestinal tract was frozen at -20°C and the solid mass cut and chopped with a large knife into small cubes, 2-5 mm in size, before digestion. Only in the Day -15 and Day 0 controls were the abomasa digested. Digestion was completed at 49°C in 6-8 hours instead of at 37.5°C in 12 hours.

(c) The total worm burdens of *H. contortus* and *T. colubriformis* in the controls were estimated by the usual dilution technique[5]. Elsewhere total worm counts were carried out.

TABLE 1.—EXPERIMENTAL DESIGN

Day	Number of infective larvae dosed to each sheep			
	G. pachyscelis	*O. columbianum*	*H. contortus*	*T. colubriformis*
—63	180			
—56		68		
—50		111		
—46		84		
—42		74		
—36		42		
—32		40	151	300
—28		39	147	300
—25		40	147	300
—21		41	158	316
—18		42	150	300
—15		42	148	310
	Slaughtered Day —15 Control Sheep 1.			
—13		36	156	304
—11		39	158	296
— 8		36	142	290
— 6		40	157	295
— 3		89	312	614
— 1		84	297	610
0	Slaughtered Day 0 Control Sheep 2 Dosed Sheep 6, 7 & 8 at 10 mg/K Dosed Sheep 9 & 10 at 15 mg/K			
+ 8	Slaughtered Day +8 Controls Sheep 3, 4 & 5 Slaughtered Treated groups Sheep 6 to 10			

TABLE 2.—RESULTS OF CONTROLLED ANTHELMINTIC TEST.

Species	Sheep No. / Stage	Controls						Treated								
								10 mg/K *per os*					15 mg/K *per os*			
		@1	2	3	4	5	Average	6	7	8	Average	Reduction %	9	10	Average	Reduction %
H. contortus	Third	0	20	0	0	0	0	0	0	0	0	—	0	0	0	—
	Fourth	63	264	210	97	129	145	36	70	79	62	57.2	6	5	5.5	96.2
	Fifth	211	425	610	341	291	414	7	17	103	42	89.9	4	0	2	99.5
	Adult	96	971	1,087	1,124	1.210	1,140	16	14	72	34	97.0	1	0	0.5	99.9
	Total	370	1,680	1,907	1,562	1,630	1,699	59	101	254	138	91.9	11	5	8	99.5
T. colubriformis	Third	0	40	0	0	0	0	0	0	0	0	—	0	0	0	—
	Fourth	0	40	0	0	0	0	0	0	0	0	—	0	0	0	—
	Fifth	163	260	570	481	268	440	0	5	0	1.6	99.6	0	0	0	100.0
	Adult	144	680	1,540	1,069	1.072	1,227	0	1	30	10.3	99.2	0	0	0	100.0
	Total	307	1,020	2,110	1,550	1,340	1,667	0	6	30	12	99.3	0	0	0	100.0
G. pachyscelis	Fifth	81	33	0	0	0	0	0	0	0	0	—	0	0	0	—
	Adult	0	0	58	72	40	56.6	0	0	0	0	100.0	0	0	0	100.0
	Total	81	33	58	72	40	56.6	0	0	0	0	100.0	0	0	0	100.0
O. columbianum	Third	0	88	0	0	0	0	0	0	0	0	—	0	0	0	—
	Fourth	30	134	93	46	107	82	7	19	36	21	74.4	6	12	9	89.0
	Fifth	58	52	207	220	177	201	3	17	0	7	96.5	1	1	1	99.5
	Adult	166	234	124	85	309	173	1	4	0	2	98.8	0	1	0.5	99.7
	Total	254	508	424	351	593	456	11	40	36	30	93.4	7	14	10.5	97.7

@ Sheep 1 & 2 indicator controls: Sheep 3 to 10 slaughtered Day +8.

(d) Larval stages were identified microscopically according to the descriptions of Veglia[6 7], Ortlepp[8] and Douvres[9] but classified in the various larval stages[4].

Results

These are summarized in Table 2.

Controls

No third stage larvae were recovered from the Day -15 control unexpectedly, but the other stages present indicated the viability of the original larval doses.

The Day 0 control showed that all larval stages were present at the time of treatment, including third and fourth stage larvae of *O. columbianum* in the gut wall.

The Day +8 controls had fairly uniform worm burdens with variable distribution of larval stages. Fourth stage larvae of *O. columbianum* were recovered from the intestinal wall of two of these sheep (viz. sheep 3 & 5).

Treated with I.C.I.50,627.

10 mg/K.

This dosage rate shows a low efficacy against fourth stage larvae of *H. contortus* and *O. columbianum,* only removing 57.2% and 74.4% respectively. Against fifth stage and adult worms in all species its efficacy varied from 89.9% to 100.0%.

15 mg/K.

Efficacy rose against fourth stage larvae both of *H. contortus* (to 96.2%) and of *O. columbianum* (to 89.0%); similarly it rose to 99.5% - 100.0% against fifth stage and adult worms of all species.

Regardless of the dosage rate no fourth stage larvae of *O. columbianum* were recovered from the gut wall of any treated sheep.

Discussion

Anthelmintic

I.C.I.50,627 dosed at the rate of 15 mg/K fulfills all the requirements of an excellent anthelmintic, but at 10 mg/K it is not as effective against fourth stage larvae of *H. contortus* and *O. columbianum.*

Further trials against worms of other common genera such as *Ostertagia, Nematodirus, Cooperia, Bunostomum, Chabertia,* should be carried out to establish the range of parasites susceptible to the drug.

Larval anthelmintic test

For some years attempts have been made to establish a standard method of testing anthelmintics against all parasitic stages of the life cycle of nematodes of sheep[3 4 5]. Evidence has been presented to show that a controlled test, with the inclusion of an indicator control on the day of treatment, is the most satisfactory[5].

The name "larval anthelmintic test" is suggested to avoid confusion with the standard controlled anthelmintic test of Moskey and Harwood[10].

Reinecke *et al.*[5] have defined almost all the basic requirements and details of carrying out an anthelmintic test of this nature, but the following three considerations ((1), (2) & (3)) need further elucidation:

(1) *Uniform worm burdens*

Confidence in the interpretation of the results, in experiments of this nature, is largely dependent on the uniformity of the worm burdens in the control animals. The greater the variation, the more difficult is it to interpret results and the more essential is it to increase the number of controls. The worm burdens in the moderate number of controls in this experiment, were fairly even, although not completely uniform (Table 2). This uniformity was probably due to dosing smaller numbers of larvae during the period of infestation (Table 1), but was not achieved in the previous experiments[3 4 5] in which massive doses of larvae were used. These uniform worm burdens eliminated the need for large numbers of controls and increased the confidence in the interpretation of results.

Where worm burdens were lower than 600 the dilution technique for estimating worm burdens[5] proved unreliable, hence total counts were carried out.

(2) *The rôle of the indicator control*

The Day -15 control, although it indicated the viability of the initial larval doses, is actually redundant to the experiment.

The Day 0 control gives the numbers of the various larval stages on the day of treatment. On Day +8 all third stage larvae of *H. contortus* and *O. columbianum* had reached at least the fourth stage, while all *T. colubriformis* had already reached the fifth stage[6 7 9]. This progressive development is clearly illustrated in Table 2. In the Day +8 controls, third stage larvae had disappeared, fourth stages decreased, while fifth stage and adult worms had increased. Anthelmintic efficacy, is estimated by comparing the worm burdens of the treated sheep with those of the controls slaughtered on the same day. The estimation is valid when dealing with adult worms, which do not develop in the interval between treatment and slaughter, but not with larval stages which continue to develop, hence cognisance must be taken of the conditions obtaining in the control on the day of treatment. This continued development is particularly important when assessing whether an anthelmintic is equally effective against all stages.

A good example is the low efficacy of I.C.I.50,627 at 10 mg/K against fourth larval stages of *H. contortus,* when comparing the worm burdens of the treated sheep with those of the controls killed on the same day. Treatment took place eight days previously when these larvae were still in the third stage. Since the fourth moult takes place on the ninth to the eleventh day (Veglia[6]) it is logical to conclude that the anthelmintic was not effective against the third stage larvae.

The low efficacy against the fourth stage larvae of *O. columbianum* is more difficult to interpret as various factors must be taken into account:

(a) Experimental infestation and the life-cycle.

A total of 173 infective larvae were dosed on Day —3 and Day —1 to ensure adequate numbers of third stage parasitic larvae on Day 0. The fourth stage, i.e. from five to 25 days after infestation, was covered by a total of 316 larvae divided into fairly even doses at regular intervals from Day —6 to Day —25.

(b) The histotrophic phase.

The larvae remain in the intestinal wall from the first till the seventh day after infestation, although many fourth stage larvae are delayed in the histotrophic phase[7].

Third and fourth stage larvae were present in the Day 0 control, and fourth stage larvae in two of the three Day +8 controls in the gut wall. None were recovered from any of the treated sheep killed on Day +8, regardless of the dosage rate. This has not been noted with other compounds, in spite of their having some effect on the histotrophic phase[5].

(c) Fourth stage larvae 18 days or older.

Larvae 18 days old at treatment would have reached the fourth moult 8 days later and been classified with fifth stage larvae at slaughter[4]. Efficacy was high against these stages when compared with Day +8 controls.

It seems logical to conclude that I.C.I.50,627 was effective against third stage, and fourth stage larvae in the histotrophic phase, but not as effective against fourth stage worms, aged between 7 and 18 days, in the lumen.

(3) *Gaigeria pachyscelis*

This parasite does not lend itself to repeated infestation at short intervals, as only the first larval dose is viable[11]. The fate of subsequent larval doses is unknown and it is, therefore, an unsuitable species for anthelmintic tests of this nature. Consequently only a single dose of infective larvae was administered so that the anthelmintic was tested against the fifth stage only. Similar tests against earlier stages of the life-cycle are necessary[1,2,5].

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CYSTICERCOSIS, HYDATIDOSIS AND COENUROSIS IN THE REPUBLIC OF SOUTH AFRICA

ANNA VERSTER

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SUMMARY

1. The incidence of cysticercosis was determined from data submitted by 121 abattoirs covering a period varying from four to six years.

a. *Cysticercus bovis* has an average incidence of 3.031% in the Republic, with the highest average incidence, 6.946%, in the Transvaal Bushveld.

b. *Cysticercus cellulosae* has an average incidence of 1.479%, with the highest regional incidence, 2.907%, in the Middleveld.

2. The incidence of hydatidosis, based on data from 40 abattoirs for periods varying from 8 to 24 months, is about 1% in all species of slaughter stock. The following regions have the highest average incidences:

a. Eastern Cape Province, 13.67%, in cattle.

b. Western Cape Province, 3.55%, in sheep and 5.93% in pigs.

3. *Coenurus cerebralis* is most prevalent in the South-western Free State and Karoo. A survey of 85,300 sheep in the Bredasdorp District (Western Cape) showed an incidence of 0.78%.

4. Methods of control are described and discussed.

INTRODUCTION

Cysticercosis, hydatidosis and coenurosis are common parasites of livestock in the Republic of South Africa. Apart from the economic losses they cause the livestock breeder, the cystic stages of three of these may also parasitize man with serious consequences.

CYSTICERCOSIS

Cysticercus bovis of cattle, the cystic stage of *Taenia saginata* Goeze, 1782 of man, and *Cysticercus cellulosae* of pigs, the cystic stage of *Taenia solium* Lin., 1758 of man, occur in all parts of the Republic, their prevalence varying in different regions.

ORIGIN OF DATA

Data, collected from 1957 to 1962, were submitted by various abattoirs to the Department of Health; only data from abattoirs which employed health inspectors for at least four years are considered in this publication.

Data from 103 centres slaughtering less than 10,000 cattle per annum, are grouped to determine the incidences in individual regions, that of the country as a whole being determined from 18 larger abattoirs slaughtering more than 10,000 cattle annually.

REGIONAL INCIDENCES

The data pertaining to the nine regions (as shown in Fig. 1) are summarized in Tables I to IX.

INCIDENCE THROUGHOUT THE REPUBLIC

The incidences at the 18 large centres, which draw their slaughter stock from all the major cattle and pig rearing regions of the Republic, are summarized in Table X. The incidences at these centres, treated as a unit, and those in the individual regions are summarized in Table XI.

DISCUSSION

Both *C. bovis* and *C. cellulosae* occur throughout the country; *C. bovis* is more prevalent, having an average incidence of 3%

TABLE I.—INCIDENCE OF *Cysticercus bovis* AND *Cysticercus cellulosae* IN THE WESTERN CAPE PROVINCE

	Cysticercus bovis			*Cysticercus cellulosae*		
	Number Slaughtered	Number Infested	Percentage	Number Slaughtered	Number Infested	Percentage
Caledon	5,684	20	0.352	5,705	40	0.701
De Doorns	4,640	97	2.091	21,983	30	0.137
Franschhoek	1,808	4	0.221	1,440	8	0.556
Hermanus	5,748	141	2.453	5,122	34	0.664
Kuilsrivier	6,978	13	0.186	2,834	13	0.459
Malmesbury	12,607	110	0.873	21,914	54	0.246
Mooreesburg	3,395	14	0.412	4,604	17	0.369
Napier	1,272	4	0.315	1,391	1	0.072
Paarl	38,014	751	1.976	27,299	144	0.528
Piketberg	2,820	33	1.170	2,153	44	1.044
Porterville	1,025	8	0.781	896	15	1.674
Riversdale	4,164	39	0.937	2,342	2	0.171
Robertson	11,995	90	0.750	10,603	59	0.557
Simonstown	1,397	9	0.644	—	—	—
Stellenbosch	29,367	574	1.955	15,980	21	1.314
Strand	57,685	726	1.405	66,534	212	0.319
Swellendam	829	10	1.206	691	4	0.579
Tulbagh	2,461	96	3.901	1,552	21	1.314
Vredenburg	1,300	10	0.769	810	4	0.494
Wellington	8,885	116	1.306	5,705	40	0.701
Worcester	28,871	428	1.483	18,429	65	0.353
Total and Average	224,945	3,293	1.464	217,038	800	0.369

TABLE II.—INCIDENCE OF *Cysticercus bovis* AND *Cysticercus cellulosae* IN THE COASTAL BELT

	Cysticercus bovis			*Cysticercus cellulosae*		
	Number Slaughtered	Number Infested	Percentage	Number Slaughtered	Number Infested	Percentage
George	11,670	66	0.566	11,142	25	0.224
Humansdorp	2,955	57	1.929	1,052	31	2.947
Mossel Bay	7,663	46	0.522	4,967	22	0.443
Uitenhage	18,131	327	1.804	6,476	31	0.479
Total and Average	40,419	490	1.212	23,637	109	0.461

TABLE III.—INCIDENCE OF *Cysticercus bovis* AND *Cysticercus cellulosae* IN THE KAROO

	Cysticercus bovis			*Cysticercus cellulosae*		
	Number Slaughtered	Number Infested	Percentage	Number Slaughtered	Number Infested	Percentage
Aliwal North	26,098	27	0.104	21,017	68	0.324
Beaufort West	9,892	86	0.869	5,303	16	0.302
Burgersdorp	2,680	20	0.746	1,610	21	1.304
Calvinia	1,379	4	0.290	737	4	0.543
Carnarvon	1,228	5	0.407	543	1	0.184
Colesberg	2,423	6	0.248	1,395	4	0.287
Cradock	12,839	143	1.114	5,385	31	0.576
De Aar	10,503	164	1.562	3,464	5	0.144
Graaff-Reinet	9,160	11	0.120	5,636	65	1.153
Middelburg	3,293	16	0.486	1,125	0	0
Molteno	1,429	5	0.350	111	6	5.405
Oudtshoorn	14,848	49	0.330	11,065	56	0.506
Upington	13,740	18	0.131	5,967	10	0.168
Total and Average	109,512	554	0.506	63,358	287	0.453

TABLE IV.—INCIDENCE OF *Cysticercus bovis* AND *Cysticercus cellulosae* IN THE EASTERN UPLANDS

	Cysticercus bovis			*Cysticercus cellulosae*		
	Number Slaughtered	Number Infested	Percentage	Number Slaughtered	Number Infested	Percentage
Fort Beaufort	10,972	126	1.148	2,446	110	4.497
Grahamstown	24,334	1,798	7.389	18,250	317	1,737
King William's Town	17,662	446	2.525	9,830	160	1.628
Kokstad	5,664	335	5.915	3,870	130	3.359
Margate	6,375	118	1.851	3,127	2	0.064
Matatiele	4,129	95	2.301	1,511	16	1.059
Port Alfred	2,887	63	2.182	969	24	2.477
Port Shepstone	14,614	1,110	7.596	6,996	44	0.629
Queenstown	22,353	471	2.107	13,254	226	1.705
Stutterheim	5,908	124	2.099	2,821	64	2.269
Umtata	14,863	1,431	9.628	9,060	889	9.812
Umzinto	4,326	143	3.306	1,148	6	0.523
Total and Average	134,087	6,260	4.669	73,282	1,988	2.713

TABLE V.—INCIDENCE OF *Cysticercus bovis* AND *Cysticercus cellulosae* IN THE NATAL UPLANDS

	Cysticercus bovis			*Cysticercus cellulosae*		
	Number Slaughtered	Number Infested	Percentage	Number Slaughtered	Number Infested	Percentage
Colenso	3,119	70	2.244	511	19	3.718
Dannhauser	11,775	121	1.028	1,027	27	2.629
Dundee	9,649	33	0.342	2,538	15	0.591
Eshowe	7,433	448	6.027	2,117	20	0.945
Glencoe	12,995	408	3.140	2,171	6	0.276
Howick	8,277	183	2.211	1,811	0	0
Ladysmith	24,042	999	4.155	5,119	92	1.797
Newcastle	10,595	550	5.191	1,760	35	1.989
Piet Retief	5,618	478	8.508	1,921	69	3.592
Stanger	6,559	60	0.915	129	0	0
Vryheid	15,424	894	5.796	4,373	62	1.418
Total and Average	115,486	4,244	3.675	23,477	345	1.470

TABLE VI.—INCIDENCE OF *Cysticercus bovis* AND *Cysticercus cellulosae* IN THE HIGHVELD

	Cysticercus bovis			*Cysticercus cellulosae*		
	Number Slaughtered	Number Infested	Percentage	Number Slaughtered	Number Infested	Percentage
Balfour	13,515	97	0.718	18,306	508	2.775
Bethal	29,336	345	1.176	4,779	146	3.055
Bethlehem	28,737	338	1.176	17,031	496	2.912
Delmas	11,870	156	1.314	3,088	73	2.364
Ermelo	12,737	166	1.303	3,382	38	1.124
Ficksburg	8,394	54	0.643	4,361	168	3.852
Harrismith	8,971	108	1.204	3,962	93	2.347
Heidelberg	13,958	179	1.282	35,917	422	1.175
Heilbron	3,768	83	2.203	986	27	2.738
Middelburg	12,008	335	2.790	6,546	278	4.247
Reitz	3,441	30	0.872	1,346	43	3.195
Residensia	10,781	90	0.835	79	1	1.266
Standerton	19,836	219	1.104	5,889	232	3.940
Volksrust	9,010	283	3.141	2,890	79	2.734
Total and Average	186,362	2,483	1.332	108,562	2,604	2.399

TABLE VII.—INCIDENCE OF *Cysticercus bovis* AND *Cysticercus cellulosae* IN THE MIDDLEVELD

	Cysticercus bovis			*Cysticercus cellulosae*		
	Number Slaughtered	Number Infested	Percentage	Number Slaughtered	Number Infested	Percentage
Brandfort	2,759	16	0.580	1,437	52	3.619
Fochville	4,222	32	0.758	1,929	40	2.074
Henneman	8,054	142	1.763	3,628	77	2.122
Kroonstad	36,476	715	1.960	11,606	302	2.602
Lichtenburg	17,273	283	1.638	3,583	193	5.387
Mafeking	19,421	626	3.223	9,219	236	2.560
Odendaalsrus	33,354	1,050	3.148	4,631	195	4.211
Orkney	30,448	482	1.583	3,245	117	3.606
Parys	14,403	400	2.777	11,932	317	2.657
Sasolburg	16,501	199	1.206	4,507	55	1.220
Schweizer Renecke	5,486	56	1.021	2,020	114	5.644
Vaalharts	4,344	82	1.898	1,054	5	0.474
Vryburg	1,811	55	3.037	139	6	4.317
Wolmaransstad	5,263	82	1.558	1,679	53	3.157
Total and Average	119,815	4,220	2.112	60,609	1,762	2.907

TABLE VIII.—INCIDENCE OF *Cysticercus bovis* AND *Cysticercus cellulosae* IN THE TRANSVAAL BUSHVELD

	Cysticercus bovis			*Cysticercus cellulosae*		
	Number Slaughtered	Number Infested	Percentage	Number Slaughtered	Number Infested	Percentage
Duiwelskloof	1,706	149	8.734	357	7	1.961
Groblersdal	6,885	535	7.771	1,211	17	1.404
Louis Trichardt	15,866	1,509	9.511	2,595	48	1.850
Messina	12,714	94	0.739	3,162	43	1.360
Nylstroom	9,128	695	7.614	3,905	68	1.741
Phalaborwa	2,677	193	7.210	372	13	3.495
Pietersburg	42,899	3,521	8.208	64,599	1,454	2.251
Potgietersrus	13,653	658	4.820	4,532	91	2.008
Warmbad	8,329	555	6.663	3,154	50	1.585
Total and Average	113,857	7.909	6.946	83,887	1,791	2.135

TABLE IX.—INCIDENCE OF *Cysticercus bovis* AND *Cysticercus cellulosae* IN THE LOWVELD

	Cysticercus bovis			*Cysticercus cellulosae*		
	Number Slaughtered	Number Infested	Percentage	Number Slaughtered	Number Infested	Percentage
Barberton	21,712	460	2.119	2,524	37	1.466
Lydenburg	9,430	456	4.836	2,897	108	3.728
Nelspruit	16,698	583	3.491	4,898	112	2.287
Sabie	11,692	158	1.351	2,206	55	2.493
White River	9.984	139	1.392	3,021	70	2.317
Total and Average	69,516	1,796	2.584	15,546	382	2.457

TABLE X.—INCIDENCE OF *Cysticercus bovis* AND *Cysticercus cellulosae* IN THE LARGE CENTRES

	Cysticercus bovis			*Cysticercus cellulosae*		
	Number Slaughtered	Number Infested	Percentage	Number Slaughtered	Number Infested	Percentage
Benoni	160,360	66,853	3.163	109,365	18,154	1.437
Bloemfontein	107,366	2,058	1.917	82,673	664	0.803
Cape Town	809,581	9,879	1.220	231,497	1,868	0.807
Durban	567,887	17,333	3.052	399,938	7,275	1.819
East London	122,237	6,550	5.358	72,453	1,156	1.596
Germiston	251,008	4,840	1.928	54,901	555	1.011
Johannesburg	2,113,481	66,853	3.163	1,263,340	18,154	1.437
Kimberley	96,789	725	0.749	25,658	70	0.273
Klerksdorp	152,660	2,380	1.559	28,028	659	2.351
Krugersdorp	163,911	4,011	2.447	66,951	583	0.871
Pietermaritzburg	115,484	1,509	1.307	31,976	382	1.195
Port Elizabeth	259,739	8,450	3.253	107,318	484	0.451
Potchefstroom	82,087	2,271	2.767	18,750	449	2.395
Pretoria	472,464	37,101	7.853	181,772	4,544	2.500
Springs	146,094	3,463	2.370	81,949	676	0.825
Vereeniging	161,881	4,318	2.667	51,850	1,882	3.630
Welkom	146,921	3,471	2.363	37,103	1,009	2.720
Witbank	84,738	5,108	6.028	18,037	53	0.294
Total and Average	6,014,688	187,237	3.113	2,863,559	42,194	1.474

TABLE XI.—SUMMARY OF *Cysticercus bovis* AND *Cysticercus cellulosae* IN INDIVIDUAL REGIONS

	Cysticercus bovis			*Cysticercus cellulosae*		
	Number Slaughtered	Number Infested	Percentage	Number Slaughtered	Number Infested	Percentage
Large Centres	6,014,688	187,237	3.113	2,863,559	42,194	1.474
Natal Uplands	115,486	4,244	3.675	23,477	345	1.470
Transvaal Bushveld	113,857	7,909	6.946	83,887	1,791	2.135
Lowveld	69,516	1,796	2.584	15,546	382	2.457
Western Province	224,945	3,293	1.464	217,038	800	0.369
Coastal Belt	40,419	490	1.212	23,637	109	0.461
Karoo	109,512	554	0.506	63,358	287	0.453
Eastern Uplands	134,087	6,260	4.669	73,282	1,988	2.713
Highveld	186,362	2,483	1.332	108,562	2,604	2.399
Middleveld	199,815	4,220	2.112	60,609	1,762	2.907
Total and Average	7,208,687	218,486	3.031	3,532,955	52,262	1.479

while that of *C. cellulosae* is 1.5%. It is of interest that the relative incidences of these parasites have changed in the last 30 years; at the Cape Town, Pretoria, Durban and Johannesburg abattoirs the average incidence of *C. bovis* increased from 1.39% in 1931 to 3.97% in 1961, while that of *C. cellulosae* decreased from 4.67% to 1.93% during the same period[1].

Cysticercus bovis

The only region in which the average incidence is high is the Transvaal Bushveld (6.946%) where seven of the nine abattoirs recorded incidences above 5%. (The incidence at Messina — 0.739% — differs markedly from those at other abattoirs in this region, and should, therefore, be treated with reserve). Average incidences in excess of 5% are recorded at three large abattoirs (Pretoria, Witbank, East London), at four abattoirs in the Eastern Cape Uplands (Umtata, Port Shepstone, Grahamstown, Kokstad), and at four abattoirs in the Natal Uplands (Piet Retief, Eshowe, Vryheid, Newcastle).

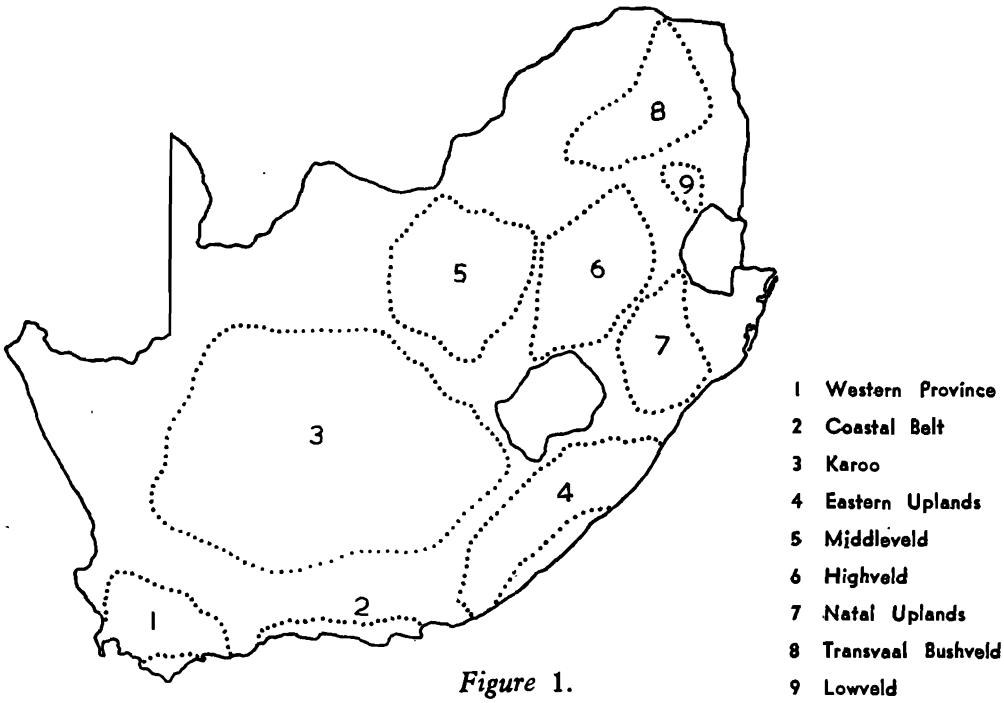

Figure 1.

The only region in which the average incidence was below 1% was the Karoo (0.506%), only two of 13 abattoirs recorded higher incidences, viz. Cradock (1.114%) and De Aar (1.562%).

Cysticercus cellulosae

The average incidence of *C. cellulosae* is below 5% in all regions. The highest regional incidence is that recorded in the Middleveld, 2.907%, followed by the Eastern Cape Uplands, 2.713%. Three individual abattoirs recorded incidences above 5%, viz. Umtata (9.812%), Schweizer Reneke (5.644%) and Molteno (5.405%).

Regional incidences below 1% were recorded in the Western Province (0.369%), Coastal Belt (0.461%) and Karoo (0.453%).

Hydatidosis

Verster[2] has shown that hydatidosis in domestic livestock in the Republic may be due to any one of four subspecies of *Echinococcus granulosus* Batsch, 1786, viz. *E. g. granulosus* Batsch, 1786 , *E. g. africanus* Verster, 1965; *E. g. lycaontis* Ortlepp, 1937; *E. g. ortleppi* Lopez-Neyra and Soler Planas, 1943. The subspecies, *E. g. felidis* Ortlepp, 1937 of the lion, *Panthera leo*, has not been recorded from domestic livestock.

Origin Of Data

The incidence of hydatidosis is based on data collected at 40 abattoirs in various parts of the country, for periods varying from eight to 24 months.

As these data are fully discussed elsewhere[2], only a summary will be given here.

Regional Incidence

The incidence of hydatidosis in six regions and at the large consumer centres is summarized in Table XII.

Incidence Throughout The Republic

The incidence of hydatidosis in all species of slaughter stock is approximately 1% (Table XIII).

Discussion

Although the incidence of the parasite is low (1%) in all species of livestock, it is clear from Table XII that it is high in some species in some regions.

TABLE XII.—THE INCIDENCE OF HYDATIDOSIS IN THE REPUBLIC OF SOUTH AFRICA.

Region	Cattle		Sheep		Goats		Pigs	
	Number Slaughtered	Percentage Infested	Number Slaughtered	Percentage Infested	Number Slaughtered	Percentage Infested	Number Slaughtered	Percentage Infested
Large Centres	1,582,211	0.68	*5,122,399	0.85			607,096	0.84
Western Province	16,344	4.72	102,564	3.55	935	0	11,699	5.93
Karoo	15,946	1.18	114,376	0.86	3,806	1.21	5,899	0.88
Eastern Province	32,425	13.76	114.752	1.51	11,874	3.18	17,034	2.82
Highveld	33,146	1.52	52,376	1.27	1	0	9,579	0.40
Orange Free State	7,608	2.38	27,998	0.69	0		2,377	0.30
Transvaal Bushveld	18,740	8.24	19,314	2.17	829	0.84	21,078	0.99

*Sheep and goats treated as a unit.

TABLE XIII.—AVERAGE INCIDENCE (PERCENTAGE) OF HYDATIDOSIS: LARGE CONSUMER CENTRES COMPARED WITH THAT OF ALL THE CENTRES

Species	Large Centres		All Centres	
	No. Slaughtered	Percentage Infested	No. Slaughtered	Percentage Infested
Cattle	1,582,211	0.68	1,706,420	1.08
Sheep & Goats	5,122,399	0.85	5,571,224	0.92
Pigs	607,096	0.84	674,762	0.98

Cattle

The highest average incidence in cattle was recorded in the Eastern Cape Province, 13.76%. Six abattoirs in this region participated in the survey. The incidence was below 10% at Umtata (5.45%) and Grahamstown (9.92%); between 10% and 20% at Uitenhage (11.83%) and Queenstown (16.94%); above 20% at Fort Beaufort (20.14%) and King William's Town (22.99%). The high incidence in cattle in this region may be due to the predominance of older animals slaughtered. Thus at Grahamstown 73.6%, and at Queenstown 93.9%, of the cattle slaughtered are 6 and 8 tooth animals.

In the Transvaal Bushveld the average incidence at Pietersburg, Potgietersrus and Tzaneen was 8.24%; that at Potgietersrus being 16.17% and that at Tzaneen 12.60%. It is possible that some of the animals become infested with material originating from wild carnivores which are still prevalent in this region.

Sheep

The highest average incidences in sheep were recorded at Welkom (14.50%) and Vryburg (12.45%). No explanation can be given to account for the high incidence at Welkom. That at Vryburg is probably due to a combination of several factors. Vryburg, in the Northern Cape, is in a predominantly cattle ranching region where, until recently, no attempts were made to control the black-backed jackal, *Canis mesomelas* Schreber, 1775. This carnivore is too small to prey on cattle, but is a very successful predator of sheep. The sheep in this region are, therefore, protected from predation by kraaling at night. This practice brings the sheep into contact with the dogs at the homestead on their way to and from the kraal. This practice has also resulted in an increase in the number of sheep dogs used for herding purposes. These factors tend to increase the incidence as the sheep are exposed to possible infestation from pastures and water sources contaminated by wild carnivores and sheep dogs by day, and also twice daily are exposed to possible infestation from the dogs at the homestead.

Pigs

The average incidence in pigs is higher in the Western Cape Province (5.93%), than in any other region, and that at Worcester (13.67%), the highest recorded in this survey. The prevalence of the parasite in swine is probably related to the practice of turning these animals out on free range in the vineyards at certain times of the year.

The incidence at Potgietersrus (9.94%) in the Transvaal Bushveld (average 0.99%) is probably related to a similar practice, that of running the animals in the peanut lands.

COENUROSIS

Coenurus cerebralis of sheep, the cystic stage of *Taenia multiceps* (Leske, 1780) of the domestic dog and other carnivores, is a common parasite in sheep rearing regions.

ORIGIN OF DATA

The geographical distribution of the parasite has been determined from letters written by farmers to the Institute, and on records of infested sheep, sent in either for diagnosis or for research purposes.

It is not possible to determine the incidence of this parasite in the Republic. Replies to questionnaires sent to farmers in the Bredasdorp district in the Western Province, give an indication of the incidence there during a three year period.

GEOGRAPHICAL DISTRIBUTION

The districts in which this parasite has been recorded are indicated in Fig. 1.

This parasite has been recorded on 82 farms outside the Western Province. These farms are mainly in the Karoo and South Western Free State. The majority of the farms (44) are in an area extending from Fauresmith (Koffiefontein) in the North, to Richmond in the South West and Steynsburg in the South East. It appears to be most prevalent in the Colesberg district where it has been recorded on 19 farms.

INCIDENCE IN BREDASDORP DISTRICT

The replies received from 51 farmers to whom the questionnaire was circulated, may be summarized as follows:

Coenurosis occurred on 42 of the farms. The number of sheep infested in a three year period varied from 1 to 140 per farm. The percentage so infested on the 51 farms was 0.78% of 85,300 sheep.

There were a total of 221 dogs on these farms, the number varying from 0 to 40 per farm. On 19 farms some of the dogs are dewormed from once to 12 times per annum, but all the dogs are dewormed on only 13 farms. On the majority of the farms it is the sheep dogs that are treated while the homestead and/or labourer's dogs are but rarely treated.

DISCUSSION

The data can only give an indication of the parasite's geographical distribution as it probably also occurs on many farms in other regions which have not been reported. In the Transvaal cases have been recorded on two farms in the Ermelo district[3]; as yet it has not been reported from Natal.

The incidence recorded in the Bredasdorp district (0.78%) is probably biased in that only a few farmers completed the questionnaire, the greater majority of whom had lost animals from coenurosis. The cases recorded were diagnosed by the farmer himself and thus cannot be confirmed.

CONTROL

Both the sexual and the cystic stages of these four tapeworms can be effectively controlled by curtailing the availability of infestive material to both the definitive and the intermediate hosts. This entails the complete destruction of the cystic material, or at least its treatment by boiling or freezing, to render it harmless to the definitive host. At the same time the definitive host must either be removed or treated for tapeworms to eliminate this possible source of infestation. Unfortunately treatment with an effective taenicide is only a temporary measure unless precautions are taken to prevent reinfestation.

CONTROL OF THE CYSTIC STAGES

Control measures must be directed at destroying the cystic stages of the parasites to curtail their availability to the definitive hosts.

a. *Cysticercosis*

Carcases of cattle and pigs should be examined by a competent person before any meat is issued for human consumption. Should cysticerci be present, heavily infested carcases should be destroyed, lightly infested carcases may be treated either by cooking or by freezing to render the cysticerci harmless.

b. *Hydatidosis*

Raw offal, especially the liver and lungs, should not be fed to dogs; if fed it must be thoroughly cooked to kill any hydatid cysts that may be present.

c. *Coenurosis*

Sheep showing symptoms of coenurosis should be slaughtered as soon as the parasite is diagnosed. The head and/or spinal cord must be burnt so that dogs and other carnivores do not have access to the infestive material.

CONTROL OF THE SEXUAL STAGE

The sexual stages of these parasites utilize man (cysticercosis) and dogs and other wild carnivores (hydatidosis; coenurosis) as definitive hosts.

Cysticercosis

The farmer, his family and entire staff should be examined for tapeworm infestation by a medical practitioner. Should any be parasitized they must be treated, under medical supervision, with an effective taenicide. However, this is merely a temporary measure and efforts must be made to prevent reinfestation.

Hydatidosis, Coenurosis

All the dogs on farms must be treated with an effective taenicide at regular intervals. It must be stressed that *all* the dogs, whether they be sheep dogs, homestead dogs or dogs belonging to labourers, must be treated. Further, as dogs often wander from one farm to another, dogs on adjacent farms also must be treated.

The above measures to curtail the availability of infestive material will also prove effective in cases where wild carnivores may act as definitive hosts. The effect will, however, only be apparent after a fairly long period, i.e. when the older, previously infested livestock, have been slaughtered out.

ANIMAL HUSBANDRY METHODS

Certain farming practices, or combinations of them, appear to be correlated with a high incidence of hydatidosis. These practices are those that tend to increase contact between domestic livestock and dogs or other carnivores; these same practices would tend to increase the incidence of coenurosis.

1. The practice of feeding raw offal to dogs is common and widespread as is shown by the high incidence of *Taenia hydatigena* in dogs (unpublished data). This practice is particularly dangerous on farms where sheep dogs are used for herding purposes.

2. In many parts of the country livestock are kraaled overnight to protect them from predators. The livestock are thus brought into close contact with the homestead dogs, possible disseminators of tapeworm ova, on their way to and from the kraal. This factor is probably partly responsible for the high incidence of hydatidosis (12.45%) in sheep in the Vryburg district.

3. The practice of rearing pigs on "free range" appears to be correlated with a high incidence of hydatidosis in some regions. This practice exposes pigs to infestation originating from wild carnivores as well as from stray dogs. At Worcester the pigs are turned out on free range in the vineyards, while at Potgietersrus they are allowed out on the peanut lands; the incidences of hydatidosis are 13.67% and 9.94% respectively at these abattoirs.

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THE PROBLEM OF THE 'MILK FEVER' SYNDROME IN THE WESTERN PROVINCE

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SUMMARY

The results of a preliminary investigation into the incidence of the "milk fever" and "downer cow" syndromes in the Western Cape Province are reported. The most significant findings are in regard to plasma minerals. No cases of uncomplicated hypocalcaemia were encountered but various combinations of hypocalcaemia, -phosphataemia, -kalaemia, and -magnesaemia. These findings are compared with those of other workers in this field and their significance discussed especially with regard to prophylaxis and treatment.

INTRODUCTION

In view of numerous reports, especially from the winter rainfall area, that relapses occurred after conventional treatment of milk fever, and that cases of milk fever not associated with parturition were often encountered, a preliminary investigation *in loco* was carried out. The mobile laboratory was used for this purpose and the work was done in collaboration with local veterinarians. Practitioners reported that in most cases cows showing typical signs of milk fever responded to the first treatment but often relapsed and subsequent treatments gave indifferent results. Animals were often unable to rise for various lengths of time and some died. Where the attacks were not associated with calving, they were invariably connected with grazing on green cultivated crops, usually oats. In these animals the response to treatment was similar to that shown in cases occurring soon after calving.

RESULTS

The findings on nine cases showing signs of "milk fever" are presented in Table 1.

These cases can be grouped according to the combinations of minerals showing abnormally low concentrations in the plasma.

Group 1.

Three cases showing hypophosphataemia and hypocalcaemia.

Case No. 1 was a Jersey cow, in good condition, which calved two days before the attack. The animal responded immediately to an intravenous injection of 350 ml of a 25 per cent calciumborogluconate solution to which four per cent of magnesium chloride was added.

The next three cases in this group occurred at the same time on the same farm. According to the farmer they represented different stages of the typical attacks seen on green oats grazing. All three cows had been in milk for some time and had been on green oats for nearly a month. It is interesting to note that the owner's classification of these cases was in accordance with the clinicochemical findings.

Case No. 2 was unable to rise but quite alert. She had severe ruminal stasis, hypothermia (97°F) and alkalosis (HCO_3 = 36.7 m.eq./L). She recovered completely after an injection of 350 ml of M.F.C. solution*.

Case No. 3 was an old Jersey cow showing signs of incoordination. Milk yield had decreased the previous day. The most important signs were complete ruminal stasis, hypothermia (97°F) and the plasma bicarbonate was elevated (41.2 m.eq./L). She responded equally well to treatment with M.F.C. solution.

* M.F.C. solution—May and Baker Ltd.

TABLE I.—BLOOD VALUES FROM AFFECTED AND CONTROL ANIMALS.

Group	Case No.	Locality	Description	Ca mgm %	PO_4 mgm %	Mg mgm %	K mEq/L
1	1	Swellendam	Attack two days *post partum*				
			Before treatment	5.3	0.51	3.0	4.8
			After treatment	11.6	1.53	1.6	4.4
		Riversdale	Animals grazed on green oats, various stages of lactation.				
	2		Paralysed but alert.	7.8	1.54	2.2	4.0
	3		Paretic, rumen stasis	6.8	2.35	2.6	4.5
	4		Constipated	9.5	4.14	2.2	5.5
2		Riversdale	Attack two days *post partum*				
	5		Before treatment	4.7	0.63	2.2	3.5
			After treatment	12.5	2.18	1.6	3.9
3		Riversdale	Paralysed for six weeks. Green oat grazing				
	6		No treatment	13.4	1.02	1.1	5.6
4		Heidelberg	Calved a month ago. Green oat grazing				
	7		Before treatment	13.5	1.1	0.8	—
			After treatment	19.5	1.8	0.8	3.5
*	8	Riversdale	Attack six days after calving	10.5	0.63	1.0	3.25
5	9	Swellendam	Attack 27 days after calving. Green oat grazing				
			Before 1st treatment	12.5	0.74	2.0	—
			After 1st treatment	19.5	2.9	1.5	4.5
			Before 2nd treatment	12.5	1.7	2.5	2.7
			After 2nd treatment	13.0	2.6	2.2	3.0
	10	Caledon	Control	14.0	1.75	1.2	6.1
	11	Caledon	Control	15.0	2.25	1.2	6.4
*	12	Riversdale	Control	11.6	1.48	1.4	5.2
*	13	Riversdale	Control	11.5	2.5	1.2	6.4
Normal values				9–12	3–6	18–30	4.1–5.5

* Cows on the same farm.

Case No. 4 also had a history of a sudden decrease in milk yield. The animal was constipated with hard, mucous covered faeces. There was improvement after administration of M.F.C. solution and she eventually recovered without any further treatment.

Group 2.

One case showing combined hypo-phosphataemia, -calcaemia, and -kalaemia.

Case No. 5 developed a typical attack of milk fever two days after calving. When the first blood sample was taken 12 hours after the attack started, the animal was still in a sternal position and not comatose, but showed a marked torticollis. After an intravenous infusion of 320 ml of a 10 per cent acid sodium phosphate solution, the torticollis disappeared and the animal became very alert. Thirty minutes later it became depressed and developed the typical signs of milk fever with coma and the head on the side. She recovered rapidly and completely after an intravenous injection of 350 ml Myrilos*.

Group 3.

One case showing hypo-phosphataemia and -magnesaemia.

Case No. 6 was an old Jersey cow grazing on green oats and receiving a little lucerne hay and oat straw. She had calved a month before, showed inco-ordination on the previous evening, and was unable to rise the next morning.

On examination the animal was lying comfortably, appeared alert, but was totally un-

* Myrilos—Burroughs Welcome.

able to move even when stimulated. Complete ruminal stasis and hypothermia were present. There was no clinical improvement after the administration of 350 ml M.F.C. solution intravenously but she was, however, able to rise 24 hours later without any further treatment.

Group 4.

Two cases with a combination of hypo-phosphataemia, -magnesaemia and -kalaemia.

Case No. 7. The animal had gone down suddenly six weeks before while grazing on green oats. At the beginning of her illness she had been treated with M.F.C. solution on two consecutive days without response. After lying on her side for the first three days she was able to maintain a sternal position. With careful nursing she was eventually able to rise with assistance. The blood sample was collected at this stage.

Case No. 8 had been placed on green oats grazing immediately after calving. Six days later she developed an attack of milk fever and responded immediately on being treated with 350 ml of a 25 per cent calcium boro-gluconate solution. A relapse occurred after 24 hours and there was no response to two further injections given on the two subsequent days. The blood specimen was collected on the fourth day of the attack. She was dosed with eight ounces of bonemeal daily and was able to rise on the twelfth day. From the third day after the initial attack she was able to 'crawl' for short distances.

Group 5.

One case with hypo-phosphataemia and -kalaemia.

Case No. 9. This animal was also on green oats from calving and showed milk fever on the second day. She responded to treatment with calcium boro-gluconate but had to be treated again the following day. She had two further attacks on the 26th and 27th days of lactation but on both occasions responded to ca-boro-gluconate therapy. Although the plasma potassium was not determined initially and was normal after the first treatment it was definitely low on the following day. The animal is, therefore, included in a separate group.

Group 6.

Clinically normal animals.

Cases 10, 11, 12 *and* 13 were clinically normal animals in herds where milk fever occurred. It is of interest to note that in all these animals the levels of phosphate and magnesium were below normal.

Discussion

The occurrence of a variety of plasma mineral imbalances in the milk fever syndrome is in accordance with the findings of Egyed[1]. Reporting on 168 cases showing typical symptoms he found that:—

85.1 per cent had hypophosphataemia;
75 " " " hypocalcaemia;
46.5 " " " hypermagnesaemia;
45.2 " " " eumagnesaemia;
8.3 " " " hypomagnesaemie;
2.4 " " " normal P, Ca and Mg figures.

The various combinations of findings in Egyed's series, in order of frequency, can be seen from Table 2.

Table 2.—Combinations of findings in Egyed's Series in order of frequency

Order of Frequency	Ca	P	Mg
1	—	—	0
2	—	—	+
3	—	—	—
4	0	—	—
5	0	—	+
6	0	—	0
7	0	0	0

+ = hyper-state
— = hypo-state.
0 = eu-state.

As will be noted, the cases studied in the Western Province fall into the same groups as found by Egyed and pending studies on a larger number, it can be assumed that the positions in the Western Cape and in Israel are very similar. Hypophosphataemia was

most commonly encountered and attention has been drawn to the fact that the clinically normal animals in the series showed low plasma inorganic phosphate levels.

Uncomplicated hypomagnesaemia (lactation tetany) is extremely rare in the Western Cape. The three cases (6, 7 and 8) with a low magnesium concentration in the plasma, all showed a hypophosphataemia with normal calcium levels. All three cases were associated with grazing on green oats and the attacks were not directly associated with parturition. It is interesting to note that in some countries lactation tetany is also known as "wheat pasture poisoning" and commonly occurs during inclement weather[2] as did cases 7 and 8 in this series.

Egyed did not report on the plasma potassium levels in his studies. The significance of hypokalaemia found in the present series cannot be assessed but Kronfeld[3] describes the hypokalaemic cow as "a milk fever patient which, after calcium therapy, picks up her head, looks alert, stretches her neck out, creeps on the front elbows and knees, but has no control of her flaccid hindquarters and thus remains down".

In the cases investigated it was impossible to distinguish clinically or chemically between milk fever attacks associated with parturition and similar syndromes occurring on green grazing at any stage of lactation. As these cases are identical, the widely accepted notion that milk fever is caused through some change necessarily associated with parturition must be queried.

The finding of profound ruminal stasis in all these cases is significant. Moodie and Robertson[4] have shown that induced intestinal stasis in the lactating cow rapidly causes hypocalcaemia and conversely, hypocalcaemia leads to intestinal stasis. In hypocalcaemia, therefore, a vicious cycle will be established which can be overcome with calcium therapy. Amounts insufficient in themselves to restore calcium balance, may have this effect by restoring calcium absorbtion.

The exact rôle of gastro-intestinal stasis in milk fever associated with parturition and attacks occurring at other times needs further elucidation. The stasis may be the cause or the effect in one or both conditions. The unpredictable variation of different plasma levels, however, leads to the suspicion that a gastro-intestinal stasis of unknown origin is the cause of the syndrome in both instances.

The multiplicity of mineral imbalances which may be present in an individual case and the inability to distinguish between them clinically, makes rational replacement therapy extremely difficult. The prevalence of hypophosphataemia found in both affected and control cows in the Western Province indicates an absolute phosphorous deficiency and the administration of this element should always be considered as a supplement to calcium therapy. That the present methods are not entirely satisfactory is indicated by the number of relapses encountered. With our knowledge at present it would appear advisable to use solutions containing calcium, phosphorus and magnesium as a routine. The question of dosage and the danger of induced hyper-states are pertinent. Daykin[5] recommends the following dosages: Ca-boro-gluconate 120 g, sodium acid phosphate 30 g and magnesium sulphate 60 g. Most commercial compounds contain about half these amounts. In some of the latter solutions, however, the magnesium and phosphorus are included as magnesium hypophosphite. Hypophosphites are physiologically unavailable and are excreted unaltered in urine[6 7 8] in as soon as two hours[9]. The fate of magnesium in these compounds is unknown.

Wehner[10] claims very good results with a mixture of 100 g Ca-boro-gluconate 35 g Mg-gluconate and 13 g Methionin (to stabilize the mixture). This is dissolved in 750 cc water and constitutes one dose. He also claims that 4 infusions within 60 hours caused no toxicity.

In the light of our findings it is suggested that the above parenteral treatments be followed by 90 g (3 oz) NaH_2PO_4 *per os* every 8 hours.

At present the treatment of milk fever by insufflation of the udder is regarded as archaic and dangerous. However, the efficacy of this

treatment, where infusions have failed, has withstood the test of time, and it should be considered when a case does not respond to other measures. Marshak[11] has demonstrated a marked increase in blood calcium, phosphorous and sugar and a slight rise in blood magnesium following udder insufflation. Clinical improvement was noticeable within 15 minutes, and most animals regained their feet within 2-3 hours. By instilling an intramammary antibiotic before insufflation, he proved the perfect safety of this procedure in over 2000 quarters treated.

The advantage of udder insufflation over mineral infusions is that the physiological balance of all blood minerals tends to be restored independently of the imbalance present.

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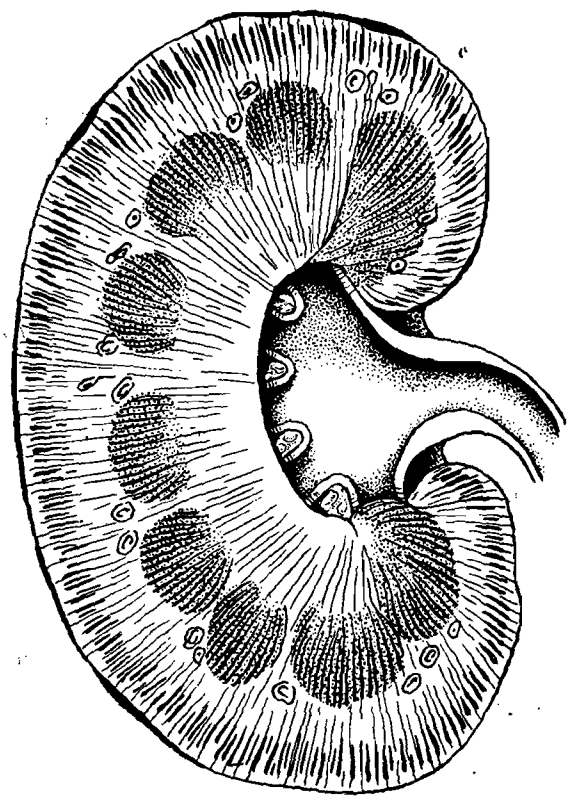

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INTRANUCLEAR INCLUSIONS IN THE HEPATOCYTES IN ENZOOTIC ICTERUS IN SHEEP

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SUMMARY

Eosinophilic intranuclear inclusions were demonstrated in the hepatocytes in enzootic icterus in sheep in 24.3% of cases examined. An account is given of the morphology and some histochemical features of the inclusions. Histochemical procedures for the demonstration of nucleic acids, lipids, carbohydrates, specific protein groups and minerals were used on formalin-fixed, paraffin-embedded liver sections. The possible nature and significance of the inclusions are discussed and the conclusion is reached that they are probably of non-viral origin.

INTRODUCTION

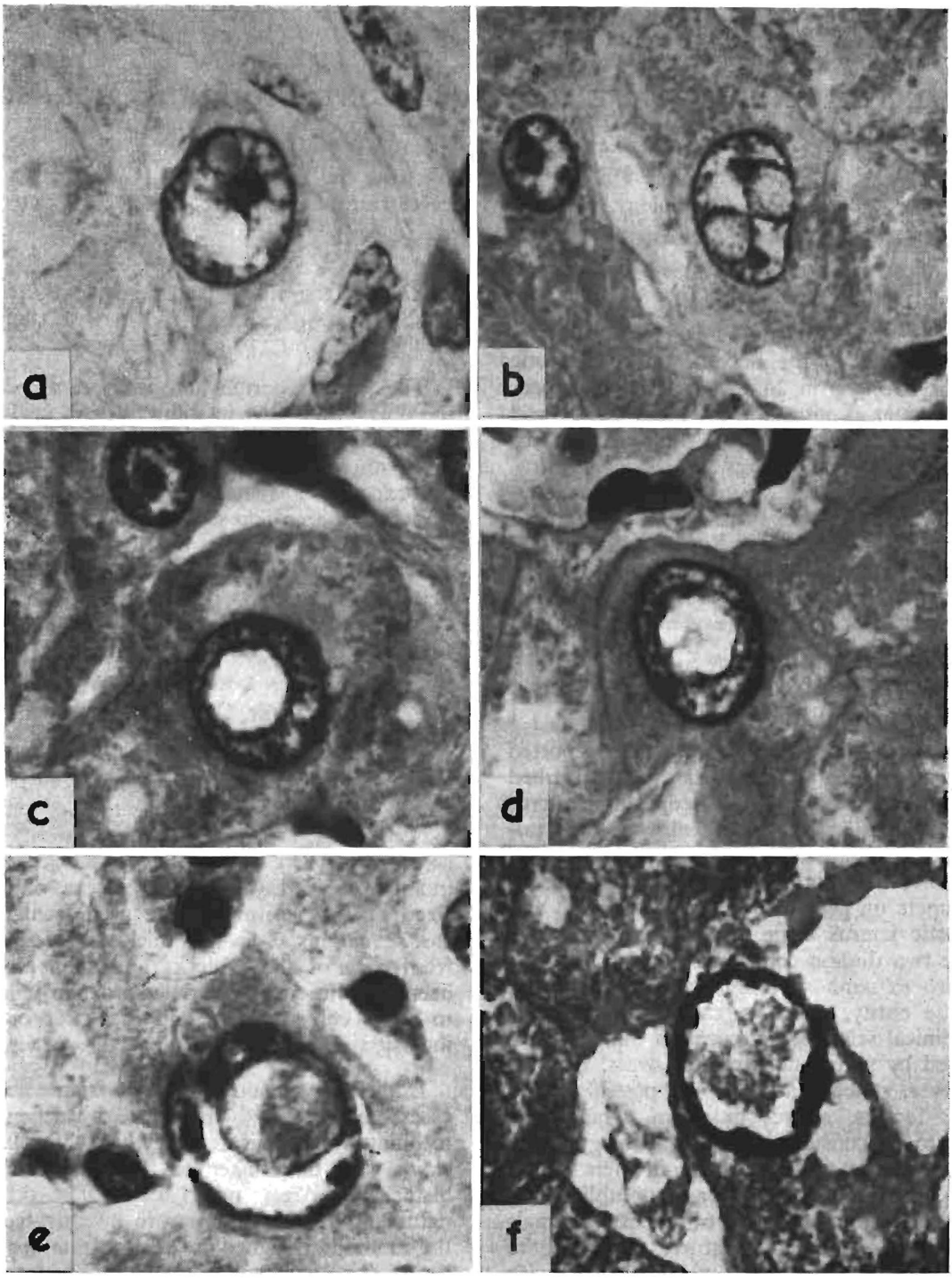
Enzootic icterus in sheep was first reported in South Africa by de Kock[1] who described the symptomatology, macroscopic and microscopic pathology of the disease. He was unable to determine the aetiology of the condition. Brown[2-6] and Brown *et al*[7 8] in a series of papers on geeldikkop (Tribulosis ovis) and enzootic icterus came to the conclusion that these two disease conditions can be regarded as two extreme syndromes of a single basic disease entity, which might be a low grade subclinical selenium intoxication that is precipitated by severe non-specific stress. Brown[5], however, stated that there are possibly many other factors, as yet unknown, which may have to be considered in the aetiology, since neither of the two syndromes in their typical form can be considered as resembling the classical chronic seleniosis of domestic animals as described in the literature. According to him, if selenium is in any way concerned with the aetiology of geeldikkop and enzootic icterus, it probably causes a certain amount of damage to various enzyme systems, particularly those supporting the various transfer mechanisms and the glycolytic cycle.

The study reported here was prompted by the observation that in some cases of enzootic icterus seen during routine examination of histopathological specimens, eosinophilic intranuclear inclusions resembling virus inclusion bodies were present in the parenchymal cells of the liver. It was decided to determine the frequency of occurrence of these structures in this disease and to investigate their histochemical properties in an attempt to ascertain their significance.

MATERIAL AND METHODS

The material examined consisted of seventy cases diagnosed as enzootic icterus over the past 37 years from the collection of pathological cases at this institute. Twenty-two cases, originally studied by de Kock[1], were included. Paraffin-embedded blocks of liver fixed in 10% formalin were used from each case. Sections 3-6μ in thickness were cut from each of these blocks and stained with haematoxylin-eosin and haematoxylin-phloxin to study cell structure and incidence of the inclusion bodies.

Additional sections were cut from selected cases and stained by the histochemical techniques as indicated in the table. Lipid stains were undertaken on 8 cases, the other histochemical tests on 11 cases. The protein nature of the inclusions were investigated by the methods designed to demonstrate specific protein groups in tissue sections. The tetrazotized benzidine procedure, as described by Lillie[9], was applied to duplicate sections.



Coupling was effected with H-acid (the monosodium salt of 8-amino-1-naphthol-3, 6-disulfonic acid) and β-naphthol. As suggested by Thompson *et al*[10],[11] the benzoylation time in 10% (v/v) benzoyl chloride in pyridine, to differentiate between nucleic acid and other protein groups, was reduced to one hour. This proved long enough to prevent any staining of proteins other than nucleic acid.

In the staining procedures with dinitrofluorobenzene (DNFB) the techniques as described by Thompson *et al* (loc. cit) were followed and H-acid was used as coupling agent throughout. Specific groups of proteins; tyrosyl groups, amino groups and sulphydryl groups reacting with DNFB were subsequently blocked by diazotized p-nitroaniline, nitrous acid and hydrogen peroxide respectively (table). By using two blocking agents consecutively only one of the protein groups is left free to react with the stain.

Unembedded formalin fixed liver material from a limited number of cases containing intranuclear inclusions were available for the preparation of frozen sections.

No attempt at virus isolation was made.

Results

In 17 (24.3%) of the seventy cases of enzootic icterus examined, morphologically similar intranuclear inclusions were found in the hepatocytes. The number of inclusion bodies varied from very few per section, in most cases, to very numerous. Three of the 22 cases available for examination from the original material examined by de Kock[1] contained intranuclear inclusions.

Inclusion bodies appeared as roughly round to oval pale eosinophilic structures in the H & E sections (fig. k & l, plate II). Irregular or elongated cigar shaped forms were also observed to a lesser extent. They were frequently finely vacuolated. Nuclei containing inclusions had undergone margination of chromatin and a clear zone or 'halo' separated the inclusions from the peripherally situated chromatin. In some nuclei, bearing inclusions, the nuclear chromatin was only partially marginated. Very delicate eosinophilic threads, stretching from the inclusion to the periphery of the nucleus were present in some instances. Inclusions occurred mostly singly within the nucleus but in rare instances two were noticed to be present. One, or more, nucleoli were usually still recognisable in the marginated chromatin. Although mostly centrally placed in the nucleus, inclusions at times lay to one side, touching the nuclear membrane.

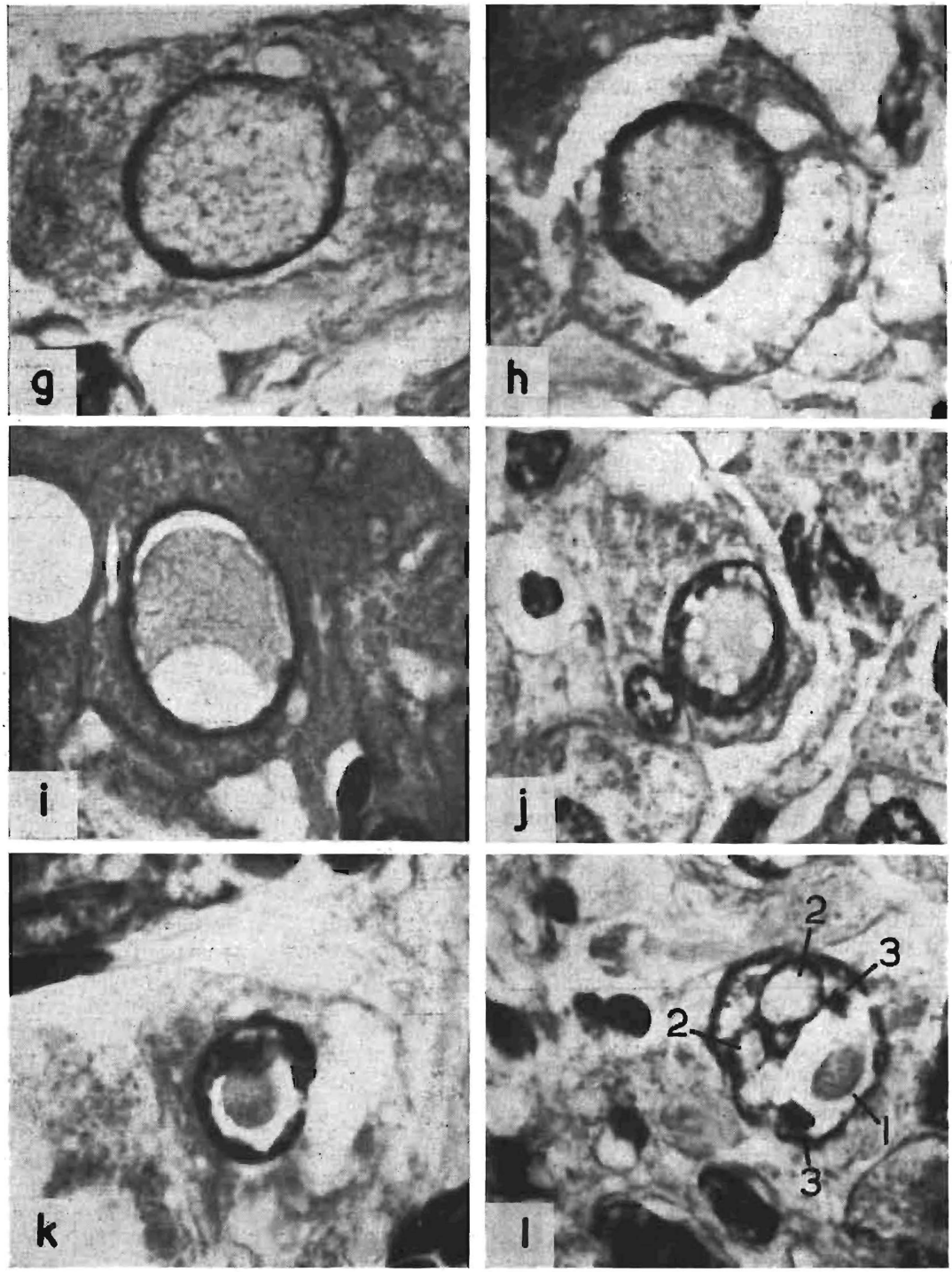
Finely vacuolated, round to oval globules of varying size were also observed intranuclearly in the parenchymal cells of all cases in which inclusions were found (fig. a-d, plate I). The globules were also present in the majority of cases in which inclusions were absent. They occurred singly, but more frequently were multiple and sometimes present with inclusion bodies in the same nucleus (fig. 1, plate II). The smaller globules were chromophobic and distinctly vacuolated. With increase in size and presumably of age, the vacuolation of the globules became indistinct and the globules attained a faint eosinophilic appearance in the centre (fig. d & e, plate I), with a very dark basophilic peripheral rim. The impression was gained that these globules apparently coalesce to form large vacuoles within the nuclei. A fine, granular eosinophilic material, sometimes filling the whole vacuole, then appeared in the centre of the vacuole (fig. f, plate I & fig. g, plate II). This is followed by the granular eosinophilic material assuming a more hyaline appearance (fig. h, plate II) and starting to retract from the peripheral chromatin (fig. i & j, plate II) to form the inclusion, lying free in a clear space within the nucleus (fig. k & 1, plate II).

A striking histological feature in virtually every case of enzootic icterus was parenchymal

PLATE I.

Haematoxylin & pholxin X 1300.

a) Hepatocyte with a small chromophobic intranuclear globule and a dark staining nucleolus.
b) Two finely vacuolated intranuclear chromophobic glovules with a dark staining basophilic peripheral rim. Two nucleili are visible.
c) Large chromophobic globule with distinct vacuolation.
d) Intranuclear globule with small amount of eosinophilic material in the centre.
e) Large intranuclear globule containing granular eosinophilic material. Three nucleoli are present.
f) Nucleus with complete margination of chromatin and containing granular eosinophilic material.



cytomegaly and karyomegaly of varying degree, some of the hepatocytes attaining gigantic proportions. This enlargement of the liver cells was generally more pronounced in the periportal regions of the lobules but in many cases the lobules were more or less uniformly affected. The occurrence of intranuclear inclusions and globules were mainly restricted to these abnormally large nuclei; although present, they were less frequently seen in the smaller nuclei of more normal size. No mitotic figures were seen in nuclei showing karyomegaly.

Histochemical characteristics of the inclusions are summarized in the table. No demonstrable lipids, lipofuscins, carbohydrates, iron, calcium, haemoglobin or any of its iron-containing derivatives were present. Deoxyribonucleic acid or ribonucleic acid could not be demonstrated in the inclusions. That the inclusions have a protein nature were demonstrated by the positive staining reactions with tetrazotized benzidine and dinitrofluorobenzene (DNFB).

With the pyronin-methyl-green method the centre of the intranuclear globules gave a negative staining reaction while nucleoli within the same nucleus stained strongly. The basophilic peripheral rim of the globules was Feulgen positive. Identical staining reactions to the intranuclear inclusion bodies were obtained with the eosinophilic material within the globules.

Discussion

It is generally accepted that inclusion bodies either represent aggregates of actual virus particles, or degenerative changes within the cell, which are the result of viral infection but which do not consist of viral particles. Inclusion bodies, however, are by no means restricted to viral diseases. This is amply illustrated by their intranuclear occurrence in parenchymal cells of the liver and kidney in plumbism and in experimentally induced cases by injections of aluminium hydroxide, ferric hydroxide, carbon and by bismuth, irradiation and treatment with thioacetamide[12].

The results of the histochemical examination of the inclusions reported here point to a non-viral nature. From the negative Feulgen reaction it can be deduced that the inclusions do not contain deoxyribonucleic acid. Positive staining with tetrazotized benzidine indicates that the inclusions contain protein or nucleic acid. Blocking of tissue sections by benzoyl chloride and subsequent staining with tetrazotized benzidine only leaves the nucleic acid reactive. A negative result obtained with the inclusions of enzootic icterus after pretreatment of sections with benzoyl chloride further indicates the absence of nucleic acid as also does the negative result obtained by the methyl-green-pyronin method. Although not conclusive, the absence of nucleic acid in the inclusions seen intranuclearly in the hepatocytes in enzootic icterus strongly suggest a possible non-viral origin. Most viral inclusions which have been examined histochemically react positively to stains for nucleic acid[11]. The demonstration of tyrosyl, amino and sulfhydryl groups by the DNFB stain in the inclusions point to the presence of protein or polypeptides.

Despite the abovementioned findings, the possibility that a virus may still be implicated in the development of the intranuclear inclusions in enzootic icterus cannot be ruled out completely without an adequate virological search being made in cases in which inclusions are present. Rift Valley fever and Wesselsbron disease are two important virus diseases occurring in sheep in the Republic of South Africa in which intranuclear inclusions have been reported to occur in the hepatocytes[13,14]; both may occur as complications in enzootic icterus. The inclusions of Rift Valley fever are negative for deoxyribonucleic acid (DNA), ribonucleic acid (RNA), mucopolysaccharides and basic protein[15]. Inclusions of enzootic

PLATE II.

Haematoxylin & phloxin X 1300.

g) Nucleus with margination of chromatin and completely filled with granular eosinophilic material.
h) Similar to (*g*), eosinophilic material has a more dense and hyaline appearance.
i) & *j*) Nuclei showing retraction of eosinophilic material from peripherally situated chromatin.
k) Eosionphilic intranuclear inclusion body.
l) A nucleus containing an eosinophilic inclusion body (1), chromophobic globules (2) and nucleoli (3).

TABLE: STAINING CHARACTERISTICS OF INTRANUCLEAR INCLUSIONS IN HEPATOCYTES IN ENZOOTIC ICTERUS

Test for presence of	Reagents and methods	Results
LIPIDS	Oil Red O[9]	Negative
	Sudan IV[27]	Negative
	Sudan Black[28]	Negative
LIPOFUSCINS	Schmorl's Method[28]	Negative
CALCIUM	Von Kossa's Silver Nitrate[9]	Negative
	Dahl's Alizarin Red S[28]	Negative
IRON	Gomori's Iron Reaction[29]	Negative
	Masked Iron Reaction[29]	Negative
ACID FASTNESS	Ziehl-Neelsen, long method[28]	Negative
CARBOHYDRATE SUBSTANCES	P.A.S.[27]	Negative
	Alcian Blue, short method[27]	Negative
HAEMOGLOBIN AND DERIVATES	Ralph's Haemoglobin Stain[27]	Negative
NUCLEIC ACIDS	Feulgen Reaction[28]	Negative
	Kurnick's Methyl-Green-Pyronin[28]	Negative
	Brachet's Methyl-Green-Pyronin[28]	Negative
PROTEIN histidine, tryptophane, tyrosine and purines and pyrimidines of nucleic acids	Tetrazotized benzidine[9]	Positive
Purines and pyrimidines of nucleic acids	Tetrazotized benzidine after pretreatment with benzoyl chloride[9]	Negative
Tyrosyl, amino and sulphydryl groups	Dinitrofluorobenzene (DNFB)[11]	Positive
Specific demonstration of tyrosyl groups	DNFB. Blocked by nitrous acid and H_2O_2	Positive
Specific demonstration of amino groups	DNFB. Blocked by diazoaniline and H_2O_2	Positive
Specific demonstration of sulfhydryl groups	DNFB. Blocked by diazoaniline and nitrous acid	Positive

icterus reported here differ from those of Rift Valley fever in giving a positive staining reaction for protein. In Wesselsbron disease le Roux[13] reported the presence of eosinophilic intranuclear inclusions in only three instances out of a number of cases examined by him. No study has been reported in the literature on the incidence, histochemistry and significance of these structures.

Of particular significance is the occurrence of parenchymal cytomegaly and karyomegaly in the liver in enzootic icterus. The development of very large hepatic parenchymal cells has been described by Bull[16], Bull *et al*[17], Bull and Dick[18], Dybing and Erichsen[19] and Schoental and Magee[20 21] in chronic pyrrolizidine alkaloid poisoning. Bull[16] termed this phenomenon megalocytosis. It is regarded as characteristic for chronic pyrrolizidine alkaloid poisoning by the abovementioned authors. Peterson[22] studied the effects of the pyrrolizidine alkaloid, lasiocarpine N oxide, on nuclear

and cell division in the liver of rats. He found that a single injection of this alkaloid greatly reduced the capacity of the liver parenchymal cells for mitotic division with the result that regeneration of these cells was accomplished by an increase in the size of the cells and the ploidy of their nuclei, and not by an increase in their number. Megalocytosis, however, has also been described in aflatoxicosis in domestic animals, caused by the toxin formed by the fungus *Aspergillus flavus*. Link et Fries[23 24 25 26].

Schoental and Magee[20] observed intranuclear globules in megalocytic hepatocytes in rats poisoned experimentally by a single dose of lasiocarpine, one of several alkaloids isolated from *Heliotropium lasiocarpum* L. (Menschikoff, 1932) and *Heliotropium europeum* L. (Culvenor, Drummond and Price, 1954). Bull *et al*[17] experimentally showed this latter plant to be the cause of enzootic jaundice of sheep in Australia. These globules reported by Schoental and Magee[20] differed in their staining reactions: sometimes unstained, sometimes faintly eosinophilic and sometimes neutrophilic with a narrow basophil peripheral rim, which stained by the Feulgen method. The central part of the globules were Feulgen and pyronin negative. Megalocytosis and intranuclear vacuoles, which they regarded as abnormally enlarged nucleoli, were reported by Dybing and Erichsen[19] in liver cells of rats experimentally poisoned by *Senecio aquaticus*. These vacuoles were eosinophilic in the centre, the larger ones having a narrow peripheral basophil 'membrane', they were pyronin positive and Feulgen negative. In experimental aflatoxicosis in pigs Harding *et al* (loc. cit) reported the presence of misshapen amphophilic inclusions in the enlarged nuclei of liver cells.

Judging from these reports there seems to be a definite relationship between the occurrence of karyomegaly in the liver cells in chronic pyrrolizidine alkaloid poisoning and chronic aflatoxicosis and the presence of intranuclear 'globules', 'vacuoles' or 'inclusions' as described by the authors quoted above. Morphologically the intranuclear globules in the hepatocytes in enzootic icterus resemble these structures (cf. figs. 6 & 7, Harding *et al* (loc. cit)). The staining reactions of the intranuclear globules in enzootic icterus are also very similar to those reported by Schoental and Magee[20]. The negative staining of these globules with the methyl-green-pyronin method indicates that they are probably not hypertrophied nucleoli. They occurred constantly in all cases in which intranuclear inclusions were found in enzootic icterus and in the majority of cases in which inclusions were absent. Globules were mainly confined to the enlarged liver cell nuclei. The evolution of eosinophilic inclusions and the fact that the amorphous eosinophilic granular material in the bigger globules reacted identically to histochemical tests as the inclusions, point to a possible connection between the intranuclear globules and the intranuclear inclusions in the hepatocytes in enzootic icterus. We have concluded that the globules are probably an earlier developmental phase of the inclusions.

The genesis of the intranuclear inclusions in enzootic icterus remains obscure. At present the evidence available seems to indicate that these structures are abnormal products of an altered nuclear metabolism of the hepatocytes induced by a non-viral agent or hepatotoxin and causing morphological changes in these cells very similar to those resulting from chronic pyrrolozidine alkaloid poisoning and chronic aflatoxicosis.

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TRADEMARK

EQUIZOLE

(EQUINE THIABENDAZOLE)

HIGHLY EFFECTIVE

Strongyles:	Strongylus vulgaris 100%	50 mg./Kg.
	Strongylus equinis 100%	
	Strongylus edentatus 90%	
	Small strongyles 100%	
Pinworm:	Oxyuris equi 100%	
Ascarids:	Parascaris equorum 100%—100 mg./Kg.	

NO SIDE EFFECTS

"In the toxicity trials, doses of 600 mg./Kg. and below did not produce untoward clinical effects."

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ADVANCES IN GEELDIKKOP (TRIBULOSIS OVIS) RESEARCH

7. A PRELIMINARY NOTE ON THE INFLUENCE OF ICTEROGENIN ON THE POLYETHENOID FATTY ACID COMPOSITION OF LIVER CELL MEMBRANES

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Summary

The influence of icterogenin on the polyethenoid fatty acid composition of rabbit liver lipids is described. It has been found to cause a fair to marked decrease in the amount of linolenic acid present particularly in the neutral lipids of liver cell walls and associated structures. This is associated with a rapid decline in bilirubin excretion. The effects of pentobarbitone sodium anaesthesia on liver cell lipids is also described and compared with those of icterogenin.

Introduction

The pentacyclic triterpene acid, icterogenin (22, β-Angeloyloxy-24-hydroxy-3-oxo-olean-12-en-28-oic acid), has for long been known to induce a syndrome in sheep clinically similar to natural geeldikkop yet differing from it in many important respects[1,2,3].

The icterus produced in these animals has been classified as an intrahepatic cholestasis[4,5] and the essential biochemical lesion produced by the compound is believed to be a decreased permeability of the liver cell wall towards compounds like bilirubin glucuronides, porphyrins, bromsulphalein, copper ions etc[3,6,7]. The icterogenic triterpenes, including icterogenin itself, have proved valuable tools for studies on biliary secretion in the sheep and rabbit and their action has been shown to be highly stereoisomer specific[8,9,10]. The suggestion has been advanced by inference from the results of studies on biliary secretion in natural geeldikkop and triterpene intoxication that excretion of bile pigments, bile acids, porphyrins and sulphonephthalein dyes through the hepatic cell membrane is an active energy dependant process[7,11]. Decreased permeability of the hepatic cell membrane towards the compounds mentioned is believed to result from failure of the energy supplying mechanisms associated with the transport systems[3,7,11]. Previous studies[7] have indicated that the activity of succinic and glyceraldehyde-phosphate dehydrogenases is markedly depressed in the liver cell during icterogenin intoxication. The fact that the icterogenic activity of triterpene acids like icterogenin is highly stereo-isomer specific would seem to indicate an equally specific site of action on the liver cell membranes. The purpose of the present paper is to report some observations on the effects of icterogenin on the lipid components of hepatic cell membranes.

Materials and Methods

The icterogenin used was kindly provided by our co-workers Dr. W.T. de Kock and Mr. L.A.P. Anderson of the National Chemical Research Laboratories, Pretoria. All the experiments cited were performed on young male black or grey rabbits whose weight varied between 2- and 3 Kg. Each animal used was provided with an external biliary fistula for the purpose of following the progress of the intoxication by noting the hourly excretion of bilirubin conjugates as reported elsewhere[8]. Two types of experiment were performed viz.: (a) where liver tissue removed from each animal at the time of introduction of the cannula in the common bile duct and administration of icterogenin was compared with liver tissue from the same animal at the end of the test period[7] and (b) where liver tissue from poisoned animals without prior partial hepatectomy was compared with that from control rabbits who were provided with biliary

cannulae at the same time as the test animals. In all instances pentobarbitone sodium anaesthesia was used for introduction of the biliary cannulae at a dosage level of 1/5th of a grain per lb. bodyweight. Icterogenin was administered intraperitoneally as previously described[8] in 2% "celofas" suspension at a dosage rate of 100 mg per Kg bodyweight. Control animals received "celofas" solution only.

In the first type of experiment 5g of liver was removed from each animal prior to the administration of icterogenin and 25g was taken following euthanasia at the end of the test period which lasted from 7-9 hours. The duration of the test period was determined by the time taken for the appearance of unequivocal suppression of bilirubin excretion[8]. Thirteen animals were used in this group of experiments. Liver lipids were isolated and fractionated as follows: Liver tissue, once it was removed from the animal, was homogenized immediately in a "Servall Omnimix" homogenizer with ten volumes of ethanol: ether 1:3. The homogenate was allowed to stand for 30 minutes and was then filtered, Filtrates were retained and the residues were rehomogenized with a further ten volumes of the ethanol: ether mixture. This second homogenate was then filtered and both filtrates were combined and taken rapidly to dryness at 40°C *in vacuo* in a rotary type evaporator. The crude lipid so isolated was dissolved in the smallest volume of light petroleum (B.P. 40-60°) and the solution repeatedly washed with small volumes of water until no more pigment or colloidal material was removed. (Any emulsions which formed at this stage were broken by addition of small amounts of ethanol or by centrifugation.) The light petroleum extracts were then dried with small amounts of anhydrous sodium sulphate and after filtration were concentrated to a small volume *in vacuo* at less than 40°C. Fractionation into neutral lipid and phospholipid fractions was achieved by addition of ten volumes of acetone and allowing to stand at −15°C for two hours. The phospholipid precipitate was filtered off and washed with small volumes of ice cold acetone. The acetone washings were added to the filtrate and the bulk was then taken rapidly to dryness *in vacuo* at less than 40°C to yield the neutral lipid fraction. The purity of each fraction was followed by chromatography on silicic acid impregnated paper[12] and four separate chromatograms were usually made of each fraction for use with the following location sprays: 0.2% ninhydrin in water saturated butanol, phosphomolybdic acid and stannous chliride[13], 0.001% aqueous Rhodamine 6B and iodine vapours. Some minor mutual contaminants were removed by chromatography on silicic acid columns[14,15].

Polyethenoid fatty acids were determined by the micro-method of Herb and Riemenschneider[16] and results were expressed as percentages of linoleic, linolenic, arachidonic, eicosapentaenoic and docosahexaenoic acids present in each fraction. In general the weights of each fraction used in the isomerization procedure varied between 2-7 mg depending on the amount of liver tissue used for lipid extraction.

In the second group of experiments sixteen animals were used of which half served as undosed controls. The same dosage level of icterogenin was used as before. After the test period of 7-9 hours the rabbits were slaughtered and 30g of liver were removed at once from each of the test and control animals. It was usual to do only one test and one control experiment each day. All livers were homogenized without delay in ice cold 0.25M sucrose (ten volumes) in the "Servall Omnimix" homogenizer and the homogenate subsequently fractionated by the method of Hogeboom[17] into cell debris, mitochondrial and supernatant fractions. Lipids were isolated from each subcellular fraction and separated into neutral lipids and phospholipids as above. Polyethenoid fatty acids were determined as before and the results were expressed in the same manner. The extraction and purification of lipids from the final supernatant cell fractions proved to be difficult and entailed considerable loss of material. This fraction was therefore rejected in most of the experiments and work was confined to the lipids extracted from the cell debris and mitochondria.

All rabbits were maintained on green

lucerne *ad libitum* prior to the experiments. All chemicals used were Analytical reagent grade, all solvents used were redistilled, isomerization of polyethenoid acids was conducted at 190°C in an oxygen free nitrogen atmosphere, silicic acid used for column chromatography was from Sigma Chem. Co. (300 mesh for lipid chromatography) and pure samples of lecithins, cephalins, phosphatidylserine, phosphatidyl ethanolamine, sphingomyelin, various triglycerides, cholesterol esters and the polyethenoid fatty acids used to standardize the various techniques which were employed were obtained from the Sigma Chem. Co. Bile samples were analysed as described elsewhere[8].

Results

Before the experiments with icterogenin were commenced a study was made of the effects of the anaesthetic and the operation technique as a whole on the composition of the poly-unsaturated fatty acids of the lipids in the livers of eight control rabbits. The results presented in Table 1 are representative of those from the whole group. The abbreviation "pre" used in this table refers to the 5g of liver removed at the commencement of the operation, while the abbreviation "post" refers to the 25g of liver removed at the end of a seven hour test period.

TABLE 1—CHANGES IN THE POLYETHENOID FATTY ACID COMPOSITION OF RABBIT LIVER LIPIDS FOLLOWING PENTOBARBITONE ANAESTHESIA, PARTIAL HEPATECTOMY AND CANNULATION OF THE COMMON BILE DUCT

Fatty Acid Composition	Neutral Lipids						Phospholipids					
	8 Pre-	8 Post	9 Pre	9 Post	10 Pre	10 Post	8 Pre	8 Post	9 Pre	9 Post	10 Pre	10 Post
% Linoleic acid.....	13.77	17.71	10.16	13.58	9.08	15.64	8.01	15.86	12.18	16.95	10.12	9.70
% Linolenic acid.....	5.77	7.87	5.42	7.58	7.43	14.26	1.66	3.45	6.34	4.52	4.14	4.20
% Arachidonic acid.....	0.90	1.29	1.73	0.76	0.04	0.82	3.20	5.33	5.67	5.69	3.10	3.11
% Eicosenoic acid.....	0.03	0.43	0.84	0.24	0.15	0.63	0.80	1.53	2.80	2.17	1.34	1.24
% Docosenoic acid.....	0.90	1.65	3.68	1.61	3.05	1.76	0.54	1.90	7.26	2.46	1.09	0.96

Note: Eicosenoic and Docosenoic refer to eicosapentaenoic and docosahexaenoic acids respectively.

The procedure as a whole appeared to affect the poly-unsaturated fatty acid composition of the neutral lipid fraction, there being in nearly all cases a marked increase in the percentage of linoleic acid and in general a mild increase in the percentage of linolenic acid. Values for the other three fatty acids at the end of the test period in each animal were rather variable and on the whole no significant change was apparent. In the case of the liver phospholipids of these animals no significant alterations in the polyethenoid acid values appeared either. Values obtained for linoleic acid were elevated in a few instances, (e.g. animals 8 and 9 in Table 1) but in most cases little significant alteration was observed.

The results obtained from animals poisoned with icterogenin are represented by those given in table 2. The abbreviations "pre" and "post" are used in the same context as above.

From this table it is clear that icterogenin brings about some quite marked changes in the unsaturated fatty acid composition of liver lipids. The most constant findings are in the figures obtained from analyses of the neutral lipid fractions. Linoleic acid is variably affected, but the values for linolenic acid are almost constantly lower at the end of the intoxication than they were at the start of each experiment. Values for the three minor acid constituents do not show any significant variations. The same pattern is discernable in the phospholipid fractions from each liver.

In the second series of experiments where livers from separate control and poisoned animals were fractionated into subcellular

TABLE 2.—CHANGES IN THE POLYETHENOID FATTY ACID COMPOSITION OF LIVER LIPIDS FOLLOWING ICTEROGENIN ADMINISTRATION TO RABBITS WITH BILIARY CANNULAE

Fatty Acid Composition	Neutral Lipids								Phospholipids							
	11 Pre	11 Post	12 Pre	12 Post	13 Pre	13 Post	14 Pre	14 Post	11 Pre	11 Post	12 Pre	12 Post	13 Pre	13 Post	14 Pre	14 Post
% Linoleic Acid.....	17.39	17.66	13.38	12.23	12.11	10.06	11.68	17.93	19.66	19.21	11.27	17.81	11.97	12.91	18.35	17.87
% Linolenic Acid.....	5.56	3.15	2.28	1.79	2.16	5.50	5.71	5.03	3.33	2.62	1.52	0.87	7.24	5.60	3.86	2.83
% Arachidonic Acid...	1.38	1.82	1.70	1.33	0.65	0.68	2.31	1.53	6.10	6.54	4.76	7.73	3.87	4.82	6.74	5.77
% Eicosenoic Acid.....	0.59	0.54	0.21	0.41	0.11	0.12	0.16	0.30	2.04	2.03	1.24	2.08	1.62	1.81	2.13	1.92
% Docosenoic Acid....	1.69	1.77	0.70	0.57	0.21	0.22	0.25	0.16	2.56	2.48	1.46	2.37	0.52	1.26	1.39	2.09

Note: Eicosenoic and Docosenoic refer to eicosapentaenoic and docosahexaenoic acids respectively.

particles, pairs of rabbits were done each day and the rabbits in each pair were so selected to be as close as possible in bodyweight and condition. Representative results from this series of tests are presented in Tables 3 and 4. The results are so grouped that rabbits 16 and 17 or 18 and 19 etc. constitute pairs. The results of each pair are directly comparable for the reasons mentioned above and by virtue of the fact that the liver lipids from each pair were put through the same daily batches of analytical techniques. The duration of the test period was for practical purposes identical in the case of each pair.

TABLE 3.—THE POLYUNSATURATED FATTY ACID COMPOSITION OF THE NEUTRAL LIPID FRACTION FROM SUBCELLULAR PARTICLES OF LIVER CELLS FROM CONTROL AND POISONED ANIMALS

Material	16 Control	17 Ictero-genin	18 Control	19 Ictero-genin	20 Control	21 Ictero-genin	22 Control	23 Ictero-genin
CELL DEBRIS								
% Linoleic Acid.....	9.3	14.4	9.6	8.5	13.7	5.7	18.1	6.8
% Linolenic Acid.....	6.3	3.8	3.4	2.1	15.1	3.7	4.1	1.5
% Arachidonic Acid.....	2.7	1.2	1.5	1.0	4.0	1.3	1.3	0.01
% Eicosenoic Acid.....	0.84	1.15	0.68	1.1	1.6	0.56	0.4	0.02
% Docosenoic Acid.....	0.29	0.47	3.4	2.3	6.8	1.14	0.5	0.03
MITOCHONDRIA								
% Linoleic Acid.....	3.7	3.46	12.3	4.9	8.6	6.7	22.1	23.0
% Linolenic Acid.....	4.2	1.61	4.0	1.3	10.7	5.7	4.9	8.5
% Arachidonic Acid.....	1.2	0.01	1.7	1.2	1.2	1.6	2.9	4.8
% Eicosenoic Acid.....	0.44	0.01	1.0	0.63	0.62	0.84	1.0	1.8
% Docosenoic Acid.....	0.39	0.02	2.8	4.3	1.9	2.2	2.6	1.07

From this group of experiments it appeared that the most marked and constant changes following icterogenin intoxication appeared in the neutral lipids of the cell debris fraction. A decrease in the percentages of all the polyethenoid acids in these lipids was a general finding; this decrease being most apparent in the case of linolenic and arachidonic acids. Values obtained from the phospholipids of this cell fraction were most variable. Apart from some apparent increase in the percentages of linoleic and arachidonic acids in some instances no general trend was discernable. In the case of the mitochondrial lipids a decrease

TABLE 4.—THE POLYUNSATURATED FATTY ACID COMPOSITION OF THE PHOSPHOLIPID FRACTION FROM SUBCELLULAR PARTICLES OF LIVER CELLS FROM CONTROL AND POISONED ANIMALS

Material	16 Control	17 Icterogenin	18 Control	19 Icterogenin	20 Control	21 Icterogenin	22 Control	23 Icterogenin
CELL DEBRIS								
% Linoleic Acid	8.1	14.6	14.9	18.2	5.9	11.1	20.7	18.4
% Linolenic Acid	7.2	5.9	3.7	4.2	6.5	7.2	5.4	1.8
% Arachidonic Acid	4.5	5.9	7.6	9.0	3.2	5.3	8.5	6.5
% Eicosenoic Acid	1.6	1.9	2.0	3.2	1.5	2.5	2.8	2.0
% Docosenoic Acid	1.4	2.2	2.2	4.1	2.5	2.4	8.7	1.0
MITOCHONDRIA								
% Linoleic Acid	9.9	13.0	13.7	5.8	9.7	8.1	11.6	21.8
% Linolenic Acid	8.2	7.0	6.4	2.8	6.9	8.8	8.1	2.8
% Arachidonic Acid	7.0	5.4	4.7	4.7	4.7	4.2	7.6	7.8
% Eicosenoic Acid	0.41	1.6	2.0	0.95	2.2	2.0	3.5	2.5
% Docosenoic Acid	2.0	1.5	2.3	1.3	2.3	2.6	1.79	1.9

in the linolenic acid content of both lipid fractions was about the only most general and constant finding.

The results presented in Table 5 illustrate the marked degree of suppression of bilirubin excretion in icterogenin poisoned rabbits. These results were obtained from rabbits 18-23, the analyses of whose liver lipids appear in Tables 3 and 4, hence these figures given for bilirubin excretion may be compared directly with the results of the lipid analyses. The values presented for the excretion of bilirubin in these animals are expressed in mg of the pigment excreted hourly over the test period[8].

DISCUSSION

The technique of comparing liver tissue removed from a rabbit before intoxication with

TABLE 5.—THE BILIARY EXCRETION OF BILIRUBIN IN ICTEROGENIN POISONED RABBITS AND CONTROL ANIMALS, BOTH PROVIDED WITH CANNULAE OF THE COMMON BILE DUCT

Test Period	Bilirubin excretion (mgm. per hour).					
(hrs)	Rabbit 18 (Control)	Rabbit 19 (Icterogenin)	Rabbit 20 (Control)	Rabbit 21 (Icterogenin)	Rabbit 22 (Control)	Rabbit 23 (Icterogenin)
1	0.31	0.31	0.30	0.14	0.35	0.13
2	0.43	0.43	0.29	0.20	0.51	0.32
3	0.39	0.28	0.32	0.09	0.44	0.09
4	0.38	0.13	0.24	0.06	0.39	0.003
5	0.36	0.07	0.26	0.03	0.32	0.005
6	0.33	0.05	0.27	0.02	0.31	0.005
7	0.54	0.02	0.28	0.02	0.32	0.002

that removed from the same animal at the end of the test period has proved an extremely useful one in our studies on the icterogenic triterpene acids. At least 5-6g of liver can be removed successfully from a 2-3Kg rabbit, the partial hepatectomy is attended by very little bleeding from cut liver surface, and in general recovery is uneventful. In the present studies the amount of liver tissue that could be successfully removed and the sensitivity of the method for the analysis of the polythenoid acids constituted distinct limiting factors. The yield of lipids after isolation and appropriate purifications is very low. In the later series of experiments, where the whole livers of both test and control animals were available for fractionation into subcellular particles, the yield of pure lipids was still low particularly in the case of the mitochondria. In the latter instance amounts of chromatographically homogenous phospholipid as low as 2mg had to be used for the isomerization in the estimation of the polyunsaturated acids. Amounts as low as these are probably marginal as regards the sensitivity of the method particularly in the case of the minor acid fractions, viz. arachidonic, eicosenoic and docosenoic acids. This may account for the considerable variations seen in the results pertaining to these acids.

In general sufficient pure material was usually obtainable from the dell debris fraction of each liver to give reasonably consistent results, particularly in the case of the neutral lipids, where amounts of 5-7mg of pure lipid fraction were readily obtained.

The cell debris fraction consists mainly of cell walls, endoplasmic reticulum and nuclear fragments. There seems little doubt from the results presented here that icterogenin has a marked influence on the neutral lipid components of these structures. This is seen mainly as a fair to marked decrease in the linolenic acid content of this lipid fraction. The neutral lipids of liver cells consist mainly of hydrocarbons, pigments, sterols and sterol esters and mono-, di- or triglycerides. Some preliminary attempts have been made to separate these components on silicic acid columns, in order to determine the exact site of icterogenin's action. Such chromatography entails considerable losses of these components and with the amount of liver tissue used in these experiments the final yield of pure individual lipid from each subcellular fraction is too low for accurate estimation of the various polyethenoid acids by the isomerization procedure. A few experiments have been performed, however, using pooled pure lipid components from a number of chromatographic runs. These indicate that the sterol ester fraction might be primarily concerned. Further work on this aspect will be reported in a later paper.

Mitochondrial lipids appear on the whole to be far less affected by icterogenin than those of the cell walls, nuclear fragments etc. The same general decrease in linolenic acid was observed in both lipid fractions. These findings agree in the main with previously reported respiratory enzyme studies in which mitochondrial integrity was believed to be largely unaffected[7].

The results presented in Table 1 indicate that the operation for introduction of common bile duct cannulae has a rather profound effect on the polyethenoid acid composition of the neutral lipids of rabbit livers, this being indicated mainly by a rise in the linoleic and linolenic acid content of these lipids. Since it is difficult to imagine how the purely surgical manipulations involved can affect the composition of these lipids, it is most likely that these changes were brought about by direct action of pentobarbitone sodium itself, or were consequent to the general metabolic effects of anaesthesia. On the whole these effects appear to be suppressed by icterogenin, the main effect of which is to bring about a decrease in the polyunsaturated acids of the neutral lipids and particularly in the linolenic acid content.

While the exact biochemical mechanisms involved in the action of icterogenin and similar compounds remains as yet undefined, the present and previous studies have narrowed down the field somewhat. By virtue of its chemical nature and solubility properties, it seems reasonable to suppose that the site of action of icterogenin would be at some lipid area in the cell membrane. The present study lends some weight to this idea.

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The authors wish to thank the Chief, Veterinary Research Institute, Onderstepoort for permission to publish this paper, and our technicians Messrs. R. J. J. Briel and P. J. de Wet for their considerable assistance in the preparation of the rabbits for surgery, the administration of anaesthetics and in the operations for introduction of the biliary cannulae.

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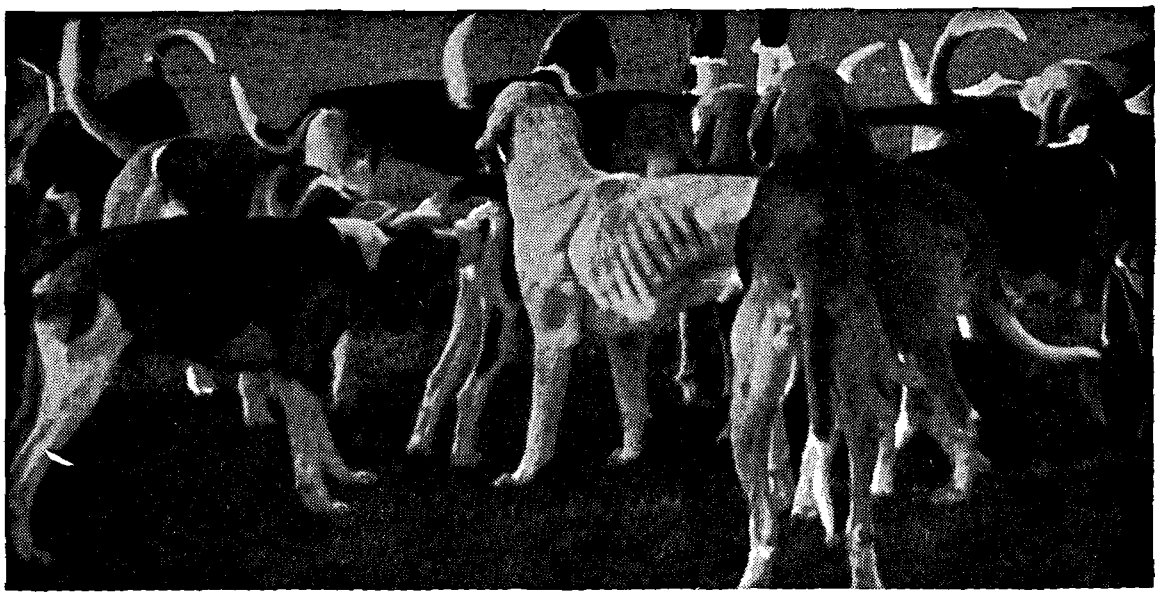

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A PRELIMINARY NOTE ON THE ISOLATION AND PHARMACOLOGICAL ACTIONS OF THE TOXIC PRINCIPLES OF *HOMERIA GLAUCA* (W. and E.) N.E. Br.

(*Natal Yellow Tulp*)

T. W. NAUDÉ* AND D. J. J. POTGIETER**

SUMMARY

By using very mild isolation procedures a new bufadienolide cardiac aglycone and several minor, thus far unidentified, toxic components were isolated from *Homeria glauca*. It is the first recorded isolation of a member of the cardiac glycoside group from the Iridaceae.

The subcutaneous LD50's for the guinea-pig and mouse were determined.

In spite of being one of the digitalis group, this compound causes marked nervous rather than cardiac symptoms in these animals and furthermore proved to be a relatively potent local anaesthetic.

Tulp poisoning, due to members of the genera *Homeria* and *Moraea* of the family Iridaceae, has been recognised for more than a century; the earliest recorded case being that of Ecklon in 1830. All attempts to date at isolating the toxic principles from these plants have been unsuccessful[1].

By deviating completely from the accepted, classical chemical isolation procedure and employing very mild techniques combined with selective semiquantitative toxicity tests on guinea-pigs (*per os*) at each isolation step, a series of toxic principles has now been demonstrated in *Homeria glauca*.

The extraction procedure consisted of shaking the dried, finely ground plant material, suspended in 0.2M acetic acid solution, with chloroform at room temperature. The neutralised, dried chloroform extract was evaporated under reduced pressure at 40°C to a tarry residue from which the toxic principles were removed by extraction with 50 per cent (v/v) ethanol in 0.2M citrate buffer, pH 3.25. The resulting solution was extracted with chloroform. After evaporation of the solvent the mixture was separated by column chromatography on silica gel packed in an organic phase consisting of a mixture of chloroform, ethanol and water (950 : 25 : 25, v/v) and developed with the same phase. The individual components were collected and identified by thin layer chromatography on silica gel. The chromatograms were developed in 7.5 per cent ethanol in chloroform and sprayed with one per cent ceric sulphate in 4N sulphuric acid.

The main toxic component was the tenth of the first twelve practically pure components eluted and was toxic at 0.4 to 0.5 mg/Kg, whereas a further five of these components were toxic at between 10 and 40 mg/Kg. The column was then eluted with ethanol and this fraction, containing a whole series of more polar components, proved to be lethal at 10 mg/Kg.

The main toxic component was crystallized from ethanol and recrystallized from benzene and chloroform and resulted in a yield of 0.04 per cent by weight of the original, dry plant material. These crystals were toxic at 0.25 mg/Kg, a 1200 times increase in toxicity by weight in comparison with the plant material, and accounted for 52 per cent of the original toxicity of the material extracted.

Subcutaneous LD50's were determined ac-

* Veterinary Research Institute, Onderstepoort.
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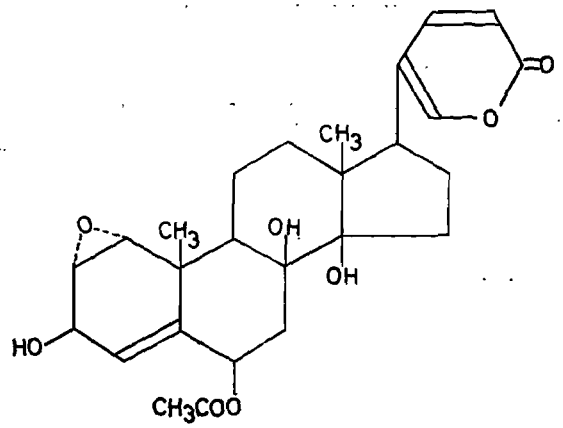

cording to the method of Litchfield and Wilcoxen[2] and were found to be 0.194 ± 0.01 mg/Kg for the guinea-pig and 3.6 ± 0.9 mg/Kg for the mouse.

The symptoms observed were those of a nervous poison. In the guinea-pig an initial hypersensitivity was followed by a curare-like paresis going over into paralysis with death due to respiratory paralysis. With minor variations, the same symptoms were also observed with the original plant material as well as with the six other toxic fractions mentioned above.

In mice a certain percentage died during respiratory distress and in the others this was followed by a considerable degree of recovery. A severe convulsive state then appeared accompanied by rolling and circling, hyperaesthesia and severe ataxia, resulting in further deaths.

During the isolation a numbing effect was experienced whenever the active fractions accidentally came in contact with the mucous membrane of the mouth. A relatively potent local anaesthetic action was confirmed by the human intradermal as well as the rabbit's cornea methods[3]. In the former method concentrations as low as 0.0025 per cent (w/v) in normal saline gave definite anaesthesia, but moderately painful side reactions were encountered. In the latter method complete anaesthesia was obtained in five out of eight rabbits at a concentration as low as 0.05 per cent and no untoward effects were observed with even a one per cent solution. In rabbits a very marked myosis was observed during these tests, whereas in guinea-pigs mydriasis was encountered.

The main toxic component has been identified by Enslin and van Wyk[4] as the aglycone of a bufadienolide cardiac glycoside named 1α, 2-Oxidoscillirosidine (see fig. 1). This is the first recorded isolation of a cardiac glycoside from the Iridaceae.

The convulsive symptoms observed in mice are in close accordance with those described[5],[6] for scillirosidine and scilliroside, the active principles of red squill, *Urginea maritima* var. *rubra*.

In a preliminary trial, however, a digitalis action on the heart could be demonstrated in a rat by electrocardiography. It was masked however by respiratory paralysis. Further experiments on cats to determine the relative digitalis potency of this compound in comparison with the known glycosides and aglycones are envisaged.

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J. S. Afr. vet. med. Ass. 37 (1) 1966

SOME FIELD OBSERVATIONS ON AFLATOXICOSIS IN THE POTGIETERSRUS VETERINARY AREA

L. R. HURTER

Veterinary Investigation Centre, Potgietersrus

The bushveld of the Northern Transvaal is primarily suitable for cattle ranching. Due to the post-war boom period, the temptation arose to augment farming incomes from ranching with crop production. Extensive bush clearing was undertaken and especially groundnuts were intensively planted. Within a relatively short period ranching was replaced by mixed farming. Groundnut hay was found to be an invaluable feed in the fattening of slaughter cattle.

During the winter of 1963, mortality in cattle, small stock and pigs was investigated in the area. It was established that the feeding of fungus infected groundnuts and nubbings was the cause. Conditions most suitable for the development of fungi occurred during inclement weather after stacking, injury to pods during lifting, or when the nuts were left in the ground too long before harvesting.

Further investigations proved that aflatoxin, produced by *Aspergillus flavus*, was to blame. Aflatoxicosis in poultry, dogs and pigs was already known, the toxin having been demonstrated in groundnuts, oil cake, dog biscuits, fowl mash and rarely in groundnut hay.

A number of samples of groundnuts which caused per-acute mortality in pigs after *ad lib* feeding, were submitted from the Potgietersrus Veterinary area. The fungi isolated were:—

1. *Aspergillus flavus*
2. *Aspergillus awamori*
3. *Penicillium implicatum*
4. *Penicillium steckii* (?)
5. *Penicillium chrysogenum*
6. *Penicillium variabile*
7. *Other Penicillium spp.*

The aflatoxin content in the 24 specimens submitted ranged from 2-300 parts per million, with an average of 66 parts per million. During the same period eight samples of groundnut hay were analysed with an aflatoxin range of 0-16 p. p. m., and an average of 4 p. p. m. It was only in three samples, however, that 2 p. m. m. was exceeded, and normally groundnut hay of average quality must be regarded as having little aetiological significance in aflatoxicosis.

SYMPTOMS: Symptoms may be divided into (i) *Per-acute* and *Acute*, mainly in pigs and small stock. (ii) *Chronic*, as seen in cattle. There appeared to be no breed susceptibility — Jerseys and Afrikaners were equally susceptible, symptoms depending on the p. p. m. aflatoxin present in the infected material fed.

The following mortality investigations were carried out:—

No.	*Species involved*
10	Pigs
3	Cattle
2	Goats
1	Sheep

(i) *Per-acute and Acute Aflatoxicosis*:
Mortality occurred in pigs 12 hours after *ad lib* feeding of mouldy groundnuts. Paralysis, extreme weakness, haemorrhagic diarrhoea and injected mucous membranes were observed.

(ii) *Chronic Aflatoxicosis*:
In cattle aflatoxicosis was characterized by extreme emaciation, watery diarrhoea, marked ascites and hydrothorax, eventual icterus and inability to rise.

POST-MORTEM EXAMINATION AND HISTO-PATHOLOGICAL FINDING:

(i) *Acute Aflatoxicosis*:

(a) *Post-Mortem*: *Congestion, haemorrhage* and diffuse necrosis of the liver, haemorrhagic gastro-enteritis, petecheae and echymoses of parenchymatous organs, oedema of the lungs.

(b) *Histo - pathological examination*: Generalised central lobular necrosis, bile duct proliferation and fatty degeneration of the peripheral hepatic cells, with stasis and hepatic-haemorrhages.

(ii) *Chronic Aflatoxicosis*:

(a) *Post-mortem*: Extreme emaciation and cachexia, marked ascites and hydrothorax, cirrhosis of the liver and eventual icterus, were the pathological anatomical changes usually seen.

(b) *Histo - pathological examination*: The liver showed fatty degeneration, necrosis, intralobular cirrhosis and bile duct proliferation. Nephrosis of the kidneys and fatty degeneration of the myocard was observed.

DISCUSSION

Attention is drawn to a serious threat to the livestock industry of the Northern Transvaal. The incidence and the economic losses caused in cattle, small stock and pigs is much higher than reflected in this report. Stock owners usually only report heavy mortality, but enquiries have confirmed that many sporadic cases are not brought to the attention of the Investigation Centre.

Every effort will have to be made to ensure that groundnut crops are correctly harvested and stored to eliminate unnecessary risk to both man and animals.

ACKNOWLEDGEMENTS

Thanks are due to Drs. T. F. Adelaar and J. D. Smit, both of Onderstepoort, for toxicological and histopathological examinations respectively and to Mr. D. J. S. du Toit for the collection of samples. The Chief, Division of Veterinary Field Services is thanked for permission to publish this article.

Diseases of Feedlot Cattle

R. JENSEN, M.S., D.V.M., PH.D., Dean, College of Veterinary Medicine, Colorado State University, U.S.A., and D. R. MACKEY, D.V.M., Practitioner, Greeley, Colorado, U.S.A.

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POISONING OF CATTLE FOLLOWING ACCIDENTAL SPRAYING WITH THIODAN*

G. E. THOMPSON

Cooper & Nephews S. A. (Pty.) Ltd.—East London

SUMMARY

Approximately 50 out of 250 animals accidently sprayed with 0.1% Thiodan emulsion showed acute toxic symptoms, and 11 of the affected animals died.

The clinical symptoms observed and the treatments applied are described and discussed.

HISTORY

During late summer a 5% Thiodan miscible oil concentrate was accidentally used in place of a benzene hexachloride miscible oil concentrate to prepare an emulsion wash for spraying cattle. A dilution rate of 1 in 300 gave a wash concentration of approximately 0.12% Thiodan. About 250 head were sprayed with this wash by means of a mechanical sprayrace in the early morning. They were subsequently returned to their respective camps.

First signs of toxicity were noticed at about noon by the farm labour but not reported to the farm owner until about 3.30 p.m. The owner called for help after discovering the extent of the problem at 4.45 p.m. and assistance arrived on the farm at about 6 p.m. Upwards of 50 animals were affected in addition to four which had already died.

All available animals were kept under observation. Two herds were grazing in distant camps and could not be located before nightfall.

On the following morning a further six animals were found dead in these camps. Several others required treatment and toxic symptoms continued to develop up to 48 hours after the application of the toxic spraywash.

SYMPTOMS

The symptoms are similar to those observed in chlorinated-hydrocarbon-insecticide poisoning, although chemically Thiodan is not strictly a chlorinated-hydrocarbon. These were listlessness, blind staggers, light restlessness, hyperexcitability, muscular spasms, goose-stepping and violent fits.

The onset of these fits was very sudden, usually without warning or obvious outside stimuli. Cessation of the fits was equally rapid, and the duration varied considerably, from a few seconds to several minutes. On some occasions peacefully grazing animals dropped to the ground as though pole-axed and went into violent epileptiform fits. Some of these recovered spontaneously, stood up, shook themselves, and continued grazing. Others stayed in the fits until treated with sedatives. Fits included pronounced nystagmus, salivation, champing of the jaws, and violent incoordinated leg movements. Other symptoms noted were pseudo-grazing with salivation, purging, grunting, groaning and teeth grinding.

POST-MORTEM EXAMINATION

Very few changes were found on post-mortem examination. There was mild to extensive bruising over the bony prominences of the body, early congestion and oedema of the lungs accompanied by froth in the trachea.

*Thiodan: hexachloro, hexahydro-methano-benzo-dioxathiepin oxide.

Treatment

The urgent problem was to sedate animals in severe spasms or animals suffering a succession of fits. Pentobarbitone sodium* was administered intravenously. It became immediately apparent that the animals were unexpectedly sensitive to the drug, and that small doses effected deep sedation. A third to a half of the calculated anaesthetic dose produced deep narcosis. The drug was therefore subsequently administered very slowly until satisfactory sedation was achieved. Treated animals varied from about 80 to about 600-lbs. in bodyweight, but the largest dose of pentobarbitone sodium given was 15 grains.

It is well-known that in combating dipping accidents the most beneficial first-aid treatment is thorough washing of the body with plenty of water. As labour was available, animals showing toxic symptoms were washed in an effort to remove the deposit of Thiodan on the skin. Water was used at first, but subsequently the addition of a detergent** was found to give more satisfactory results. Many affected animals recovered rapidly after washing and required no further treatment. Nineteen animals required sedation and were given supportive therapy in the form of large doses of Vitamin B12† and Calcium borogluconate§. Ten animals were found dead in the camps, and a further animal died despite treatment.

Trial Application of Thiodan Miscible Oil to Cattle

As some doubt existed as to the method of use of the Thiodan Miscible Oil in the accident, a small trial involving 20 head of healthy young steers was carried out using Thiodan 35% Miscible Oil, 1 part in 400 parts water to give 0.09% active ingredient. A careful watch was kept over these animals after treatment. No untoward symptoms were observed for nearly 10 hours, when excitatory symptoms were noted in two of the twenty animals. Both recovered, one after being washed down, and the other spontaneously. On the following morning one animal was found dead in the camp. A close watch was kept on the remaining 19 animals, and at about 1 p.m. that day one of them suddenly had a fit. Immediately thereafter a further animal was affected. Both were washed down and no further symptoms appeared. Thereafter the other 17 animals were thoroughly shampooed and rinsed. No further trouble occurred.

Five days later these trial animals were routinely passed through a plunge dipping bath containing 0.25% Chlorinated camphene***. They subsequently showed mild to severe symptoms of toxicity, but recovered after washing and rest. Other animals of similar age and condition showed no sensitivity to the Chlorinated camphene wash at this or any other dipping.

Discussion

Thiodan is an insecticide in common use as a crop protectant especially on cotton. The product under discussion had a physical appearance and odour strongly resembling some BHC Miscible Oils commercially available in South Africa. Cotton planting and cattle ranching are often carried out in close proximity in hot and/or humid climates. In such areas cotton pests and cattle ectoparasites abound and insecticidal control measures are essential. Accidental spraying of cattle with toxic substances such as Thiodan is a real danger.

The wide range of symptoms observed during this incident is of interest. Furthermore, the rapid response to treatment and the ease of recovery in severely affected animals encourages the hope that with reasonably prompt attention losses can be cut to a minimum or avoided. Animals that are well washed down (a procedure much facilitated by the use of a detergent) usually recover without further treatment. Sedation is important as in addition to the danger of self-inflicted injuries and inhalation pneumonia the fits are exhausting and increase the possibility of shock.

* Sagatal: May & Baker.
** Teepol: Shell.
† Cytamen '100': Glaxo-Allenbury.
§ K.B.G.: Cooper & Nephews.
*** Coopertox: Cooper & Nephews.

It should be stressed however that Thiodan produced symptoms not dissimilar to those one would expect from BHC or Dieldrin poisoning. In this connection it is interesting to note the empirical formulae of these three chemicals: —

Thiodan = $C_9.H_6.Cl_6.O_3.S$
BHC = $C_6.H_6.Cl_6$
Dieldrin = $C_{12}.H_8.Cl_6.O$

Of particular interest was the sensitivity to pentobarbitone sodium. Logic points to an hyperexcitable subject requiring a large dose, and in the heat of an emergency a relative overdose of barbiturate could be administered.

The long period between application of the insecticide and the onset of symptoms was also contrary to previous experience of toxicity with cyclic chlorinated hydrocarbons. Animals continued to react up to 48 hours after spraying. It would thus be advisable to wash down all animals involved, even at considerable inconvenience, as it is impossible to keep an efficient 48 hour watch over animals under average South African farming conditions.

Apparently, Thiodan causes a sensitivity to other cyclic chlorinated hydrocarbon compounds. Animals recovering from Thiodan poisoning should not be treated with chlorinated hydrocarbon containing dips for at least two weeks after recovery.

J. S. Afr. vet. med. Ass. 37 (1) 1966

IMMOBILISATION OF THE TRANSVAAL GIRAFFE (*GIRAFFA CAMELOPARDALIS GIRAFFA*) USING AN ORIPAVINE DERIVATIVE

S. M. HIRST

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SUMMARY

Thirty seven Transvaal giraffes (*Giraffa camelopardalis giraffa*) of varying ages and sizes were immobilised using the oripavine derivative M183 (Reckitt). The drug was administered by means of projectile darts shot from a crossbow.

Reactions were favourable, and subsequent capture and loading were easily accomplished. Optimal dosage rate was 1 mg per 200 to 400 lbs. live weight. Animals came under the maximum effects from 4 to 8 minutes after drug administration. Nalorphine hydrobromide was used as an antagonist, and recovery took from 20 to 110 seconds after intravenous administration.

M183 is regarded as a valuable advance in immobilising drugs, particularly for ungulates such as giraffes which are normally difficult to capture and handle.

INTRODUCTION

For the past twelve consecutive years, the Nature Conservation Branch of the Transvaal Provincial Administration has been engaged in capturing giraffes on private farms in the Transvaal and translocating them to provincial and private nature reserves in this and other provinces. Capture was normally accomplished by skilled horsemen pursuing the animals in teams and catching them manually, usually by the tail! Such methods have resulted in negligible mortality as far as the giraffes are concerned, but entail considerable risk of life and limb for the horses and the riders.

Capture of giraffes in South Africa by the use of drug immobilising techniques has been accomplished, using principally Phencyclidine ("Sernyl" — Parke-Davis), Gallamine triethiodide ("Flaxedil" — May and Baker) and Benzodioxane hydrochloride ("Quiloflex" — Boehringer)[2,3,4]. Because of the poor resistance of giraffes to a stress condition such as immobilisation, and their anatomical awkwardness when under the effect of such drugs, it was considered that until now drug capture has not offered a practical alternative to mechanical capture. Even the use of Diethylthiambutene ("Themalon" — Burroughs Wellcome), which was singularly successful in the immobilisation of the square-lipped rhinoceros[5], resulted in the deaths of three out of four giraffes.

The M-series of compounds recently synthesised by Reckitt and Sons, England, has aroused much interest amongst wildlife biologists. These compounds are synthetic derivatives of the opium alkaloid thebaine, which itself is a therapeutically useless by-product in the manufacture of morphine. Two of the drugs from this series, M99*, which is 6:14 endoetheno — 7 — α(-2-hydroxy-2-pentyl)-tetrahydrooripavine hydrochloride, and its acetyl derivative, M183*, are of particular interest as they are 10,000 times more active analgesically than morphine and possess a very favourable therapeutic index. M99 and M183 are currently undergoing clinical trials in man (Colman Green, personal communication), and because of their novel structure and therapeutic index, represent a notable advance in pharmacological research[6]. The drugs pos-

* At present available for experimental investigation only, on application to Reckitt and Sons, Ltd., Hull, England.

sess a remarkable cataleptic activity which renders them particularly valuable as animal immobilising agents.

M99 was first introduced in animal immobilisation work in 1963 on the suggestion of Sir John Gaddum, F.R.S. It has since been tested on domestic animals[7], and on a variety of wild ungulates[8]. The latter authors note several advantages offered by M99 over immobilising agents used previously. These include:

1. A wide safety margin, which means that the weight of the animal does not have to be accurately estimated — always a difficult task in the case of the giraffe.
2. High potency coupled with a high rate of absorption, which means a small fluid bulk. Small projectile darts can be used, thereby increasing the range of the projector and flattening the trajectory of the dart.
3. Rapid onset of catalepsy; drugged animals can be approached and roped before they have time to move into dense bush.
4. The availability of effective antagonists, such as Nalorphine hydrobromide ("Lethidrone" — Burroughs-Welcome) or the experimental M — series antagonist, M285 (Reckitt)†.

M99 and M183 could conceivably be used alone to capture an ungulate such as a giraffe, but in practice it is found that nervous animals retain a sufficient residue of their flight reaction to evade final capture. It is therefore desirable to combine these drugs with a promazine traquilliser such as Acetylpromazine[8]. Hyoscine hydrobromide is added to the mixture to prevent excessive salivation and possible bronchial effusion.

Although M183 differs from M99 only in the addition of an acetyl group, Van Niekerk (personal communication) reported a heavy mortality when immobilising giraffe with M99, and found M183 to be safer for this species. Accordingly, it was decided to replace mechanical capture of giraffes by drug capture using M183 as the principal immobilising agent.

Materials and Equipment

M183 was supplied by Reckitt and Sons in the form of a crystalline powder. Being a hydrochloride, the drug can be dissoved in a weak hydrochloric acid solution or a good acid buffer solution. To save space in the projectile dart, it was found advantageous to dissolve the drug in the Acetylpromazine solution, thereby making use of the buffering and preservative properties of the latter. Strength of M183 solutions used was 2.5 mg./ml. The Acetylpromazine solution was used at a strength of 10 mg./ml. and hyoscine hydrobromide solution at a strength of 100 mg./ml.

The projection equipment used was the Van Rooyen crossbow and precision-made projectile darts. The capacity of the darts was 1.5 ml. and the length of the needles $1\frac{1}{4}$ inches. Using these darts, the effective range of the crossbow was 130 yards — more than adequate in wooded terrain.

Because of the limited capacity available, a complete dose of Acetylpromazine could not always be added. In practice however, it was found that the amounts present were sufficient to effect capture.

Technique

All the animals immobilised frequented private farms in the Pilgrim's Rest district. The terrain in all cases was sub-tropical lowveld wood-land of varying density. Many animals were encountered in open clearings characterised by short grass and large isolated trees.

Young immature giraffes were preferred to others, since experience has shown that these are more suited to handling and transportation. Four of the animals captured had not yet been weaned. With two exceptions, all the animals immobilised were in good condition.

Most animals were reasonably tame, and could be approached to within a distance of

† At present available for experimental investigation only on application to Reckitt and Sons, Hull, England.

50 to 90 yards by a motor vehicle. They were "darted" in various sites, it not always being possible to select a specific site. Most were "darted" in the shoulder, rump, flank or rib area. The length of the needle ensured that the drug was always injected intramuscular and never penetrated into the pleural or peritoneal cavities. In two cases the animals moved suddenly after the darts had been fired, and were struck in the upper fore- and hind-limb respectively. They reacted essentially in the same way as did the others. No cases of failure of the dart injection mechanism occurred.

Girrafes vary considerably in their normal temperament, and their reactions to drugs differ accordingly, as has been found when using tranquillisers[9]. However, certain features of the reaction to the M183 drug mixture were shared by all animals.

The first indication of the drug's action was a lowering of the ears. This occurred from one to five minutes after administration of the drug. Many animals shook and waved the head or thrust it against the neck of a neighbour. The next stage was holding the head high with the eye-lids half-closed and moving slowly forwards with short waltzing steps — a typical "hackney gait". At this stage the animals moved slowly away from the rest of the herd, and could be approached and roped. The time which elapsed between the dart striking and the onset of the "hackney gait" was referred to as the "M183 reaction time."

Animals which were incompletely under the effects of the drug could be approached to within about twenty yards and would then move off in a clumsy canter. A notable feature of the behaviour of drugged animals was that they always moved forward, even when confronted with an object such as a small tree or a member of the catching team.

Once roped, the giraffes were cast as rapidly as possible in order to administer the antagonist. In no case did an animal go down of its own accord. To avoid injury to the long neck and limbs, it was deemed advisable to administer the antagonist, viz., Nalorphine hydrobromide, in every case, and as soon as possible. The Nalorphine was always administered intravenously via the jugular vein. The time lapse between administration of the antagonist and the animal standing up, was referred to as the "Nalorphine reaction time".

While still lying down, the animals were blindfolded and fitted with halters with three long leading ropes. Immediately after rising they were led about for a few minutes, and then led into a special crate mounted on the back of a light delivery van.

The giraffes were first placed in a temporary pen near the site of capture to recover from the effects of drugging. They normally recovered completely from all drug effects within twenty-four hours, and were then reloaded into crates and taken some eighty miles to permanent quarantine pens. No additional tranquillisers were administered. Some animals showed residual drug effects longer than 24 hours after capture, probably due to the Acetylpromazine in the drug mixture, but this was regarded as advantageous and facilitated handling.

Results

A total of thirty-seven animals — 15 males and 22 females — were captured. The estimated weights ranged from 175 to 1,100 lbs., and the heights from $6\frac{1}{2}$ to 13 feet. All animals were classed as subadults with the exception of four juveniles which had not yet been weaned.

Two animals died shortly after being captured and before Nalorphine could be administered. Each animal had received a dose of M183 of approximately 1 mg. per 150 lbs. In one case the trachea and bronchi were filled with watery rumen contents, and investigation revealed that the animal had been on its way from a waterhole when it was given the drug. The other showed no abnormalities at post-mortem. In addition eight animals collapsed and died in the temporary pens, from six to twenty-four hours after capture. Post-mortem examination, carried out immediately after death, revealed only a body temperature of 109°F+ and general hyperaemia. It appeared that the hot and humid weather prevailing, plus the shock effects of immobilising and

handling, led to collapse and death. It was considered unlikely that the M183 alone was responsible for the mortalities, as such cases did not occur when the weather became cooler and drier.

In the cases where effective immobilisation was achieved with one dose of M183, the dosage rate was approximately 1 mg. per 200 to 400 lbs. Doses less than 1 mg. per 500 lbs. did not drug the animals sufficiently for them to be approached closely. In cases where the initial dose failed to immobilise the animals completely and additional doses were given, the total dose of M183 required was greater than 1 mg. per 200 to 400 lbs. In one case, an animal which was underdosed was left alone and was subsequently seen to have recovered completely. It was later fully immobilised and captured. It was not considered advisable to leave underdosed animals in the bush, as the possibility existed that they would fall into gullies or else wander about until they collapsed from heat exhaustion.

As indicated by Harthoorn[7], the effective dose of Nalorphine bears more relation to the size of the animal than to the dose of M183 given, and this was found to be the case with giraffes (see table below).

The primary objective of the immobilisation project was to capture as many giraffes as possible within a period of a few weeks. Opportunities for experimenting with various dosages and with various combinations of the drug mixture were not presented. Details and results pertaining to the immobilisation of twenty of the thirty seven animals are tabulated below.

IMMOBILISATION OF THE TRANSVAAL GIRAFFE (GIRAFFA CAMELOPARDALIS GIRAFFA) WITH M183 (RECKITT). DATA FOR TWENTY INDIVIDUALS.

No.	Sex.	Est. wt. in lbs.	M183 dose in mg.	Hyoscine dose in mg.	Acetyl-promazine dose in mg.	M183 Reaction time, mins	Mins. before admin. Nalorphine	Nalorphine dose in mg.	Nalorphine reaction time, mins	Remarks
Z1	M	1,000	2.0+0.5 +1.0	30+7.5 +15	12+ 3 +6	6	120	250	40	Initial dose too low. M183 reaction time refers to initial dose.
Z2	F	550	2.0	30	12	5	17	150	40	Immobilised twice, 2nd time 3 days after 1st.
			2.0	30	12	7	20	175	60	
Z4	M	700	2.0	30	12	6	16	100	30	
Z7	M	800	2.5	40	11	7	23	150	40	
Z8	F	500	2.0	30	12	5	15	100	110	
Z10	F	300	2.0	30	12	6	—	—	—	Died 12 mins. after drug admin.
Z12	M	550	2.0	30	12	4	12	150	40	
Z14	M	175	1.0	40	11	7	14	100	30	
Z15	M	350	1.5	20	9	7	14	100	40	
Z16	M	250	1.0	15	13.5	5	11	100	30	
Z17	F	1,100	2.5	40	11	?	22	250	35	Moved into dense bush.
Z18	M	600	2.0	30	12	6	14	150	40	
M1	F	800	2.0+2.0	30+30	12+12	—	32	200	40	2nd dose 13 mins. after 1st
M2	F	740	2.0	30	12	8	22	125	20	
M6	M	350	2.5	40	11	4	—	—	—	Died 12 mins. after drug admin.
F1	F	300	1.0	15	6	4	11	100	60	
F4	F	500	1.0	14	13.5	—	—	—	—	Underdosed 1st time.
			2.5	40	11	7	15	125	40	2nd attempt 2 days later.
P2	F	400	2.5	40	11	5	15	150	45	
P4	F	600	2.5	40	11	5	13	150	80	
W1	F	900	2.0	30	12	8	60	250	?	Nalorphine given while animal standing.

CONCLUSION

M183 proved to be a valuable immobilising drug for the giraffe. Coupled with experience of handling and transporting these animals, the drug immobilisation technique, using M183 or a similar oripavine derivative, is a valuable means of capturing large numbers of giraffes for translocation purposes.

ACKNOWLEDGEMENTS

The Director of Nature Conservation, Transvaal, is thanked for permission to publish this report. I am indebted to the Manager of the Pharmaceutical Research and Development Laboratories of Reckitt and Sons for his interest in the work and for much valuable information on the drugs. The Nature Conservation Officers of the Nature Conservation Branch, Transvaal Provincial Administration, are thanked for their tireless assistance in the work.

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LETERS TO THE EDITOR

The Editor,
Journal of the S.A.V.M.A.

With reference to the article "A Literature Review with some Comments on the Sheep Itch Mite" (Psorergates ovis, Womersley) published in a previous number of this Journal 36 (2) 237-243, it has come to my notice that the delta isomer of BHC was registered in South Africa for use at 0.08% prior to 1960, and that in that year the concentration was increased to 0.12% and has remained so to the present.

It will indeed be appreciated if this correction could be published in the next issue of the journal.

W. M. McHARDY.

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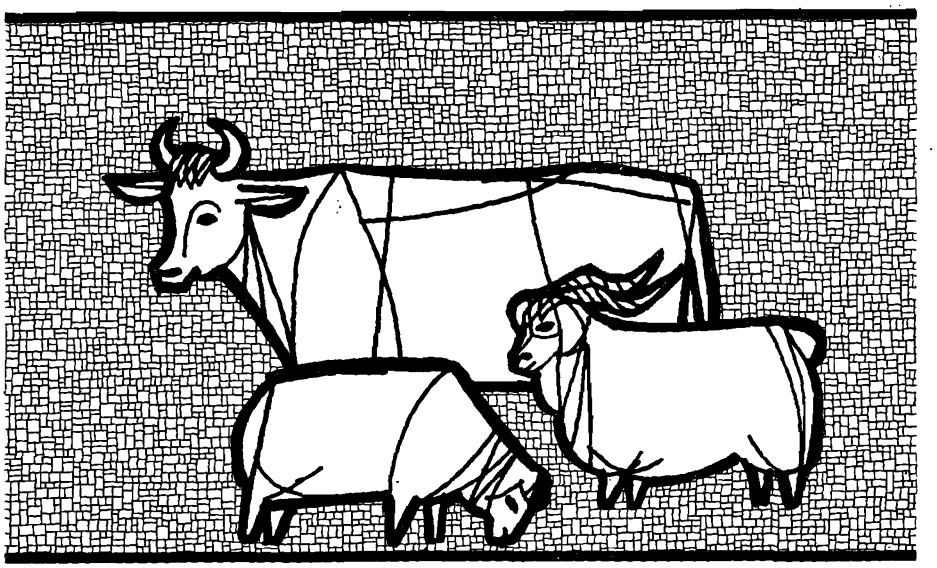

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CHRYSOMYA BEZZIANA VILLENEUVE, INFESTATION IN A BUSHBUCK

F. ZUMPT, Dept. of Entomology and Parasitology, South African Institute for Medical Research, Johannesburg

The occurrence of *Chrysomya bezziana* in Zululand was recorded in 1927[1] in the following words:

> "Larvae of this species, known as the "old world screw-worm fly" were taken from the foot of a horse and from around the anus of a bovine at Ntabanana, Zululand, in January, 1923. They were collected by H. H. Curson and determined by Major W. S. Patton."

Chrysomya bezziana Villeneuve (Fam: Calliphoridae) is an obligatory wound-parasite in man and animals in the tropical and subtropical parts of Africa south of the Sahara and in the Oriental region, extending into the Australasian region in New Guinea[2]. In southern Africa, it creates an important veterinary problem in many parts of Bechuanaland and Rhodesia, where cattle especially are affected. Records from the Republic are few.

Apart from those mentioned by Bedford, I have seen some specimens from Tolwe in the Transvaal (cattle, 1940) from Ndumu in Zululand (cattle, 1956), and 3rd instar larvae from two African patients (nose infections) in the Eshowe Hospital (IX 1963), and the Empangeni Hospital (XII. 1964), both in Natal.

It seems to be a puzzling fact that very few cases of infestation of wild animals have been recorded so far. From Subsaharan Africa, I know of only one case, namely from an elephant [*Loxodonta africana* (Blumenbach)], shot at Angodia, Uélé, Congo[3]. Larvae had been found in a suppurating wound at the base of the tail. It is therefore thought worthwhile to record another case of infestation in a wild animal, namely a Bushbuck [*Tragelaphus scriptus* (Palla)] which had to be destroyed in the so-called "corridor" between the Umfolozi and the Hluhluwe Game Reserves on the 26th October, 1965.

This antelope, which I shot in the dense riverine bush, did not appear to be sick when first seen. After it was dropped, a suppurating wound of about one inch in diameter was discovered on the back near the shoulder. A few fully grown larvae fell out, and on lifting the skin surrounding the opening of the wound, several more larvae of the 3rd instar were detected, in all about two dozen. They were isolated on sand and after 2 weeks yielded 13♂♂ and 3♀♀, so that the identification could be confirmed by the examination adult flies.

The rare records of infestation of wild animals is a "seemingly" puzzling fact, as infestations of wild animals with the larvae of *C. bezziana* must actually occur quite often. In the Ethiopian as well as the Oriental regions, reservoirs among wild animals must be present, from which domestic animals and humans have and still are acquiring their infestations. However, they are not discovered or not recorded, because persons shooting game rarely have the knowledge or interest to look for parasites, nor to preserve them for identification. It is therefore much appreciated that in recent times veterinarians and biologists have been attached to various game reserves in the Republic and abroad. Zoonoses research will in future provide us with many interesting discoveries of academic as well as economic importance.

ACKNOWLEDGEMENTS

I wish to thank the Natal Parks, Game and Fish Preservation Board for enabling us to carry out this kind of investigation, the Director of the South African Institute for Medical Research for providing the facilities to carry out a survey of arthropod parasites of vertebrates in Africa south of the Sahara, and the South African Council for Scientific and Industrial Research for financial support.

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November, 1965.

CEPORAN*: EFFICACY AGAINST *BABESIA FELIS*

J. E. DORRINGTON AND W. J. C. DU BUY

4, Kort Street, Bellville. Cape

SUMMARY

The successful treatment of biliary fever (*Babesia felis*) in cats with Ceporan is described.

MANUFACTURER'S NOTE

Ceporan [7-(2-thienyl acetamido)-3-(1-pyridylmethyl)-3-cephem-4-carboxylic acid betaine] is a semi-synthetic antibiotic obtained originally from cephalosporin and is effective against a wide range of gram positive and gram negative organisms. Ceporan is given either by the intramuscular or intravenous routes twice daily at a dosage level of 10 *to* 20 *mg per kg. After dissolving the crystalline powder, the solution retains its potency for* 12 *hours at room temperature or for four days when stored in a refrigerator.*

INTRODUCTION

Up to now our routine treatment of *B. felis* infection in cats has been the use of chlortetracyclines per os with reasonably satisfactory results. Our overall feeling has been that in our area at least, some better drug would be most welcome. The availability of Ceporan seemed to make it worthy of trial.

CLINICAL TRIALS

In all, 12 cats suffering from *B. felis* and of varying ages and weights were treated with Ceporan. The dosage given was 20 mg per kg. twice daily for 3 to 4 days. In practice we added 2 ml of Water for Injection to a 250 mg vial and gave 0.5 ml of the solution intramuscularly twice daily. All cases were confirmed by demonstrating *B. felis* in peripheral blood smears. Intramuscular injections were painless and the drug produced no visible side effects. In some cases we dosed at a rate of 40 mg/kg without any signs of toxicity. All the cases were hospitalised.

RESULTS

Ten cats treated with Ceporan showed an improved appetite within 24 to 36 hours of the commencement of treatment and were discharged as cured on the third day. Their blood smears were negative and their mucous membranes were showing a noticeable return to normal. None of these cats have to date been returned with a relapse as has been our common experience when using chlortetracyclines. One cat showed a marked icterus at the time of presentation but recovered routinely. Two cats died within 12 hours of the commencement of the treatment. These were in fact hopeless cases from the outset, being presented for treatment in a state of extremis.

CONCLUSION

Since the cost of treatment with Ceporan is no higher than the tetracyclines, we now favour the use of Ceporan in treating biliary fever in cats for the following reasons:—

1. The simplicity of administering the drug by the intramuscular route as opposed to the oral route with the tetracyclines where the resulting vomition is a constant problem.

2. The lack of any pain or discomfort on injection.

3. The rapid curative effect as is evident in the improved appetite, negative smears and satisfactory clinical improvement.

4. The absence of relapses.

* Glaxo-Allenburys (S.A.) Ltd.

BOOK NEWS

THE CLINICAL ASPECTS OF SOME DISEASES OF CATS by Joan O. Joshua; 266 pages; R4.45.

This is essentially a book for those in small animal practice. It details symptomatology and discusses methods of diagnosis and treatment. The work is based on the long clinical experience of the author, who is a recognised authority on feline diseases.

CANINE SURGERY; First Archibald Edition; 1024 pages; 1218 illustrations: R18-75.

New from cover to cover, this is the successor to the original American Veterinary Publications' Canine Surgery. Its 39 chapters contain the work of 37 of the world's foremost veterinary surgeons. Many new techniques are presented, and another new feature is the inclusion of the pathophysiology involved in most conditions to aid selection of the most rational therapy.

DISEASES OF POULTRY by Biester & Schwarte; 5th Edn.; 1382 pages; numerous illustrations; R13.75.

Besides the basic work of these two well-known authors, this latest edition has contributions from 37 other well-known authorities on breeding and diseases of poultry. Particular cogniscance has been taken of the rapidly changing trends in poultry production through the elimination of small producers in favour of large production units.

PHYSIOLOGY OF DIGESTION IN THE RUMINANT, by R. W. Dougherty; 480 pages; R10.50.

This book contains all the papers and the discussions on them, that were presented at the Second International Symposium on the Physiology of Digestion in the Ruminant that was held in Ames, Iowa, in 1964. It thus forms a valuable addition to the library of all those who are interested in this important branch of physiology.

DISEASES OF FEEDLOT CATTLE, by Jensen & Mackey; 305 pages; 147 illustrations; R11.25.

The cattle fattening industry is showing continuous and considerable expansion. The vastly different environmental and nutritional conditions under which such animals are kept cause a marked predisposition to certain diseases.

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A PRELIMINARY REPORT ON BLOOD FINDINGS IN TWENTY SPECIES OF WILD MAMMALS

E. YOUNG*

SUMMARY

Blood from twenty animal species (forty-two individuals) in the National Zoological Gardens of South Africa was analysed. The results are given in tabular form.

INTRODUCTION

Various zoological and veterinary journals and handbooks like Spector's "Handbook of Biological Data" were consulted in vain for normal blood values of wild mammals.

Normal blood values must be known in order to evaluate the results of a blood analysis of a sick animal. Blood was therefore collected from clinically healthy animals in order to determine the normal blood values for the different species. The results are incomplete in some respects, yet it is hoped that this preliminary report will serve its purpose for those concerned with the health and care of wild mammals.

MATERIALS AND METHODS

Blood was collected from the jugular vein of the antelopes, zebras and alpacas. Cardiac blood was collected from the carnivores and primates. Heparin and ethylene diamine tetra aceticacid were used as anticoagulants.

The blood was analysed according to the following methods:—

Blood sugar:	Modification of the Folin Wu method[2]
Haemoglobin:	Drabkin's cyanmethaemoglobin method[3]
Transaminase:	King method[4]
Erythrocyte sedimentation rate:	Wintrobe method[5],[10]
B.U.N.:	The method of Hench and Aldrich[6], with a few minor modifications.
Proteins:	This test included the differential plasma protein determination by fractionation by 23% sodium sulphate salting out[7] and subsequently using Weichselbaum's biuret reagent[8].
Results:	See tables (1) and (2).

CONCLUSIONS

1. Even species which are very closely related do not necessarily have the same blood values.
2. The red cell counts are lower for the Primates than for the Carnivores and Herbivores.
3. The erythrocytes of the Primates are larger than those of the Wild Carnivores and Herbivores.
4. The Red cell counts of the antelopes are, as a rule, higher than those of the domestic cow (*Bos taurus*).
5. The erythrocytes of the Alpaca are very small and have the shape of a flattened ellipse whereas the erythrocytes of the closely related One Humped Camel (*Camelus dromedarius*)[9] are of similar size but have a more rounded to oval appearance (see fig.)
6. The erythrocytes of the Impala are not only very small but they appear to be very fragile as well. As a rule, most of the erythrocytes break when a bloodsmear is made.

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TABLE 1.—ERYTHROCYTE AND LEUCOCYTE VALUES

Species	No. of Sex Male: Female	R.C.C.	P.C.V.	M.C.V.	M.C.H.	M.C.H.C.	E.S.R.	W.C.C.
Cape Eland-*Taurotragus oryx*	0:1	12.05	55	45.4	14.8	32.5	9	6,900
Lechwe—*Kobus leche*.....	1:1	10.4 (8.8–12.1)	58 (57–59)	56.7 (48.7–64.7)	22.8 (21.7–24.0)	40.8	0–11	4,050
Impala—*Aepyceros melampus*	1:0	10.89	26	23.8	9.6	40.2	—	3,800
Springbuck—*Antidorcas marsupialis*.....	1:2	14.6 (10–19)	40.5 (31–50)	28.4 (26–31)	9.5	45.5	0.3	5,250
Reedbuck—*Redunca arundinum*.....	0:1	9.09	54	59.3	25.0	42.2	1	2,000
Mountain Reedbuck—*Redunca fulvorufula*.....	1:3	8.73 (7.4–10.0)	57 (48–65)	65.5	21.4	32.8	0–19	4,850
Grey Rhebuck—*Pelea capreolus*.....	2:0	16.1 (13.4–18.6)	50.8 (46–57)	32.7 (30.9–42.5)	16.4	43.3	—	5,568
Blue Wildebeest—*Gorgon tauvinus*.....	2:3	9.4 (5.6–11.5)	—	—	15.3 (12.8–20.2)	—	0	7,700
Grey Duiker—*Sylvicapra grimmia*.....	0:1	8.4	43	51.2	15.8	30.8	0	3,300
Black fronted Duiker—*Cephalophus nigrifrons*.....	1:0	14.29	50	34.9	12.7	36.4	0	4,600
Zulu Burchell's Zebra—*Equus buchelli*.....	2:1	9.57 (9.5–9.7)	—	—	19.0	—	—	9,766
Hartman's Zebra—*Equus Zebra harmannae*.....	0:2	11.9 (11.7–12.1)	46	39.3	15.2 (14.7–15.7)	39.3	8–25	20,300
Alpaca—*Lama pacos*.....	1:2	19.8	39	19.5	—	—	0	12,167
Brown Bear—*Ursus arctos*..	0:1	6.1	44	72.1	—	—	0	7,600
Spotted Hyaena—*Crocuta crocuta*.....	1:1	9.4 (8.6–10.2)	46.7 (46.4–47)	50 (46–54)	—	—	8–16	11,100
Puma—*Felis concolor*.....	0:1	10.74	48	42.9	—	—	1.5	13,700
Cape chahma baboon—*Papio ursinus*.....	1:2	5.4	47	81.2	—	—	0.5	10,500
Vervet monkey—*Cercopithecus aethiops*.....	2:1	5.9	48	73.8	22.4	30.2	0	5,050
Samango monkey—*Cercopithecus mitis*.....	0:1	4.31	41.5	96.5	33.7	34.9	1	10,400

Abreviations:

R.C.C. = Red cell count (Million /cub. mm.).
P.C.V. = Packed cell volume (%).
M.C.V. = Mean corpuscular volume (cubic microns).
M.C.H. = Mean corpuscular haemoglobin (micromicrogram)
M.C.H.C. = Mean corpuscular Haemoglobin Concentration (%).
E.S.R. = Erythrocyte sedimentation rate (mm. per hour at 90).
W.C.C. = White cell count.

7. Many of the animals were very nervous when they were caught and the high blood sugar values of some animals might have been the result of hypersecretion of adrenalin.

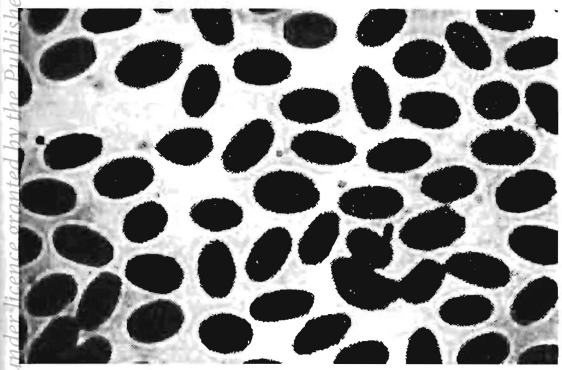
8. A great variation in the normal values for the same components can sometimes be expected in the same species. Blood from more animals of each species must be analysed before the norms for each species can be established.

TABLE 2.—CHEMICAL ANALYSIS

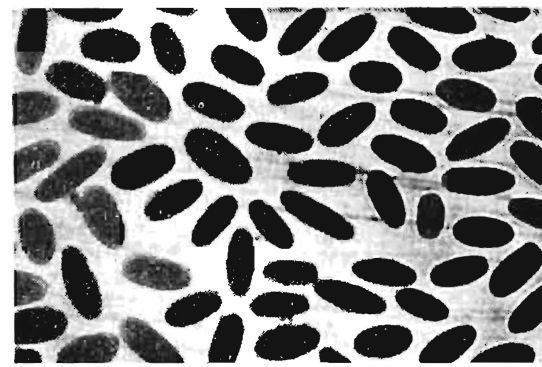
Species	No. of Sex M:F	B.U.N.	Glucose	T.P.P.	Alb.	Glob.	Hb.	SGP-T	SGO-T
Cape Eland—*Taurotragus oryx*	0°:1°	27.6	236	8.26	6.32	1.94	17.9	30	152
Lechwe—*Kobus leche*	1:1	23.0 (26–40)	33	7.8	5.9	23.7	23.7	134	96
Impala—*Aepyceros melampus*	1:0	23.9	42	7.1	4.4	2.7	10.45	98	139
Springbuck—*Antidorcas marsuplalis*	1:2	26.9 (18.4–35)	105)20–152)	7.9	4.22	3.63	18.2	86	254
Reedbuck—*Redunca arundinum*	0:1	16.6	53	8.26	3.86	4.4	22.8	—	105
Grey Rhebuck—*Pelea capreolus*	2:0	23.9 (20.2–27.6)	56.3 (40–87)	6.82	3.25	3.57	22	190	358
Blue Wildebeest—*Gorgon taurinus*	2:3	31.6 (14.6–36.8)	45.7 (28–70.6)	6.9	3.7	3.3	13.6	—	—
Black Wildebeest—*Connochaetes gnou*	1:1	26.6 (23.9–29.4)	48 (46–51)	9.1	3.9	5.2	—	—	—
Grey Duiker—*Sylvicapra grimmia*	0.1	16.6	29	7.2	2.64	4.56	13.25	30	173
Black fronted Duiker—*Cephalophus nigrifrons*	1:0	23.9	35.5	8.08	4.56	3.52	18.2	18	166
Zulu Burchell's Zebra—*Equus burchelli*	2:1	21.5 (16.6–23.9)	99 (78–111)	7.43	3.5	3.9	18.3	32	224
Hartmann's Zebra—*Equus zebra hartmannae*	01:2	19.3 (16.6–22.1)	246 (244–248)	7.65	3.8	3.8	18.1	39	233
Alpaca—*Lama pacos*	1:2	33.9 (27.6–45.5)	119 (104–138)	7.43	3.6	3.8	—	59	199
Brown Bear—*Ursus arctos*	0:1	16.6	111	9.68	5.3	4.38	—	—	—
Puma—*Felis concolor*	0:1	27.6	53	9.86	5.64	4.22	—	67	44
Cape chacma baboon—*Papio ursinus*	1:2	29.4	113	7.2	3.6	3.6	—	77	92
Vervet Monkey—*Cercopithecus aethious*	2:1	22.7 (18.4-27.6)	67	6.7	3.8	2.9	14.5	75	143
Samango monkey—*Cercopithecus mi)is*	0:1	27.6	103	8.26	4.04	4.22	14.5	208	125

Abbreviations:

B.U.N. = Blood urea nitrogen (mgm./100ml.)
T.P.P. = Total plasma protein (gm./100 ml.)
Alb. = Albumin (gm./100 ml.)
Glob. = Globulin (Gm./100 ml.)
Hb. = Haemoglobin (gm./100 ml.)
SGP-T = Serum glutamic-pyravic transaminase (King units)
DGO-T = Serum glutamic-oxalacetic transaminase (King units)



Erythrocytes of the Humped Camel.



Erythrocytes of the Alpaca.

ACKNOWLEDGEMENTS

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International Society Of Veterinary Pathologists

In conjunction with the 18 International Veterinary Congress, to be held from 17 to 22 July 1967 in Paris, there will be a meeting of the International Society of Veterinary Pathologists. This will take the form of an additional Symposinm, the particulars of which can be obtained from the program of the International Veterinary Congress.

The time for addressing the meeting is limited to 10 minutes per paper.

Any person intending to participate should notify the Secretary of the Society, Dr. L. — CL. Schulz, Institut für Pathologie der Tierärztlichen Hochschule Hannover, Bischopsholer Damm 15, 3 Hannover, Germany, of the subject/s not later than 1 March 1966.

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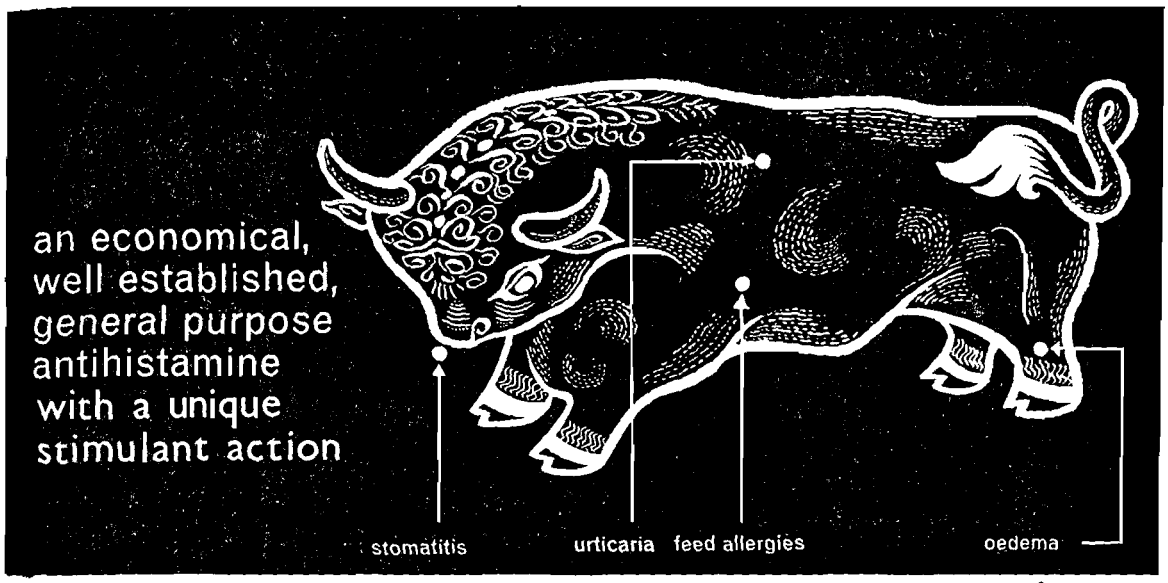

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MUSCLE NECROSIS IN CAPTIVE RED HARTEBEESTE. (*ALCELAPHUS BUSELAPHUS*)

E. YOUNG*

SUMMARY

The macroscopic and microscopic lesions in organs of Red Hartebeeste, which had died from acute heart failure, are described. The results obtained from heamatological studies on newly captured hartebeeste are also recorded. Necrosis of the myocardium and skeletal muscles and apparently high serum transaminase and blood urea nitrogen levels seem to be common features in red hartebeeste which have been caught by mechanical means. In other wild species similar histopathological lesions have been observed.

INTRODUCTION

Symptoms closely resembling paralytic myoglobinuria of horses and white muscle disease of calves and lambs have been observed in Cape Buffalo (*Syncerus caffer*)[1] Bontebok (*Damaliscus pygargus*)[2], Eland (*Taurotragus oryx*)[2], Hunters antelope (*Damaliscus hunteri*)[3], deer[4], the Greater Flamingo (*Phoenicopterus ruber roseus*) and Lesser Flamingo (*Phoeniconaias minor*)[5]. The author has also observed symptoms in Springbuck (*Anti doreas marsupialis*) and Blesbuck (*Damaliscus albifrons*).

CASE HISTORIES AND SYMPTOMS

During 1964 a number of red hartebeeste which had been caught mechanically, died after a few days in captivity. Post mortem examinations carried out on these animals revealed lesions in the heart and skeletal muscles, as well as degenerative changes in the liver and kidneys. In order to evaluate these lesions it was decided to do serial determinations of transaminase and blood urea nitrogen on a subsequent batch of newly captured red hartebeeste.

During the first half of 1965 a second group of red hartebeeste arrived at the National Zoological Gardens in Pretoria. They had been captured a few days previously in the dry north western parts of the Cape Province and had been transported by truck for about two hundred miles. The animals were stabled separately for the first night and were fed on lucerne hay, green fodder and antelope meal. They were very calm, in good condition and appeared clinically healthy. The next morning each one was caught by hand. The animals were immediately restrained and they were not allowed to overexert themselves. Blood was collected from the jugular vein; heparin was used as anticoagulant. The same procedure was repeated after a lapse of seven days.

Of the first batch one animal developed paralysis and died; seven others died suddenly without manifesting any clinical signs within the first two weeks of capture. Of the second batch one animal died suddenly within one week after arrival.

The surviving hartebeeste adapted themselves well to their new environment. One gave birth to a clinically healthy calf shortly after arriving at the Zoo.

MACROSCOPIC LESIONS

All the animals that had died were subjected to a post-mortem examination.

Skeletal muscles: The intercostal muscles and the muscles of the loin, croup and thigh were most frequently affected. Necrotic lesions of varying shapes and sizes could easily be detected macroscopically. These lesions had a

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dull whitish appearance and were frequently surrounded by extensive haemorrhages. Affected intercostal muscles usually had a streaked appearance.

Heart: Heart shewed necrotic areas in auricles and ventricles. Subendo and subepicardial petechial always present. Lungs were usually congested and oedematous. Alveolar emphysema and haemorrhages were present.

Liver: Congestion and cloudy swelling of the liver were frequently observed.

Kidneys: Nephrosis and congestion were constant findings.

Digestive system: Congestion of the abomasum and intestines gave the mucous membranes of these organs a bluish red appearance.

Incidental findings: Oestrid larvae were collected from the frontal sinuses. Cysticercus cysts were attached to the mesentery and adult conical flukes were found in the rumen.

Microscopic Findings

In six cases histopathological examination was undertaken.

Skeletal muscles: The affected muscle fibres had lost their striated appearance and were changed into a homogeneous mass which stained a deep pink with eosin. The sarcoplasma of some fibres was calcified. The nuclei of severely affected fibres showed degenerative changes. In certain areas, necrotic muscle fibres were replaced by connective tissue. There was an accumulation of macrophages, some of which contained phagocytosed cellular debris and yellowish brown, granular pigment, most probably haematogenous in nature. Giant cells, lymphocytes, neutrophils and eosinophils were also present. Hyperaemia, oedema and haemorrhages were always present in the more acute lesions.

Myocardium: Lesions, similar to those described in the skeletal muscles, were observed in sections of affected heart muscle.

Liver: Sections of the liver confirmed the diagnosis of hepatic congestion and cloudy swelling. Parenchymal and von Kupffer cells contained a yellowish brown, granular pigment, similar to that found in sections of affected muscular tissue.

Kidneys: The kidneys showed tubular nephrosis, congestion and focal haemorrhages. A few casts could be seen in the tubules.

Clinical Pathology

The blood urea nitrogen and transaminase values of five red hartebeeste were determined a few days after they had been captured and again one week later. The King method[6] was used for the determination of the transaminase values. Blood urea nitrogen was determined according to the method of Hench and Aldrich[7], slightly modified.

Blood of another red hartebeest (control animal which had ben kept in a stable for the previous few weeks) was also analysed and the results were compared with those of the new arrivals.

Blood constituent:	*Animal*:	*On arrival*:	*After seven days*:
Serum glutamic-oxal-acetic	No. 1	517	382
transaminase (SGO-T)	No. 2	463	Dead
King units	No. 3	413	173
	No. 4	373	86
	No. 5	—	119
Control animal: 119 King units.			
Serum glutamic-pyruvic	No. 1	280	98
transaminase ((SGP-T)	No. 2	245	Dead
King units.....	No. 3	248	112
	No. 4	—	26
	No. 5	—	86
Control animal: 54 king units.			
Blood urea nitrogen	No. 1	64.4	25.8
(B.U.N.)	No. 2	36.8	Dead
Mgm. %	No. 3	46.0	18.4
	No. 4	36.8	18.4
	No. 5	46.0	27.6
Control animal: 23.9 King units.			

Discussion

It is important to note that almost all the animals were apparently healthy after they had been captured and that most of them had shown no symptoms before they died after a period of one to two weeks.

From the post mortem examination it appeared that hartebeeste with extensive lesions of the skeletal muscles showed very little, if any, clinical signs of muscular degeneration. Necrotic lesions of the myocardium were probably reponsible for the peracute deaths. Other necropsy findings corresponded with the clinical diagnosis of congestive heart failure.

Muscle necrosis is probably caused by trauma and the excessive accumulation of lactic acid resulting from over exertion. It is impossible to determine at this stage why only one case developed paralysis. This symptom could be ascribed to progressive fibrosis of the initial muscle lesions. The delayed death is also difficult to interpret. It could be due to the cumulative effect of extensive tissue, including myocardial, damage. On the other hand the slow development of fatal cardiac lesions reminds one of the pathogenesis of "Gousiekte" caused by certain toxic plants.

The absence of any norms regarding serum transaminase and blood urea nitrogen values of the hartebeest and the paucity of the present data make it impossible to draw firm conclusions. It does seem, however, that the initial values are abnormally high, with a decided tendency to return to normal within a week or more. On the face of it, one would ascribe these values to the muscle damage, although hepatic malfunction cannot be ruled out. Neither the actual initial levels, nor the rate of decline, seem to be of prognostic significance.

By way of prophylaxis over exertion must be avoided during captured and transport. It may be advisable to treat all newly captured hartebeeste as though they were suffering from muscle necrosis, hepatic degeneration and nephrosis. Treatment with preparations containing vitamin E and selenium, systemic antibiotics, corticoids, antihistamines, methionine, vitamin B co. and urinary alkalizers may have potential value.

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BOOK REVIEW

THE VETERINARY ANNUAL. SIXTH ISSUE 1964/65. EDITED BY W. A. POOL. JOHN WRIGHT AND SONS. LTD., BRISTON. U.K. PRICE 63s.

The Veterinary Annual requires no introduction to veterinarians and now in the Sixth Issue continues in its service to the profession. This is a book which is of value to students, teachers, research workers and technical advisors as well as to veterinarians in the field, and is of especial value to those who do not have either extensive library facilities readily available or the time regularly to visit libraries in order to keep abreast of the rapid advances at present being made in veterinary science.

The format of the Sixth Issue is similar to previous issues and is again divided into two main sections, the first consisting of a number of articles on subjects of topical interest and the second, the larger portion of the book, being a review of the current literature.

In the first section ten articles are presented on a wide variety of subjects including Developments in Veterinary Medicine, by G. F. Boddie; Some Notes and Theories on "Scent"; Antibiotics and Supplements in the Food of Calves; Observations on the relationship between Development and Disease in the Poultry Industry in Great Britain; The Problem of Worms in Sheep; The Veterinary Surgeon and Livestock Insurance; Agriculture and the Role of the Veterinary Surgeon; The Present-day Position of Cattle Production; Developments in the Pig Industry and The Veterinary Clinical Observation Unit.

The second section of the book provides reviews, each by a different author, of the current literature of all branches of Veterinary Science. An elaborate subject index leads quickly to the information required.

A small portion of the book is devoted to a Practitioner's Index which gives information on new drugs, appliances and new books.

The Veterinary Annual not only enables one to keep informed of recent developments but also presents current trends and problems in Veterinary Science which are of international importance. The book, in fact, provides in a concise and very acceptable manner, that it is difficult to imagine one's bookshelves without it.

R.C.T.