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SURGERY OF BOVINE *IMPOTENTIA COEUNDI*

VI. 1. Surgical Conditions of the *Glans Penis* 2. Miscellaneous Developmental Defects

C. F. B. HOFMEYR*

1. Of two bulls with small, bleeding haemangiomata of the glans, one recovered with electrocoagulation and the other spontaneously.

2. Of nine bulls affected with other neoplasia of the glans, two were mature bulls with fibromata of very dense consistency. One had the tip of the penis amputated and in the other the tumour was resected. The former never served again and the latter recovered. The other seven bulls (1—2 years of age) had fibropapillomata of the glans which were removed by electrocoagulation or by sharp surgery. There was only one failure — a bull that developed more tumours after discharge.

3. Two cases of probable laceration of the glans, succeeded by gross infection are reported. One bull could not serve because of distortion and thickening of the glans after infection was eradicated; the second made a good recovery.

4. All of five bulls with prolapsed penis and mucous membrane recovered after conservative treatment.

5. Recovery was achieved in 85 per cent of 44 cases of deviated penis. This condition was seen mostly in mature bulls and was basically of two types; deviation of the body of the penis and deviation of the glans. Both forms may exist in the same animal. Deviation of the body of the penis is regarded as due to unequal filling of the *corpus cavernosum* due to organization of an internal haematoma. An operation involving resection of a D shaped piece of preputial lining over the convexity gave good results. Deviation of the glans appears to be due to dislocation of the moderator band on the dorsum of the penis. Implantation of strips of *fascia lata* onto the glans gave excellent results.

6. Three cases of miscellaneous developmental defects are mentioned. No treatment was attempted.

1. SURGICAL CONDITIONS OF THE *GLANS PENIS*

Introduction

This sixth publication on observations of 176 consecutive cases of bulls affected with surgical pathology of the penis and adnexa [1-6] deals with surgical conditions of the *glans penis*. These conditions are classified into five groups, viz.:

A. Bleeding haemangiomata.
B. Other neoplasms.
C. Abscessation and ulceration.
D. Paraphimosis.
E. Phallocampsis (deviated penis).

A. *Bleeding haemangiomata*

Pathology, prognosis and treatment

Small "haemorrhagic fistulae" communiting with the *corpus cavernosum* may be present on the glans [7, 10]. They may be lentil sized and reddish blue [9]. These openings are usually closed when the penis is at rest but bleed during erection. They sometimes ulcerate and bleed more [10].

Sometimes recovery may ensue if service is prevented for six weeks; it has also been stated that there is no definite treatment [9].

Observations on two cases in this condition, an Afrikaner and a Friesland aged 2½ and 4 years respectively, confirm those of other authors.

To establish diagnosis, pudendal block was necessary. Pinpoint elevations, which may or may not bleed, could be seen on the *glans penis*. A light tourniquet on the penis often caused blood to emerge from these elevations. Electro-coagulation was done in the usual manner in one bull.

Discussion and conclusions

The cause of the haemangiomata is un-

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known. They may represent a slight developmental defect. The report of spontaneous recovery after sexual rest by keeping the bull confined away from cows and heifers, is confirmed in one case. Electro-coagulation could have played a role in the recovery of the other case but, in the absence of a large enough series of cases, it can only be suggested as a method of treatment that might affect the outcome favourably. Even though the lesions are so slight, their significance should not be underestimated as blood in the semen adversely affects fertility.

B. Other neoplasms of the *glans penis*

Introduction

The literature refers to fibropapillomata [7, 11, 12] variously called papillomata [13] and fibromata [13]. They are of common occurrence [7, 8, 14, 15, 16], may lead to haemorrhage from the prepuce [17, 18] and occasionally to "paraphimosis" when they are large [11, 10]. Fibrosarcomata and fibropapillomata are considered rare by Bone [19]. These tumours are usually pedunculated [17, 18]. They may interfere with the flow of urine from the prepuce, with resultant stagnation and foul decomposition [18].

Fibropapillomata are infectious and may disappear spontaneously. Usually surgery is indicated. The penis may be snared when the bull jumps a cow [20], or the operation performed after epidural analgesia [18, 21, 22], general anaesthesia [18] or pudendal block [10]. Local infiltration analgesia is used where necessary [20].

The blood supply to the tumours is copious [20] but haemorrhage from them is usually not considerable when the penis is flaccid [9].

The neoplasms are removed by being twisted off, with scissors [15, 18, 20, 21] with [8] or without suturing of the skin, electrocautery [8, 16] or even by amputation of the tip of the glans [20]. It has been stated that this does not interfere with service [16], although this view has been refuted emphatically [10]. Ligation of the base of the tumours by means of rubber bands has been advocated, following which the tumours drop off after seven to nine days [9]. Wart vaccine may help [10]. It has also been stated that surgery is for salvage rather than cure [19].

Formston [20] reported on twelve bulls having penile neoplasms: five Ayrshires, three Frieslands, three Shorthorns and two Red Polls. On nine, surgical removal was done. In two bulls the distal half of the penis had been amputated previously. Another report deals with 27 cases [17].

All the reports mentioned up to this point deal with fibropapillomata. One description of an epithelioma [23] was encountered.

Material

Nine cases of neoplasia of the glans were observed. Two bulls were eight and four years respectively and each had one small hard tumour, a fibroma, on the glans. The other seven bulls were all between one and two years of age and had fibropapillomata on the glans, usually multiple (Fig. 1).

Diagnosis

The diagnosis of neoplasia is simple in that pudendal block allows visual inspection of the glans. The final histopathological diagnosis is made after examination of the excised tumours.

Treatment

The operative technique is adapted extensively to suit the requirements of each case. Where multiple, tumours are either excised with the knife or scissors and the base wholly electro-coagulated or only the bleeding points. Subsequently the skin is sutured with catgut. If the tumour is at a site close to the urethra, its base is tied off with elastic and the tumour either drops off or is reduced in size, which facilitates subsequent surgical removal. If the tumour involves the wall of the urethra, it may be necessary to remove the invaded part of the wall and then create a permanent fistula by suturing the urethral mucosa to the penile layer of preputial skin.

Discussion and Conclusions

It is patent that this group falls naturally into two divisions: two full-grown bulls of four and eight years respectively, and six young bulls all between one and two years of age.

The mature bulls each had one small, hard fibroma on the tip of the glans; there was no tendency to bleed. In one, the fibroma involved so much of the urethra that amputation of the *galae glandis* was the most feasible procedure. Even though the operation in itself proved effective, with no complications or stenosis of the urethra, the amputation must be regarded as not achieving the desired result. The bull thereafter never attempted to protrude his penis. Although this case cannot with certainty be ascribed to the amputation, it certainly lends support

to the views of those authors who consider that this operation abolishes the ability to serve.

Four of the seven remaining bulls came from the same herd, thus suggesting that the fibropapillomata are infectious. These tumours also differed from those of the adult bulls in that they bled readily. All the bulls were cured by operation except one, which developed other tumours after its discharge. There is little doubt that further surgery would have been effective in this case.

The term "recurrence" is open to criticism. One bull had to undergo repeat surgery twice and another two once. In no case was there evidence that a tumour grew again at the site where it had been excised or electrocoagulated. It appeared that new tumours grew where none had been visible before. It is entirely possible that, in such cases, submacroscopic changes were present at the sites of new growth, or that spread of infection had occurred. This emphasizes the need of repeated examination and, if necessary, reoperation. This should also be made clear to the owner.

C. Abscessation and ulceration of the *glans penis*

The available literature refers to tuberculous abscesses of the penis which may be primary [24, 25, 26] or part of generalised tuberculosis [25, 26, 27]. Milne [16] states that multiple necrotic ulcers may sometimes "extend under the *tunica albuginea* proximally to the sigmoid curve". They may bleed at service. He advises that they be excised and sutured.

The two observed cases classified under this subsection were treated on general principles. One was a Friesland and the other an Afrikaner, both two years old. Of these, one recovered and the other was a failure.

One bull had abscesses due to *Corynebacterium pyogenes* infection on the glans only. They were multiple, mostly completely separate and involved the tissues between the penile (visceral) layer of the prepuce and *tunica albuginea*. The infection was eradicated but left a legacy of fibrous tissue which distorted the glans and greatly increased its size. It is suggested that the glans was lacerated during coitus and so provided entry for the pyogenic infection (Fig. 10).

The penis of the other bull most likely had been lacerated and supervening infection caused a chronic ulcer with the formation of excessive fibrous tissue. Healing could be effected without distortion of the glans.

In a previous discussion [3] it was pointed out that the generally held, but incorrect, opinion is that laceration of the glans causes adhesions between visceral and parietal layers of the prepuce. In these two cases the visceral layer was grossly pathological and yet there was no sign of adhesion between the two layers.

D. Paraphimosis

Introduction

Limited congenital phimosis may occur in young bulls. During sexual excitement the penis is forced through the small preputial opening and cannot return because of subsequent swelling and the drawing of preputial hair into the cavity [8, 10, 13, 21]. The hair may cut the penis [13]. Paraphimosis may also result from injury during coitus. The subsequent swelling prevents withdrawal of the penis. In these injuries loss of substance of the penis [22] may rarely occur [28]. Penile lesions may be caused by the rubber band of the artificial vagina during semen collection [10, 28, 29]. Unless the rubber band is quickly removed, gangrene results [10]. Sometimes paraphimosis may be present after epidural analgesia [21].

In many cases surgery is regarded as contra-indicated: initially hot magnesium sulphate packs [8], together with lubrication to promote spontaneous withdrawal [8, 10, 16] are advised instead. Operation for phimosis is to be performed if this treatment is unavailing [8, 10]. If severe necrosis is present, the best results are said to be obtained if spontaneous separation is promoted. A burlap sack is used as a suspensory bandage after the penis has been covered liberally with cod liver oil. If stricture occurs, affected parts are to be amputated and the urethra slit open [8].

Wrapping the penis with gauze after applying petroleum jelly and replacing it into the preputial cavity to prevent adhesions has also been advised [10, 22]. Deep wounds of the penis are to be sutured [13].

Material

Five cases are discussed in this group, four of the Afrikaner breed and one a Jersey, the ages varying between 4 months and 3 years. All had paraphimosis due to swelling of and haemorrhage into the preputial layers

(Fig. 9). These cases are not included in any other category because of the distinct appearance of the pathology and the fact that the mechanisms of recovery differ.

Aetiology and pathogenesis

Although this group is very small, the fact that four out of the five were Afrikaner bulls may be significant. It is also noteworthy that all their ages were between two and three years. Where the onset of prolapse of the penis and prepuce was witnessed, an injury during service was seen. This was probably the case in all four Afrikaner bulls. In previous papers [3, 4] it was stated that penile injuries appear to be connected particularly with inexperience of the bulls. Observations on this subgroup further strengthen that opinion.

When the injury was inflicted, the haemorrhage was to all intents and purposes instantaneous, of considerable volume and, as the penis was in the extruded position, the haemorrhage occurred mostly behind the base of the glans between the penis and the temporarily reflected parietal layer of the prepuce. If this did not occur so quickly and extensively, the penis would have been withdrawn, eventually resulting in lesions dealt with previously [3, 4].

If the preputial orifice is very wide, it appears unlikely that even extensive haemorrhage could prevent withdrawal of the penis. The suggestion is thus that these Afrikaner bulls had a relatively small preputial orifice which was adequate under normal conditions. Although various authors have mentioned the drawing in of preputial hair as a cause of protruded penis, this was not seen in any of these cases. Whether the Afrikaner has a smaller preputial orifice than other breeds, cannot yet be stated with certainty. The Jersey was too young to have served. The very early sexual maturity of the Jersey breed is well known. In many other breeds at four months there is still partial adhesion between parietal and visceral preputial layers. It is suggested that the Jersey bull calf sustained prolapse of the penis during masturbation.

Treatment

The appearance of a prolapsed penis and prepuce can be quite alarming, because of swelling, contamination and even sloughing of superficial preputial layers. The natural instincts of the surgeon are to widen the preputial orifice, to replace the prolapsed parts and retain them by inserting sutures around the orifice. Besides the fact that the swelling (haematoma plus oedema) may be so large as to make replacement of the parts impossible, or almost so, even after extensive slitting of the orifice, the injured parts become inacessible if replaced. After replacement, the devitalised tissues would lie in folds and infection may wreak havoc. Hot water, acriflavine/glycerine(or other suitable ointment or oily preparation), local massage and pressure applied with a towel, if necessary, has led to recovery in all of these cases. This treatment is much simpler than advised by others. It is not one that appeals to farmers, as they prefer replacement and are not disturbed as much by any pathology which is hidden from view — and the hanging penis is unsightly and alarming. That is why this treatment should be carried out preferably at a hospital and not on the farm. It should also be emphasised that the treatment at the beginning appears to have little effect: it may even appear as if there were deterioration.

E. Phallocampsis (Deviated Penis)

Introduction

At birth the epithelial surfaces of the sheath and *glans penis* of the bull calf (i.e. the parietal and visceral layers of the prepuce) are intimately fused. Separation begins at four months and is complete at nine months [30]. During the first 3 — 6 months of life the penis cannot be protruded even when the retractor penis muscles are relaxed [31]. Under lack of hormonal influence, as in oxen, this separation may not be complete. Rupture of the frenulum, apparently due to mechanical causes, often lags behind that of the rest of the surface, so that the ventral ridge of the raphe of the penis is still joined to the raphe of the sheath. The frenulum is then very thin — only 20 to 30 microns [30]. The raphe of the penis is a remnant of the frenulum and is seen clearly [31]. The frenulum may persist, or the remnant (raphe) may be abnormally thick, fleshy and cord-like, when it is referred to as "frenum", the diminutive thus being used terminologically to indicate the physically larger structure.

Carroll, Aanes & Ball [32] found that 40 of 10 940 bulls had a persistent frenulum. It was most common in Aberdeen Angus and in Beef

Shorthorn [32], thus suggestive of being an hereditary trait. Ashdown [30] studied four other cases of persistent frenulum. In three of these cases a histological examination was made: a large blood vessel was found to traverse the persistent frenulum. Anatomical investigation suggested that the frenulum is predisposed to persist at this point. It was considered to represent a developmental anatomical abnormality and not a serious defect.

If the frenulum persists, the penis is bent by the adhesion thereof to the sheath and section of the frenulum brings about recovery [7, 9, 19, 29, 32, 33]. It consists of loose collagen, connective tissue, elastic fibres and squamous stratified epithelium [30, 31, 34]. It is claimed that no harm will come from use of bulls after operation, if the male offspring is castrated [32]. Bone [19], while incriminating the fibrous band of the frenum as a cause of phallocampsis, states that the condition does not appear to be heritable, but should not be rectified unless the progeny can be traced — and thus contradicts himself.

Fitzgerald [35] made an important contribution to the development of deviated penis of the bull. He found that most forms of deviation occur at the base of the glans or cranial termination o fthe body. Here the *corpus cavernosum* comes to a conical termination. A heavy fibrous band extension continues to the cranial extremity of the glans. This band is curved in a moderately spiral direction conforming to the normal axis of the penis. Normally it lies on the dorsal surface of the *tunica albuginea* in loose connective tissue. In deviated penises this dorsal ligament slips to the concave side of the deviation and augments the angle of the penis during erection.

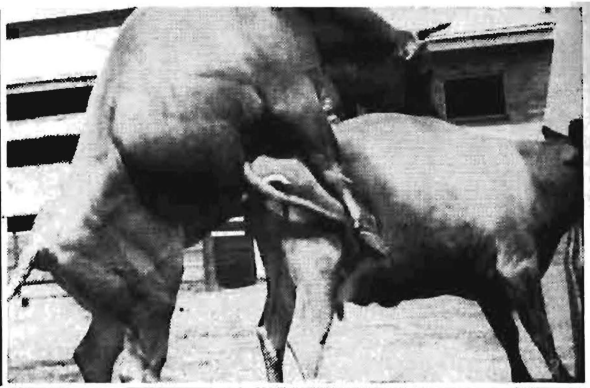
Further observations have been made on deviated penis [10, 16]. It usually occurs in the distal third or preputial portion of the penis, namely the glans. It may be hereditary. In most cases the glans is bent downwards, less commonly it is spiral. An upward deviation is rare. In older bulls it may follow on balano-posthitis, lacerations and scar tissue. In rare cases a "club" penis may be found and the prognosis then is very poor. It may be found in young bulls and in those with long service. Treatment is surgical but the prognosis guarded. Under high epidural analgesia or pudendal block the operation can be performed but skill and practice are necessary in order to become adept. A V-shaped part of the *tunica albuginea* is removed from the convex side and sutured. If the deviation is extensive, two V's may have to be removed from the convex side. Regular antiseptic oily douching and sexual rest postoperatively are indicated. In occasional mild deviation the penis may be gripped on the convex side when the bull mounts.

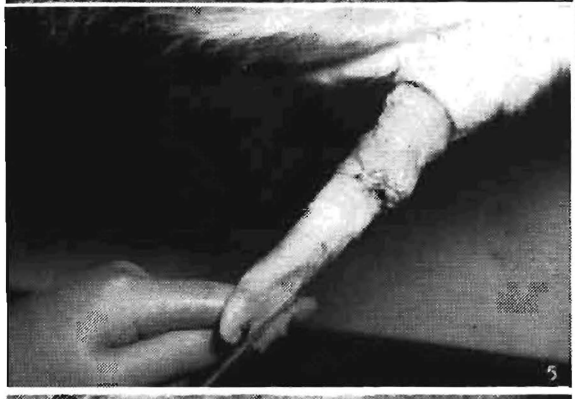
Operation for straightening the penis is also regarded as unsuccessful, when amputation of the tip of the penis is advised in some cases [21]. Bone [19] holds different views. Bent penis, apart from persistent frenulum, is held as being caused by vascular agenesis at the base of the glans, lateral deviation of the dorsal ligament and thickening of the median raphe. These developmental failures are generally associated with underdeveloped *corpora cavernosa*. This cannot be corrected surgically and is only of diagnostic interest. "Corkscrew" penis is separated into a different category from bent penis, as the penis has a thick band along the median raphe. This results from imperfect formation or closure of the urethral groove during embryonal development. It is corrected by removal of the fibrous band. It is not regarded as heritable, but should not be corrected unless the progeny can be traced.

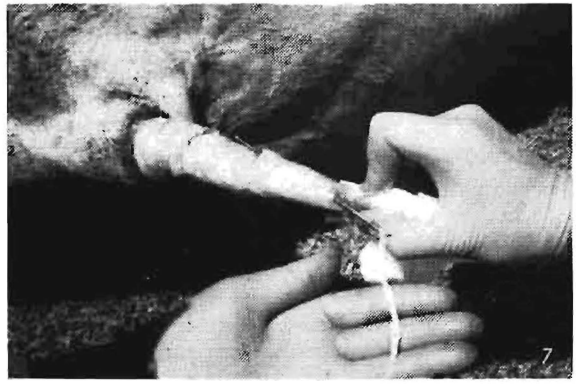
Walker [36] has come to different conclusions. He divides deviated penis into spiral, ventral and S-shaped forms. The spiral type usually occurs in bulls 2½—5 years of age, with a successful previous breeding history. The various causes are listed as:

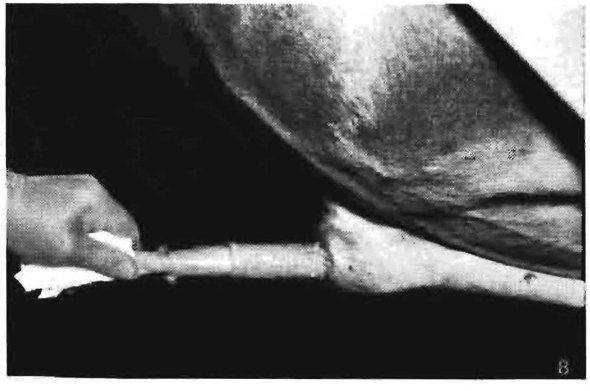
(i) trauma — with preputial laceration and haematoma of the penis, causing scar tissue and distortion or irregular filling of the *corpus cavernosum;*
(ii) congenital, possibly even hereditary;
(iii) management, i.e. bulls prepared for show;
(iv) hormonal, with testosterone causing disproportionate *corpus cavernosum* development relative to accessory structures.

Walker [36] has only corrected spiral deviation successfully. According to him the aim of the operation is to promote adhesions between the *tunica albuginea* and the "fibrous tunic" to prevent rotation of the *corpus cavernosum*. He avoids use of tranquillisers and pudendal block for fear of having the protruding penis infected after operation. In-









filtration analgesia only is used. The fibrous tissue is exposed on the dorsum of the penis from half an inch (1.25 cm) to two inches (five cm) beyond the point of preputial reflection i.e. about seven inches (about 18 cm) in all. From the preputial reflection the connective and elastic tissues are dissected laterally and backwards to expose the dorsal ligament which is incised longitudinally from the tip of the glans to the point of firm attachment posteriorly. Two strips are cut from this incision and left attached caudally. The gap in the ligament is sutured and the strips anchored to the *tunica albuginea* on each side. The skin is sutured and the bull given an antibiotic for three days postoperatively. Of eight bulls with spiral penis operated upon, seven returned to service. One case of ventral curvature was also corrected.

A simpler method involved the placing of six deep sutures through the penile layer of the prepuce and the *tunica albuginea* to cause adhesion. Three bulls recovered permanently, six recurred and three were failures.

It is evident that there is wide divergence of opinion about the causes and treatment of acquired deviated penis. Apart from dislocation of the moderator band or scar tissue formation involving the *corpus cavernosum,* the other ideas concerning the aetiology are often peculiar and unmotivated. The same applies, in general, to the treatment, the prognosis of which is given as poor. The first report seen regarding the problem of therapy more realistically is that of Milne [16], who removed portions of the *tunica albuginea* on the convexity of the curve of the penis. However, his successes were well below half of the cases. Walker [36], in the treatment of deviated glans, has employed a technique calculated to maintain the moderator band in place. On a small series of cases his results have been heartening, but the operation technique is not as simple as it might be. No author besides Milne [16] has reported an operation to rectify a curve starting proximal to the *fornix praeputii.*

Material

Forty-four cases of phallocampsis were observed, i.e. 25 per cent of the series of 176 cases — an indication of its importance in *impotentia coeundi:* 19 Jerseys, 9 Afrikaners, 7 Brahmans, 6 Friesland and one each of Shorthorn, Red Poll and Brown Swiss bulls. Their ages ranged from two to nine years, with an average of 4.7 years.

Aetiology and pathogenesis

Cases of phallocampsis can be divided into two distinct categories:

(a) Developmental aberration as represented by (i) persistent frenulum and (ii) cordlike frenum.

(b) Deviated penis from acquired causes. The deviation may be restricted to the penis behind the glans, or be confined to the glans only, or involve both body and glans of the penis. Whether in some cases there was a developmental predisposition, cannot be excluded with certainty.

(a) *Developmental aberration*

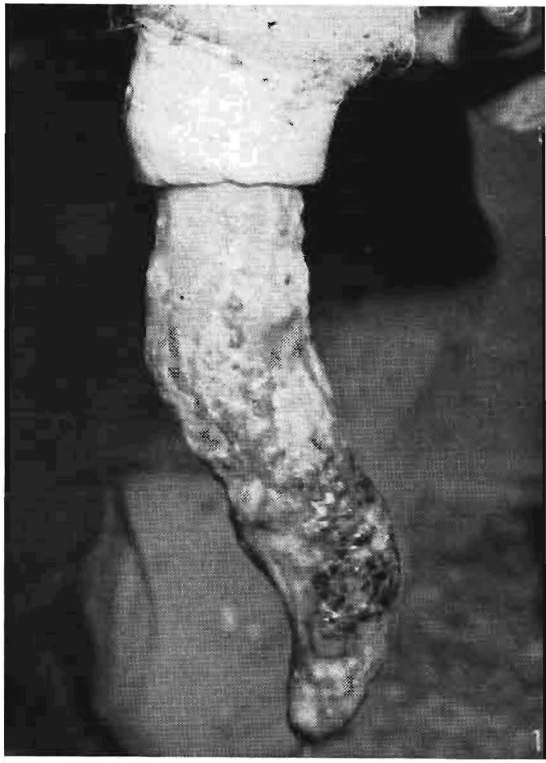
(i) Persistent frenulum.

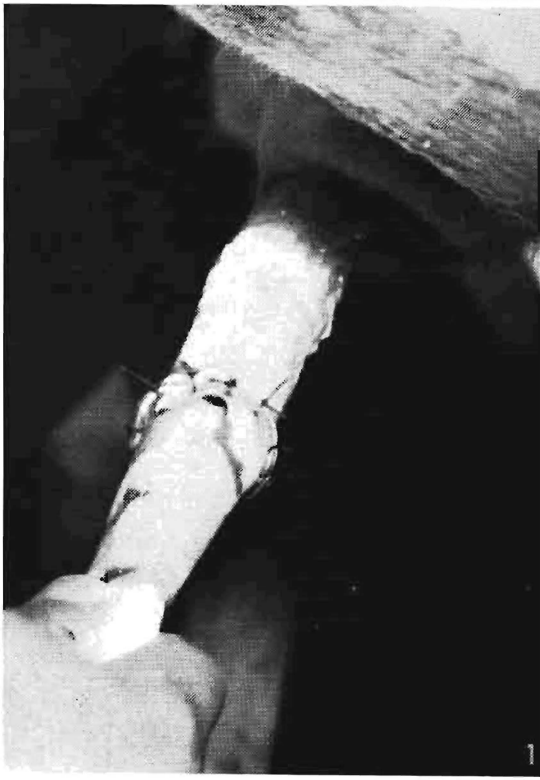
From the literature no conclusive proof can be gleaned whether this abnormality is hereditary or not. No definite information relative to this point could be obtained from the history of the one case treated by the author.

(ii) Cord-like frenum.

As before no statement can be made concerning the heritability of this condition. The presence of scarring of the frenum in the two cases seen, suggests that such a cord-like frenum would tear more readily after overstretching, than the normal raphe. A series of traumata would cause scar tissue formation, consequently the local contraction and loss of elasticity would produce phallocampsis (spiral curvature in these cases) at erection.

Fig. 1. Fibropapillomata of **galea glandis.**
Fig. 2. Jersey — semispiral **glans penis.**
Fig. 3. Removal of frenum.
Fig. 4. Persistant frenulum.
Fig. 5. See Fig. 4. — the frenulum removed surgically.
Fig. 6. Removal of D-shaped piece of skin proximal to the base of glans for deviation of the body of the penis.
Fig. 7. Transplantation of strips of **fascia lata** onto **glans penis,** showing needle probe inserted and threaded with a strip of fascia.
Fig. 8. Granulomata on **glans penis** developing where ends of **fascia lata** strips were left projecting from under the mucosa.





A well developed frenum would thus predispose to phallocampsis. The grounds for this conclusion admittedly are insecure and many more such cases must be studied.

(b) *Deviated penis from acquired causes*

(i) Displacement of the moderator band appears to be an important cause of deviation of the glans proper but not of deviation of the penis behind the glans. One case was interesting in that the penis emerged straight, but suddenly coiled into a spiral as soon as the galea glandis touched the cow, suggesting sudden dislocation of the moderator band. In such cases it is also frequently possible to cause such dislocation towards the raphe during examination. The tendency of the deviated glans to spiral is very marked. This is in accordance with expectations, more of which will be said when treatment is discussed.

(ii) Cicatricial contraction as result of scar tissue outside the *tunica albuginea* is strongly favoured by others as a cause of penis deviation. This is not borne out by the present series. In six cases only was any scarring seen and in the majority of them this was superficial, indicating the presence of a previous break in the epithelium only. In the occasional case, fibrous tissue could be palpated beneath the penile layer. In one case only did the scar tissue appear to be sufficient in volume and in distribution to be responsible for deviation. Although scar tissue can cause distortion of the penis, the idea that it is of major importance in this respect must be discarded. That injury can cause dislocation of the moderator band and simultaneous wounding of the penile layer of the prepuce, is of course possible, even likely. But the deviation would then not be caused by the subsequent scar tissue so much as by such displacement.

(iii) Vascular agenesis was not seen in this series. If it is a cause of phallocampsis (and this opinion has not been substantiated) obviously it will be apparent at the first service attempts: a history or normal service would not be given.

(iv) Unequal filling of the *corpus cavernosum* is regarded by the present author as the probable cause of deviation of the penis where the seat of pathology lies behind the glans. Obviously scar tissue in the *corpus cavernosum* in the area proximal to the glans will not be evident during ordinary clinical inspection. The presence of such scar tissue could not be verified by *post mortem* examination, as none of the bulls died while within reach of the author. However, if the erected penis is subjected to forceful bending during service, rupture of the trabeculae in the *corpus cavernosum* may occur. This would form an internal haematoma in the spongy body. When fibrous tissue is laid down, the filling of the affected part of the *corpus cavernosum* may be influenced adversely and cause deviation from behind the base of the glans.

(v) Finally, disproportionate growth of the *corpus cavernosum* as a result of hormonal influence is cited as a cause of phallocampsis. No supportive evidence is presented at all. It must be assumed that such a statement has been made as a theoretical postulate. In the series of cases here reported, such a cause certainly was not operative. If it were to be included in the aetiology at all, it would be of minor importance.

As regards pathogenesis, it is clear that in these cases of penis deviation some form of trauma, single or repeated, has played a role. Excessive bending of the penis or displacement of the moderator band has been suggested. In the former certainly, and in the latter very likely, a single trauma may result in penis deviation. Six cases had a history of injury followed by phallocampsis. The aetiological role of trauma in penis deviation is thus established, but if trauma were by far the principal cause of acquired deviation, more reference to trauma in the anamnesis would have been expected. There are several possible explanations for this apparent lack of association. The time that elapsed between the bulls failing in service and admission to hospital was usually a matter or months. The owners probably either had forgotten to mention possibly injury, or had failed to notice it, particularly as deviated penis is not usually preceded by very well marked swelling: it is undramatic and any injury causing a haematoma inside the *corpus cavernosum* may not be detectable at all from the outside. Similarly, forcible dis-

Fig. 9. Jersey — Paraphimosis.

Fig. 10. Traumatic injuries of **glans penis.**

Fig. 11. Removal of strip of **fascia lata** from thigh.

Fig. 12. See Fig. 6. — the operation completed.

location of the moderator band would cause less swelling than trauma giving rise to a large haematoma. It thus appears that injury preceding phallocampsis is unlikely to be observed, or, if seen, might be regarded as too insignificant to be of importance, the more so if a series of microtraumata had been responsible.

It is notable that all the bulls in this subgroup had penises deviated towards the right. The probable explanation is as follows. The raphe of the penis is not in the midline — it follows a gentle curve to the right of the median plane. It is quite strong and resists extension to a greater degree than the rest of the glans. If, for any reason, the half erect, or erect, glans should come into forceful contact with a relatively unyielding surface, the raphe, as result of its position and resistance, would cause the glans to bend to the right. Injury, then, would take place in this position and dislocation of the moderator band towards the raphe would be facilitated.

In two previous papers[3,4] it was reported that bulls under three years formed half (and more) of the cases. The aetiology in all was injury. As injury appeared to be the major cause in acquired phallocampsis, a comparison between those groups and the present subgroup is of interest. In the latter were 10 bulls three years of age and less, ten bulls 3—4 years and 24 bulls four years and over: a complete reversal of age distriobution as compared to that described in previous papers[3,4]. The apparent contradiction can be resolved inasmuch as the observed pathology in phallocampsis (apart from that due to persistent frenulum) indicates that microtraumata in most cases had occurred over a period of years to cause weakening of the moorings of the moderator band and eventual dislocation and phallocampsis in contract to a single and not necessarily repetitive injury operative in the previous groups. This would explain the shift in age of the group mainly concerned: from under three years to over four years.

Grouping of cases according to breed incidence probably could provide significant information: Jersey, 19; Afrikaner, 9; Brahman, 7; Friesland, 6; Red Poll, 1; Shorthorn, 1; and Brown Swiss, 1.

There can be no doubt that the large number of Jerseys in this group must be related to the vigorous service and excellent libido characteristic of the breed, as there are no extraneous factors which can offer an explanation. The energy of service might weaken investing tissues of the moderator band, even if they were of normal strength, but more readily were they inherently weak. With the data at hand, no final decision can be taken on this point.

The Afrikaner is the most popular breed in South Africa. Nine cases cannot regarded as significant. The same applies to the Friesland, the most popular dairy breed. The Brahman, third in the above table, gives rise to some suspicion about proneness towards phallocampsis, as the breed is represented by small numbers in this country. Here, too, it is too early to draw conclusions.

Treatment

The various surgical methods employed were as follows:

Technique 1 is applicable where there is a frenum, i.e. a thick cord instead of the normal thin raphe. Under pudendal block the penis is held at the galea, withdrawn and rotated to bring the whole of the frenum into view. It is grasped with tissue forceps at one extremity and dissected off while avoiding the penile layer on either side (Fig. 3). Postoperative treatment involves the daily instillation of acriflavine-glycerine.

Technique 2[30, 31, 37] is confined to the cases with a persistent frenulum. After blocking of the *N. pudendus,* the *galea glandis* is held with a swab. The frenulum, which forms a broad band running from the base of the glans along the raphe to the right ventral surface on the galea, is exposed by suitably rotating the penis. It is attached at its two extremities with a gap in the middle, where the raphe is free (Fig. 4).

Overlapping, interrupted through-and-through sutures are inserted from side to side from the edge of the gap posteriorly to the caudal extremity of the frenulum. These sutures are arranged parallel to the long axis of the penis, effectively to control haemorrhage from the big blood vessels traversing the frenulum. The same suturing procedure is applied from the anterior end of the gap to the cranial extremity of the attachment. The parts of the frenulum between the two suture lines are then resected (Fig. 5). Postoperative treatment consists of instillation of acriflavine-glycerine.

Technique 3 is employed to rectify curvature of the penis from behind the glans. A D-shaped incision is made behind the glans on the convexity of the curvature as seen at erection (Fig. 6). The straight part of the incision is made along the base of the glans and encompasses about one-third of the circumference. The curved incision is about five cm wide. The reflected parietal layer of the prepuce enclosed in the incision is dissected away, and the resultant raw area closed by interrupted chromic catgut sutures, the suture line lying across the long axis of the penis (Fig. 12). Postoperative treatment consists of daily instillation of acriflavine-glycerine into the preputial cavity.

Technique 4 is designed to correct deviations of the glans. Anaesthesia is obtained by the injection of tranquilliser, if indicated, pudendal block and local infiltration analgesia over the *fascia lata* at the site described below. The operation is done with the patient either standing or in lateral recumbency.

After suitable preparation of the surgical area, a line is taken from the *tuber coxae* to about 10 cm behind the lateral lip of the trochlea of the femur. Subcutaneous infiltration is effected for about 15 cm on this line over the bulge made by the quadriceps muscle. An incision is made on this line for about 12 cm, penetrating the skin only. The bleeding points are dealt with and the subcutaneous fascia divided and reflected to expose the *fascia lata*. A linear cut, the length of the skin incision, is made through the *fascia lata* without injuring the underlying muscle (Fig. 11). Four more such cuts are made parallel to the first and about 0.5 cm from it. The *four* strips of *fascia lata* thus isolated are divided at their ends and placed between two swabs moistened with penicillin. The gap in the *fascia lata* thus created is sutured with catgut and the skin closed by interrupted silk sutures.

The penis is withdrawn and surgically prepared. A five mm transverse incision is made through the penis layer immediately behind the galea on the right side of the glans. The point of a needle probe is inserted and pushed backwards between this layer and *tunica albuginea* parallel to the long axis of the penis until the point causes a bulge just behind the reflection of the penile layer. A short transverse incision is made at this point and the probe pushed through (Fig. 7). One *fascia lata* strip is threaded through the eye of the needle probe which is slowly drawn backwards so as to cause the fascial strip to lie between penile layer of the prepuce and *tunica albuginea*. Excess *fascia lata* is snipped off, whilst care is taken to bury the ends of the inserted strip completely. The same procedure is repeated three more times over the dorsum and left side of the glans so that four *fascia lata* strips are buried, parallel to each other, and arranged from the left to the right side of the glans over the dorsum. The small incisions are not sutured.

Postoperative treatment consists of daily instillation of acriflavine-glycerine.

Evaluation

Of the forty-four cases treated there were six failures in all and two where the final outcome could not be assessed: a recovery rate of about 85 per cent, with two cases undecided.

Technique 1 was applied in two cases only. One recovered while one case improved but required operation according to Technique 4 to straighten the penis completely. Any conclusions at this stage would be presumptuous. Technique 1 appears to be of use but may have to be boltstered up by a further operation. It is not contra-indicated, but cases have to be selected properly.

Technique 2 was carried out only on the one case which recovered completely. The technique is simple and straight-forward and should always be effective in this type of condition.

Technique 3 was regularly used for deviation behind the glans. It was designed on the theory that phallocampsis should improve if some fibrous tissue formation could be caused on the convexity of the curve by removing some of the reflected preputial cover. The parietal layer, enveloping the penis at extension, is of necessity loose. Fear was thus entertained that the correcting forces would be insufficient to achieve the objective.

This technique was employed 15 times. It was used as the first operation on two cases. One was a failure: the resection of the parietal layer possibly had been too conservative. Extraneous circumstances forbade correction of this error. In the second case the operation was also too conservative, but when repeated it led to the desired result.

In six cases the curvature affected not only the glans but also the penis proximal thereto. Transplantation of *fascia lata* was done simultaneously with operation Technique 3. In one case the latter operation had to be repeated. One bull had to be discharged before he had any erection in hospital; his fate has not been verified. The other five all made good recoveries.

In another five cases Technique 3 was used after the transplantation of *fascia lata*, as it was not evident at the first erection observed that there was a curve behind the fornix in addition to deviation of the glans. One case caused great difficulties, as not only had transplantation of *fascia lata* to be done twice in addition to resection of parietal preputial layer, but also Milne's operation. The bull recovered fully but it is impossible to know which operation, or combination, was effective. Circumstances attending another case also precluded a definite conclusion. The bull was operated upon many miles away and could serve normally after operation. He was seen once, a few months after operation: erection was indifferent and no proper swelling of the penis took place. As he was in poor condition it was impossible to determine whether the incomplete erection was due to indifferent libido or a physical result of the operation. The other three cases made a good recovery.

Thus of the 15 cases on which Technique 3 was used, there was one definite failure, one inconclusive and one unknown result. These results are encouraging as compared to those obtained by Milne[16] who claimed 25 per cent success. By his technique one runs the risk of introducing infection into the *corpus cavernosum* and it is difficult to judge the extent of the resection, while the possibility of causing adhesions to the penis has to be faced. One such operation done nearly had unfortunate sequelae from these complications.

With Technique 3, operation is quick, clean and simple. The main problem is to create sufficient tension to correct the curvature. This problem has not been solved to perfection as the operation sometimes does not succeed in effecting a perfectly straight penis at erection. In these cases service may not always succeed at the first attempt. Apart from that of Milne[16], Technique 3 appears to be the only method available for correction of deviation behind the glans.

Technique 4 is indicated only for deviation of the glans itself. It has been in use for the past eight years.

The very first bull operated upon in this manner had only one strip of *fascia lata* implanted. Immediately after recovery he could serve normally, but the penis deviated again after three successful services. Repeat operation could not be carried out because of practical circumstances.

Seventeen bulls had three strips of *fascia lata* implanted. In one bull the operation failed as the excessive scar tissue resulting after previous operations done elsewhere caused permanent deformity of the glans. In one case this operation was done after resection of the frenum. In another bull transplantation of fascial strips was done on two occasions. This was the only case where fascial strips had been left protruding from the incisions. The formed granulomata (Fig. 8) and the glans assumed a peculiar shape, probably associated with the granulomata. These were removed. Finally recovery was obtained but it is evident that fascial strips should always be buried completely. In one bull, where curvature involved glans and body of penis, the insertion of three strips effected an improvement but straightening was not complete. Further operation (Technique 3) could have led to success, but the owner demanded return of the bull.

Thus, of the 17 cases in which three strips of fascia lata had been grafted, there were 15 recoveries, two of these after other operations had also been performed, and two failures.

Twenty-two bulls had four strips of *fascia lata* grafted on to the penis and one had five strips. In seven of these cases Technique 4 was supplemented by Technique 3, in one case twice. Another bull did serve normally but the penis subsequently failed to erect fully; the final results in one bull are still awaited. Let it be assumed that these were failures. Recovery was therefore achieved in 20 (two partial) out of 22 bulls which had had *fascia lata* grafted in four or five strips.

Recent work by Walker[36] involved anchoring the moderator band. In a small series of cases his results have been promising but his operation technique is much more elaborate and potentially subject to more complications than the fascial transplant technique.

The technique of fascial transplantation, alone or in combination with other opera-

tions, thus led to recovery in 35 (two partial) out of 40 cases. It is applicable where surgical facilities are lacking and gives promising results, combined, if necessary, with resection of the parietal preputial layer, which is also easily performed, and of material value in those cases where deviation involves the body of the glans.

2. MISCELLANEOUS DEVELOPMENTAL DEFECTS

Cases of developmental aberration which could be conveniently classified elsewhere (e.g. persistent frenulum) are not included here. In this group there were only three cases, details of which will be mentioned very briefly:

1. Afrikaner bull, one year old, with hypoplasia of the penis: the glans lay at the ischial arch and the scrotum was absent; the testes were palpable subsutaneously: i.e. a pseudohermaphrodite.

2. Jersey bull, four years old, with hypoplasia of the *glans penis* which was half normal thickness and length.

3. Charolais, two years old, with hypoplasia of the sigmoid curve of the penis and short *Mm retractores penis.*

In none of these cases was treatment attempted: they are merely recorded as part of the total series.

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LEVELS OF PLASMA GLUTAMIC OXALACETIC TRANSAMINASE IN SHEEP FROM THE CAPE MIDLANDS

J. A. ERASMUS* AND J. DE VRY*

SUMMARY

Normal values have been established for the activity of glutamic oxalacetic transaminase in the blood plasma of Merino sheep from four farms in the Cape Midlands. The "normal" range was found to be between 120.0 — 220 units per 100 ml plasma.

INTRODUCTION

One of the main biochemical lesions occurring in geeldikkop appears to be a decreased permeability of the hepatic cell wall towards compounds such as bilirubin glucoronides, porphyrins, bromosulphalein, bile salts and copper ions[1,2]. The histopathological changes of the liver include severe bile pigmentation and some degree of fatty infiltration, with usually no signs of cellular destruction[3].

The activity level of glutamic oxalacetic transaminase (GOT) in the plasma of affected animals was found to be markedly raised in the earliest stages of the disease, but after the second day of illness the activity of GOT and other enzymes tend to decline in this medium[4]. Ross, Dow and Todd[5] have suggested that an increase in GOT activity of serum should provide a logical procedure for estimating the parenchymal liver damage caused by young migrating flukes during the acute phase of fascioliasis, but in practice this test gave disappointing results in both cattle[6] and sheep[5]. In sheep, artificially infected with various strains of bluetongue virus, the plasma levels of GOT have been found to rise after the subsidence of the febrile reation to the virus[7,8].

Although the enzyme occurs in both the liver parenchyme and muscular tissue of sheep[9], an increase in plasma GOT is more pronounced in myopathies than in parenchymal liver damage[10]. The increase in the case of geeldikkop, although not pathognomonic of the disease iteself, seems to be indicative of a myopathy caused by a suspected virus infection[11] which is believed to act as one stress factor in precipitating the outbreak of the disease.

MATERIAL AND METHODS

The animals used in this study were bled during April and May 1968, a period during which geeldikkop was not prevalent, on one farm each from the Murraysburg, Middelburg, De Aar and Britstown districts in the Cape Midlands. Only values from clinically normal, fully grown Merino sheep on natural grazing were considered. All animals suffered from a slight internal parasite infestation.

Levels of GOT activity were assayed according to Reitman and Frankel, cited by Wootton[12] on the plasma of heparanized blood. All readings were taken on an E.E.L. portable model "A" photo-electric colorimeter using an Illford 624 light filter. The final values were calculated as units per 100 ml plasma from a standard solution containing 11 mg sodium pyruvate per 100 ml.

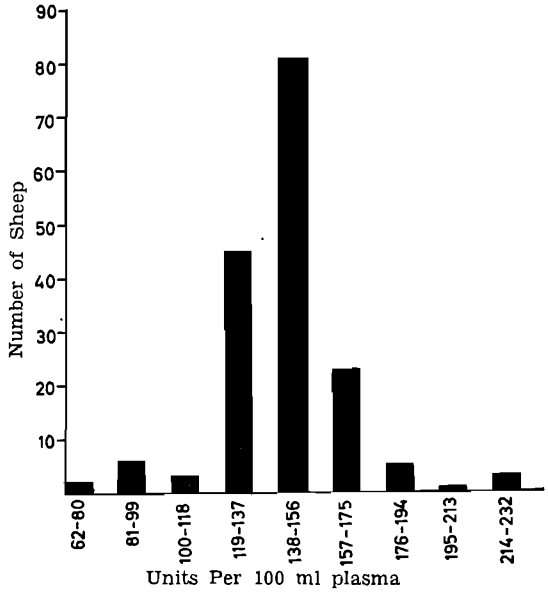

Fig. 1: Histogram of glutamic oxalcetic acid levels in ovine blood plasma

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RESULTS AND DISCUSSION

Due to a skew distribution of data (Fig. 1) the normal range by means of the Gaussian curve could not be determined. The "normal" range for plasma GOT was thus calculated using the 10 and 90 percentile values from a cumulative relative frequency curve (Fig. 2) [13, 14]. Conclusions drawn from this curve are indicated in the Table.

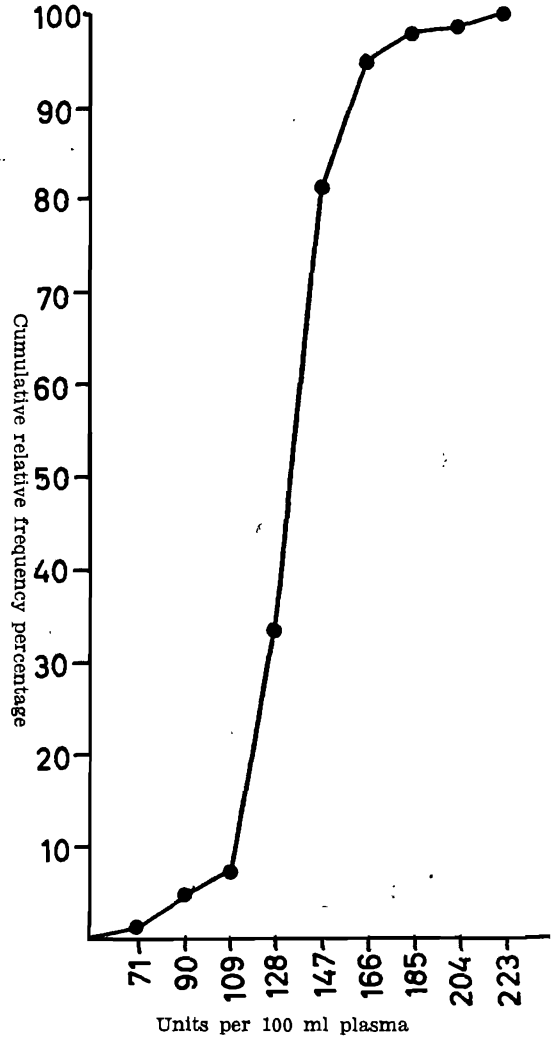

Fig. 2: Cumulative relative frequency curve for glutamic oxalacetic acid (GOT)

Table: RANGES FOR GLUTAMIC OXALACETIC TRANSAMINASIE IN OVINE BLOOD PLASMA (units/100 ml)

Figure shown by median (50%)	Ranges		
	80%	10% Lower	10% Upper
144.1 (n=70)	120.0–168.7	62.0–119.9	168.8–220.8

Values determined according to Spiegel [15].

Wootton [12] considered 80 per cent of the observed data from a population, arranged in a frequency polygon or histogram, as being the "normal" range. The upper and lower 9 per cent of the population are considered as being suspect of abnormality and the outer 1 per cent as definitely abnormal. Using these deductions, the "normal" range for plasma GOT was found to be between 120—168 units per 100 ml, while values larger than 220 units per 100 ml should be considered as definitely abnormal. Except for the lower 10 per cent values, which differ significantly, the median, 90 per cent and 99 per cent values do not differ much from GOT values calculated by Wagner *et al* [15] under experimental conditions.

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BOOK REVIEW

AN ATLAS OF CANINE SURGERY

JOHN R. ANNIS AND

ALGERNON R. ALLEN

Lea & Febiger, Philadelphia, 1967. Pp Xiii + 222, Figs. hundreds.

As the authors point out, this book is not intended to be a representation of all the operative techniques of the systems dealt with. It describes, rather, methods found most useful by the senior author, a surgeon, and illustrated by the second author, a medical illustrator.

Each alternatepage is occupied by a number of illustrations which leaves nothing to be desired as regards clarity and from which irrelevant detail has been omitted. The applicable text is on the facing page and is brief.

The first third of the book describes fundamental surgical methods and operating theatre techniques. Even the experienced surgeon should not fail to take due notice of these pages as there is always instruction to be found in the methods of others even be these methods simple and straightforward. Some differences of opinion are inevitable, of course. The reviewer prefers the first skin preparation to be done well before anaesthesia. The use of various sized and shaped plastic bags filled with sand is not shown in the book but is very useful for precise positioning.

Operations on two systems are illustrated — the gastro-intestinal and the uro-genital. Hernias have a separate section followed by a pot-pourri of procedures on dogs, with a final section on miscellaneous procedures in animals other than dogs.

In enterorrhaphy several techniques are described. Unfortunately no mention is made of an excellent method *viz.* side-to-side anastomosis employing the cutting suture.

The technique which can be employed for sterilization of the bitch can be a great deal simpler than that described. Unless the uterus is diseased its removal is also unnecessary, particularly in the young bitch.

In diaphragmatic herniorrhaphy the abdominal approach is described. Although reduction of the hernia, in many cases, is easier from the abdominal side, the surgeon will find himself in real difficulties when he has to break down intrathoracic adhesions or when he has to deal with a diaphragm torn from its costal attachments. These difficulties are inferred in the text but should perhaps rather have led to a description of the thoracic approach.

The cited disagreements must not obscure the fact that this book presents a highly desirable development in veterinary literature *i.e.* the book with a limited but clearly defined objective.

It is recommended for senior students, practicing veterinarians and medical surgeons interested in experimental surgery.

It is hoped that the authors will extend their labours to cover other body systems or regions. These two authors obviously form a very good team whose combined talents should be employed further for the good of the profession at large.

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THE LIFESPAN OF THE ERYTHROCYTES OF SOME DOMESTIC AND WILD BIRDS

L. P. NEETHLING*, J. M. M. BROWN**, O. P. M. PROSZESKY*** AND P. J. DE WET**

SUMMARY

The halflives and lifespans of the erythrocytes of the Egyptian goose, crowned guineafowl and barn owl have been estimated using chromium — 51. The same data have been obtained for the Pekin duck by using inorganic and organic selenium—75. The data obtained are compared with similar figures obtained elsewhere for fowls and turkeys.

INTRODUCTION

The authors of this paper have various interests in general and comparative physiology. The results reported here were obtained during one of the many occasions on which we pooled our various resources for obtaining information we required.

MATERIALS AND METHODS

The studies reported here were performed on two adult Egyptian geese *(Alopochen aegyptiacus* (Linnaeus) 1766, Fam. Anatidae), eleven Pekin ducks *(Anas platyrhynchos,* Fam. Anatidae), three adult crowned guineafowls *(Numida meleagris* (Linn.) 1758, Fam. Numidae) and three barn owls *(Tyto alba* (Scopoli) 1769, Fam. Tytonidae). The geese, ducks and guineafowls were maintained on a diet of mixed grain and water *ad libitum* and the owls on mice. Figures quoted here for the domestic fowl *(Gallus domesticus,* Fam. Phasianidae and the domestic bronze breasted turkey, *Meleagris gallopavo,* Fam. Phasianidae) were obtained from the literature [1, 2].

The Egyptian geese, guineafowls and barn owls received intravenous injections of chromium—51 in physiological saline via the cutaneous ulnar vein. Each bird was given 50 μC Cr.—51 as $Na_2 Cr O_4$ (specific activity, 20 mC/mg; Philips Duphar, Petten, Holland) in 0.5 ml 0.9% NaCl. Disappearance of radioactivity from the blood was followed on 0.5 ml blood samples drawn twice weekly from the birds over a period of thirty days following injection. The first samples were generally taken three days after administration of the isotope. Erythrocyte half-lives were calculated by using the method of least squares to fit the experimentally obtained points on a straight line.

Three groups of ducks were used, *viz.* Four birds aged six weeks, three birds aged eight weeks and four birds aged one year at the start of the experiment. Two birds from each age group received injections of selenium-75 ($Na_2 SeO_3$ in physiological saline, specific activity, 1.41 mC/mg). Each duck was given 60 μ C Se-75 in 0.5 ml 0.9% NaCl. The remaining ducks were given organic selenium in the form of seleno-75-methionine (Philips-Duphar, specific activity, 2.1 mC/mg) at the dosage rate of 70 μ C per bird (in 0.5 0.9% NaCl). The ducks were bled on ten irregular intervals over a period of 102 days. Two millilitres of blood were removed at each bleeding.

Heparin was used throughout as anticoagulant. Blood samples were centrifuged and counting was performed on erythrocytes after three washings with isotonic NaCl, using a Philips type PW4003 scintillation detector with a $1\frac{3}{4}$x2 inch Na/Tc crystal. The activity in the erythrocites from the ducks was plotted against time and the erythrocyte lifespan was calculated as proposed by Shemin and Rittenberg [3].

Statistical analyses were performed using standard procedures [4].

RESULTS AND DISCUSSION

The halflives found for the erythrocites of the Egyptian geese, guineafowls and barn owls are presented in Table 1, together with similar values given by other authors for the red blood cells of domestic fowls and turkeys [1, 2].

The observations regarding the lifespan of the duck erythrocyte are presented in Table 2.

It is apparent from the date presented in

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Table 1: HALFLIVES OF THE ERYTHROCYTES OF SOME WILD AND DOMESTIC BIRDS

Species	Observed $t_{\frac{1}{2}}$ values (days)	Mean $t_{\frac{1}{2}}$ (days)	Standard Error of Mean
Egyptian Goose	9.6 9.0	9.3	±0.4
Crowned Guineafowl	8.9 10.9 9.2	9.7	±1.1
Cape Barn Owl	10.1 10.1 10.2	10.1	±0.1
Domestic Fowl [1]	—	13.6	±1.3
Turkey [2]	—	12.5	—

Table 2: THE LIFESPAN OF THE ERYTHROCYTES OF PEKIN DUCKS OF VARIOUS AGES

Age of ducks at start of experiment	Lifespan in days found using seleno-75-methionine	Lifespan in days found using Na_2^{75} Se O_3
6 weeks	44 45 (mean, 45)	41 43 (mean, 42)
8 weeks	46	45 50 (mean, 47)
1 year	46 45 (mean, 46)	58 50 (mean, 49)

Mean lifespan of the duck erythrocyte as calculated from all the above observations = 45 ± 3 days.

Table 2 that it is immaterial whether the form of selenium used for *in vivo* labelling of the erythrocytes of birds is inorganic or organic. The element is incorporated directly into the haemoglobin of new cells after intravenous administration and can be isolated as selenohaemoglobin [4]. There appears to be little difference between the various age groups of ducks as regards the effectiveness of red cell labelling or the calculated lifespan of their erythrocytes.

McConnel, Portman and Rigdon [5] determined the lifespan of the erythrocytes of young ducks by using intravenous injection of labelled blood from two donor ducks and calculated a life-span of approximately 12 days. The error in their techniquqe obviously lay in *in vivo* destruction of red cells due to transfusion incompatibilities. Rodnan, Ebaugh and Spivey- Fox [6], using Cr-51, estimated the maximum lifespan of the duck erythrocyte to be 42 days. This figure is in agreement with the findings of Brace and Altland [7] in this regard. The mean lifespan calculated by us of 45 days (with a very small standard error of the mean) after using Se-75 is also in agreement with the work of Brace and Altland [7]. These findings refute the rather severe critiscisms levelled by Mollison [8] at the use of techniques such as employed in the present study and the authors cited above [7] in obtaining accurate information concerning the lifespan of animal and avian erythrocytes. Selenium- 75 labelled compounds provide an excellent and convenient way of estimation provided the observations, on which the subsequent calculations are to be based, are made at suitable intervals which cover the whole curve of rise, plateau and fall-off of red cell radioactivity levels.

We have assumed arbitrarily that the mean lifespan of the erythrocytes of the avian species studied corresponds to the time at which 95% of the labelled red cells have disappeared from the circulation. Using this value one can calculate the mean lifespan from the halflife times given in Table 1. This latter value is: ducks, 45 days; barn owls, 44 days; guinea fowls, 42 days and Egyptian geese, 40 days. These values are somewhat lower than those found for fowls and turkeys by previous workers, namely 57 and 55 days respectively [1, 2].

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THE TOXICOLOGY AND METABOLIC FATE OF SELENIUM IN SHEEP

L. P. NEETHLING*, J. M. M. BROWN** AND P. J. DE WET**

SUMMARY

Experiments are described in which rapidly fatal doses of organic and inorganic selenium for sheep are established. The distribution of the element in the body after intravenous injection of isotopically labelled selenite, selenomethionine and selenocystine is discussed. The symptoms of acute, subacute and chronic inorganic and organic selenium intoxications in the sheep are noted. The production of a hypocythaemic hyperchromic macrocytic anaemia associated with the appearance of abnormal haemoglobin C in circulating erythrocytes is mentioned. The excretion of the element by urinary and biliary routes has been studied. The isolation of Se 75-taurine formed *in vivo* from Se 75-selenomethionine is described for the first time. The incorporation of the element into plasma and tissue globulins, connective tissue, mitochondria, and enzymes like aldolase, phosphorylase and alcohol dehydrogenase has been demonstrated. The incorporation of Se 75-selenomethionine into haemoglobin is discussed. Crystalline ovine Se 75-selenohaemoglobin has been prepared apparently for the first time.

INTRODUCTION

These studies formed part of our investigations into the aetiology of the ovine diseases, geeldikkop and enzootic icterus. It was reported earlier that a chronic subclinical selenium intoxication possibly played an important rôle in this respect [1, 2, 3, 4]. This postulate has to a large extent been confirmed by recent work [5]. One of the main features of both syndromes is a persistent haemolytic state manifested by acute episodes followed by remissions in non-fatal cases [4,6, 7]. Acute haemolytic crises are followed by the appearance of an abnormal haemoglobin, designated C-haemoglobin [7] in the erythrocytes of sheep with AA or AB haemoglobin phenotypes. It has been postulated earlier that a biochemical feature of geeldikkop and enzootic icterus is an inactivation of certain sulfhydryl-group containing dehydrogenases by selenium [5, 6]. Furthermore, since from all the evidence available it is clear that the administration of organic selenium to animals results mainly in its incorporation into body proteins, we have put forward the thought that the haemolytic state, which is part of both diseases, might be the consequence of an auto-immune process resulting from the formation of abnormal body proteins [5]. It was therefore of importance to study in greater detail the toxicology and metabolic fate of organic selenium administered to sheep and to establish which of the symptoms or biochemical lesions characteristic of geeldikkop and enzootic icterus could be produced in this manner.

MATERIALS AND METHODS

Merino sheep were used throughout in this work. The ages and sexes of the animals concerned are indicated in the appropriate places below. All the animals were fed lucern hay, teff hay and water *ad libitum*.

Haematological studies were performed using standard procedures. Heparin was used throughout as anticoagulant for blood specimens. Total plasma proteins were determined by the method of Weichselbaum [8] and protein fractions by microzone elctrophoresis as described by van Zyl [9].

Haemoglobin phenotypes were studied using techniques mentioned earlier [7].

Pure selenocystine and selenomethionine were obtained from the Sigma Chem. Co., (St. Louis, Mo.), and the selenium dioxide used was analytical reagent grade (British Drug House). Se-75 selenocystine and selenomethionine as well as Se-75 sodium selenite were obtained from Philips-Duphar (Petten, Holland). All selenium compounds were administered intravenously in physiological saline solution.

Counting was performed with a Philips

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Automatic Well-type Scintillation Detector (Type PW 4003) with a $1\frac{3}{4}$ x 2" NaI/Tl crystal.

TOXICOLOGICAL STUDIES

Experiment 1—Four adult wethers were used to compare the toxicity and distribution in the body after administration of inorganic and organic selenium.. Two of the animals, both weighing 35 kg were given 200 mg of selenium dioxide in saline solution by intravenous injection. This dose represented 4 mg selenium per kg bodyweight. In addition, one of these sheep received a simultaneous tracer dose (72 mcgSe) of Se-75 as sodium selenite (100 μC; specific activity 1.4 mC per mg).

Both animals died 20 minutes after administration of the selenium. Death was preceded by the rapid onset of hyperpnoea, cyanosis, pulmonary oedema, tremors, hypersensitivity to external stimuli, twitching of the facial muscles, opisthotonus, frequent micturition, amaurosis and a marked inclination to walk backwards until baulked by some solid object. Immediately prior to death, general paresis became apparent. Death appeared to be due to anoxia. A striking finding on autopsy (performed immediately after death) was a marked apparent interference in the oxygenation of the haemoglobin of these animals, as judged by the very dark reddish-purple colour of the blood.

The distribution of inorganic selenium in the body twenty minutes after injection was studied by monitoring body tissues from the animal which had received Se-75 selenite in addition to selenious acid. The results obtained are shown in Table 1. Very little of the element (±0.1%) remained in the blood of this animal at the time of injection. Of this 87.5% was found to be attached to the red blood cells and 12.5% was present in the plasma. Other body tissues appeared to contain similarly negligable amounts of the label.

The remaining two sheep in the group each received 300 mg of selenomethionine by intravenous injection. This represented a dosage level of 3.4 mg of organic selenium per kg bodyweight. One of the animals received a simultaneous tracer dose (50 mcg Se) of Se-75 selenomethionine (100 μC; Specific Activity, 2 mC/mg). Both animals died about ten hours after injection. Very few symptoms apart from cyanosis and pulmonary oedema were found to precede death.

The distribution of the labelled dose of selenomethionine in the tissues of the sheep concerned in indicated in Table 2. Negligible amounts of the label remained in the blood and other tissues not mentioned, at the time of death. Of the label present in blood 63% was found attached to the eythrocytes and 27% was present in the plasma.

Table 1: PERCENTAGE DISTRIBUTION OF INORGANIC SELENIUM IN THE BODY TISSUES OF SHEEP TWENTY MINUTES AFTER ADMINISTRATION

Tissue	% of label present	Tissue	% of label present
Right kidney	40.0	Spleen	6.8
Left kidney	13.9	Lungs	3.9
Adrenals	11.7	Brain	3.9
Pancreas	8.3	Heart	2.3
Liver	6.9	Diaphragm	2.2

Table 2: PERCENTAGE DISTRIBUTION OF ORGANIC SELENIUM LABEL IN THE BODY TISSUES OF SHEEP TWENTY MINUTES AFTER ADMINISTRATION

Tissue	% of label present	Tissue	% of label present
Pancreas	20.2	Spleen	5.8
Right kidney	19.4	Heart	4.8
Liver	17.5	Lungs	3.4
Left kidney	17.3	Brain	2.3
Adrenals	7.9	Diaphragm	1.6

Experiment 2—Since it was obvious from the foregoing results that intravenous doses of either inorganic or organic selenium in the order of 3—4 mg Se/kg bodyweight were almost immediately lethal, it was decided to attempt to induce chronic selenium intoxication by using much lower dosage levels. Three sheep were used in this experiment and each received organic selenium only.

The first was an adult wether, which was given an initial dose of 100 mg of selenomethionine intravenously. This represented a dosage level of 1 mg Se/kg bodyweight. At this time the animal weighed 36.0 kg. Two and a half months later the animal was given a second intravenous dose of 100 mg of selenomethionine. It died five months after the commencement of the experiment. The most prominent symptoms of the intoxication were: rapid loss of weight which proceeded to cachexia (one month after the initial injection the weight of the animal had fallen to 28.6 kg.), a marked hypocythaemic macro-

cytic, hyperchromic anaemia similar to that found earlier in experimental chronic selenosis in sheep[4] and mild hypoproteinaemia associated with hyperbeta- and -gammaglobulinaemia. Haemoglobin C was found to be present in small amounts together with haemoglobins A and B towards the end of the intoxication. Total plasma protein levels fell from an initial value of 8.23g% to 6.9g% towards the end of the intoxication. This was associated with a concommitant fall in albumins and α-globulins from 4.18g% and 1.19g% to 3.09g% and 0.80g% respectively and a rise in the β + γ-globulin fraction from 2.86g% to 3.07g%.

The remaining two sheep were ewe and ram lambs aged two months at the start of the experiment. Their initial weights were 9.90 kg and 6.98 kg respectively. The ewe was given initially 12.5 mg of selenomethionine (representing 0.46 mg Se/kg). This was followed one week later by a similar dose and three months later by a third dose of 20 mg of the amino acid (representing 0.54 mg Se/kg). The animal received thus a total of 45 mg of selenomethionine. One month after the initial injection its weight was 11.48 kg, four months later it was 17.80 kg and at the end of six months only 18.5 kg.

The ram lamb was given an initial injection of 12.5 mg selenocystine (equivalent to 0.9 mg organic Se/kg). This was followed one week later by a second similar dose and three months later by a third dose of 20 mg of the amino acid (equivalent to 1 mg Se/kg). The animal thus received a total dose of 45 mg of selenocystine. One month after the initial dose the sheep weighed 8.87 kg. Four months later its weight was 12.6 kg and at the end of six months only 13.6 kg.

Both animals developed identical symptoms. Growth and weight gain were markedly retarded and at the end of the four month period the sheep had an emaciated pot bellied and mangy appearance. Their wool could be pulled out easily from the skin and hung in tatters from them. Anorexia was marked throughout most of the period of observation, and a severe hypocythaemic macrocytic hyperchromic anaemia developed in both instances. This was especially severe in the ram which received the selenocystine. This animal which possessed originally the AB haemoglobin phenotype showed the presence of haemoglobin C in its erythrocytes as the intoxication became severe.

METABOLISM STUDIES

Experiment A: Studies on the excretion of organic selenium following its intravenous administration. The common bile duct of an adult wether was cannulated as described elsewhere[10] to permit the collection of bile during the experiment and it was provided with the standard equipment in use in our laboratory for collection of urine and faeces samples[10]. The animal was given 100 μC of Se-75 selenomethionine intravenously (specific activity: 2.1 mC/mg this being equivalent to approximately 50 mcg of organic selenium). Bile, urine and faeces were collected over a ten day period at 3, 21, 45, 69, 93, 117, 141, 165, 189, 213 and 237 hours after injection.

Approximately 1% of the total dose was excreted over the ten day period. None of this appeared in the faeces, 0.25% appeared in the bile and 0.75% was excreted in the urine. Respiratory excretion was not monitored. Of the two pathways studied urine seems to be the main way in which organic selenium can be eliminated in the sheep, but it is obvious that with small doses of the compound the rate of excretion is very slow.

Urinary excretion is maximal when biliary excretion is minimal and *vice versa*. There appears to be some alternating preferential utilization or metabolism of the selenomethionine which has as its result a cyclic excretion of different metabolites in bile and urine.

The bile which was collected from the animal was pooled, kept in the refrigerator and worked up after the ten day experimental period. Bile acid salts were isolated by a procedure which incorporated features of the methods of Ahrens and Craig[11] and Mosbach, Kalinsky, Halpern and Kendall[12]. One volume of bile was added with swirling to five volumes of ethanol. The mixture was heated and then filtered. The precipitate of proteinaceous material was washed with repeated small volumes of hot ethanol until colourless and then retained for counting. The washings were added to the filtrate and the bulk sample was taken to dryness *in vacuo* at 80°C. The residue was taken to dryness and fractionated further as described by Ahrens and Craig[11]. No activity was found in the cholesterol, cholesterol-esters, triglycerides and fatty acid fractions. Sixty percent of the label present in bile was associated

with the proteinaceous material precipitated by ethanol and the remaining 40% was found to be present in the bile acid salt fraction. The bile acid salts were separated by paper chromatography into taurocholic and glycocholic acid fractions using the various sytems proposed by Haslewood and Sjöval [13], Anderson, Haslewood and Wootton [14] and Ahrens and Craig [11]. Authentic glycocholic acid and taurocholic acid (Merck. Chem. Co.) were used to locate and identify the respective spots on the various chromatograms. All the radioactivity was found to be localised in the taurocholate spots. These were cut out, the taurocholate eluted with 2.5 N NaOH and the eluate hydrolysed in sealed tubes in a small bomb at 110 — 120°C for 5—6 hours. After cooling the hydrolysate was washed into a flask and acidified with dilute HCl, sodium chloride was added to it in excess. The mixture was stirred, left for some hours and then filtered. The precipitate which contained free bile acids contained no activity; this was all present in filtrate containing the free amino acids. After concentration this was subjected to aminoacid chromatography [15]. All the activity was found in the spot corresponding with authentic taurine (Koch-Light Laboratories). Dinitro-fluoro-benzene derivatives of the aminoacids present in the hydrolysate, and those of glycine and taurine were prepared as described by Sanger [16] and compared by paper chromatography as suggested by him. All the label was found to be associated with the spots corresponding to DNFB-taurine. It was concluded that the label present in the label present in the bile-acid fraction of bile was present as selenotaurine.

Experiment B: Studies on the localization of intravenously administered organic selenium in various body tissues. Two adult wethers received intravenous injections of 125μC of selenocystine (specific activity: 103 mC/m-Mol) and two were given 104μC of selenomethionine (specific activity: 2.1 mC/mg) in the same manner. All four sheep were slaughtered 12 days later. Blood was collected at slaughter into ACD solution and centrifuged to separate cells and plasma soon thereafter. The various organs mentioned below, or representative samples of them were removed at once after slaughter, placed in individual polythene bags and frozen quickly by placing the bag in a mixture of dry-ice and acetone. They were then stored at -15°C until they could be processed further. At the time of removal from the animals' bodies representative 1 g samples of the various organs concerned were analysed for radioactivity. The distribution of activity which was found is shown in the Figure 1.

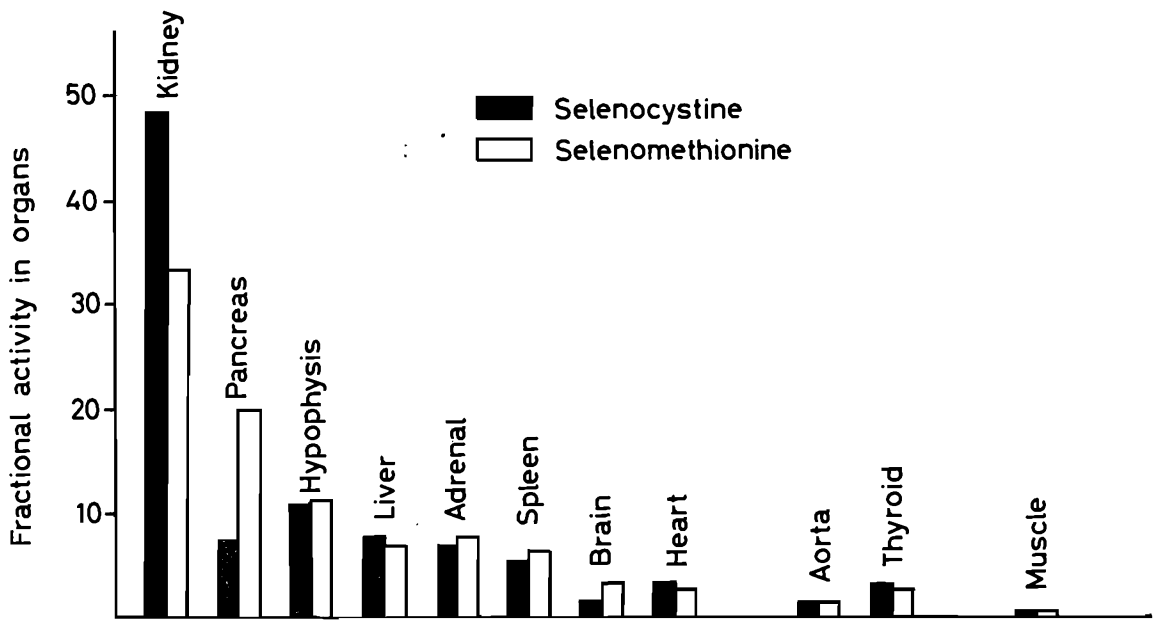

Figure: Histogram illustrating the mean percentage of total activity in one gram samples of various body tissues from sheep given Se-75 selenocystine and Se-75 selenomethionine

This histogram illustrates the mean percentage of total activity in the various 1 g organ samples from each of the two pairs of sheep. It is obvious from these data that selenomethionine and selenocystine follow the same metabolic pathways in all the tissues examined except in the case of the kidneys, in which selenocystine accumulated in the largest amounts and the pancreas which took up more of the selenomethionine label.

The large amount of activity which was localised in the kidneys of all four animals is remarkable. For this reason we decided to investigate this organ further. One kidney from each animal was thawed and separated by careful dissection into cortices and medullae. A total lipid fraction of some of each specimen was prepared by ethanol-ether extraction as recommended by Sperry [17]. The remaining material was homogonized in isotonic KCl in an allglass Potter type homogenizer. Trichloracetio acid was added to give a final concentration of 5% to precipitate the tissue proteins. After centrifugation the precipitated material and centrifugates were analysed separately for radioactivity. The distribution of label between cortices and medullae was identical in both groups of animals, namely 88% cortical and 12% medullary incorporation. Negligible activity was found in the total lipid extracts. In the case of the sheep which had received selenocystine, 99.7% of the activity present in the cortex was present in the protein precipitate and only 0.3% in the protein-free centrifugate. The corresponding figures for the cortices of the sheep which received selenomethionine were 99% in the protein precipitate and 1% in the centrifugate. A similar pattern was observed in the preparations of kidney medullae from both groups. The figures concerned were 95% and 91% in the protein precipitates from the homogenates of the selenocystine and selenomethionine labelled medullae respectively. The respective centrifugates contained 5% and 9% of the activity present.

These data show a very marked and preferential incorporation of the two labels into the proteins of the cells located in the kidney cortex.

The remaining kidneys were again separated into cortices and medullae, the latter being discarded. The cortices were homogenized in 0.02 M phosphate buffer, pH 7.4 in a stainless steel Servall "Omnimix" type homogenizer. The homogenates were then separated into cell residue, mitochondrial and

Table 3: DISTRIBUTION OF RADIOACTIVITY IN THE KIDNEY CORTICES AFTER MECHANICAL DISRUPTION AND CHEMICAL FRACTIONATION OF SMALL SUBCELLULAR PARTICLES AND SOLUBLE COMPONENTS

Selenomethionine labelled material		Selenocystine labelled material	
Cell Component	Percentage Activity	Cell Component	Percenage
Insoluble and heavy residue	56	Insoluble and heavy residues	68
Mitochondria	16	Mitochondria	11
Supernatant	28	Supernatant	21
Ammonium Sulphate Fractionation of Supernatant			
% Saturation	% of Total Activity in precipitate	% Saturation	% of Total Activity in precipitate
25	31.0	25	25.0
35	47.5	35	46.0
45	11.9	45	12.8
55	3.1	55	5.8
65	3.4	65	4.7
75	1.9	75	3.6
90	0.6	90	1.4
100	0.5	100	0.8

supernatant fractions by differential centrifugation at -10°C in a superspeed centrifuge [18]. The percentage distribution of the labels is shown in Table 3. The supernatant fraction (which contained light subcellular particles e.g. microsomes and the soluble components of the cell sap) from each group of kidney cortices was fractionated further by stepwise addition of ammonium sulphate [19] as indicated in Table 3. After each addition the mixture was allowed to stand for at least an hour before the precipitate was removed by highspeed centrifugation. The protein precipitates were dissolved in small volumes of 0.05 M phosphate buffer; pH 7.4, and monitored for radioactivity. The results found are present in Table 3 and are expressed as percentages of the total activity present in the supernatant preparations.

It is apparent from this table that both labelled amino acids are freely incorporated into the various components of the insoluble connective tissues and other heavy fragments formed on mechanical disruption of the cells. Selenocystine is incorporated to a greater extent than its methionine analogue. Mitochondrial labelling accounted for no more than one tenth to about one fifth of the total activity. The small subcellular and soluble cell sap components were well labelled in both instances. The ammonium sulphate fractionations demonstrated that labelling of the high molecular weight globulins accounted for most of the activity in the supernatant (precipitated between 25—55% $(NH_4)_2SO_4$ saturation). Somewhat more selenomethionine is incorporated into those proteins than selenocystine. The albumins and lighter proteins are poorly labelled by contrast, these fractions incorporating somewhat more selenocystine than selenomethionine.

Liver, whole hypophysis, pancreas, whole adrenals and thyroids from the two groups of animals were homogenized in isotonic KCl. A total lipid extract [17] was prepared from part of each homogenate and the remaining material was subjected to $(NH_4)_2SO_4$ fractionation. The results are presented in Table 4. These were essentially the same as obtained with the kidney cortices. Labelling of cell lipids was negligable and most of the activity was present in the proteins insoluble at half saturation with $(NH_4)_2SO_4$, namely, the globulins. The liver and pancreas are of considerable interest in that considerable amounts of the selenomethionine label remained in the supernatant after removal of the cell globulins and albumins by 100% $(NH_4)_2SO_4$ saturation. More selenomethionine than the cystine analogue appeared to be incorporated into the globulin fraction of pancreatic tissue. On the other hand the proteins of this tissue precipitating between 50% and 100% $(NH_4)_2SO_4$ saturation appeared to incorporate considerable more of the selenocystine label.

In order to demonstrate the incorporation of the organic selenium label into various tissue enzymes, aldolase and phosphorylase were isolated from muscle and alcohol dehydrogenase from liver. Aldolase (molecular weight: 147,000; 35 sulphydryl groups [20]) was isolated from 220 g of thigh muscle tissue by the method of Taylor [21] and Taylor, Green and Cori [22]. The enzyme was precipitated in the final step of preparation by addition of trichloracetic acid to a concentration of 5%, instead of allowing it to crystalize as specified

Table 4: DISTRIBUTION OF RADIOACTIVITY IN VARIOUS FRACTIONS OF SOME OTHER BODY TISSUES

Organ	Selenomethionine labelled tissues				Selenocystine labelled tissues			
	Total Lipid fraction	Precipitate from 50% $(NH_4)_2SO_4$ saturation	Precipitate from 100% $(NH_3)_4SO_4$ saturation	Supernatant from 100% $(NH_3)_2SO_4$ saturation	Total Lipid fraction	Precipitate from 50% $(NH_4)_2SO_4$ saturation	Precipitate from 100% $(NH_4)_2SO_4$ saturation	Supernatant from 100% $(NH_4)_2SO_4$ saturation
Liver	0.2	80.3	5.0	14.5	0.9	85.0	7.1	7.1
Hypophysis	—	86.5	10.0	3.5	—	90.0	7.0	3.0
Pancreas	0.1	86.0	8.0	6.0	0.8	77.0	19.4	2.6
Adrenals	—	91.0	7.0	2.0	—	91.0	7.0	2.0
Thyroid	—	88.0	5.5	6.5	—	88.0	3.0	9.0

N.B. A dash in the total lipid column indicates that no extraction of the organ concerned was performed in this instance

in the original procedures. The crude preparation so obtained contained 1.2% of the activity present in the muscle mass used for the extraction.

Phosphorylase (molecular weight: 495,000; 17 sulphydryl groups; 110 methyl-thiol groups [20]) was prepared from 350g thigh muscle by the procedure of Cori, Illingworth and Keller [23] and Green and Cori [24]. The enzyme was purified as specified in the original procedures and found to contain O.1% of the activity present in the muscle mass used for its extraction.

Alcohol dehydrogenase (molecular weight, 150,000; 19—36 sulphydryl groups [20]) was isolated from 300 g of liver by the method of Bonnichsen and Brink [25]. Material obtained after the final dialysis and ethanol precipitations of the original procedure was found to contain 0.1% of the activity present in the liver tissue used.

One gram samples of whole blood taken from the two groups of animals at the time of slaughter were counted together with the organ samples noted in Figure 1 earlier. Blood from the animals given selenomethionine contained 3.2% of the total activity counted, while that from the sheep given selenocystine contained 3.5% of the total activity counted. The distribution of label between erythrocytes and plasma was indentical in both groups of animals, namely 60% and 40% respectively.

Erythrocytes were fractionated as follows: a total lipid extract was made with ethanol-ether [17] on a suitable aliquot of cells; cell stroma was prepared by haemolysis of another cell aliquot with distilled water, followed by high speed centrifugation, washing of the stroma precipitate and recentrifugation; haemoglobin crystals were prepared in pure form as described by Drabkin [26]. The lipid and stroma fractions contained less than 0.1% of the two erythrocyte seleno-amino acid labels; more than 99.9% being found in the crystalline haemoglobin prepared from the erythrocytes of both groups of sheep.

Plasma proteins were separated by electrophoresis on filter paper strips [27]. After fixing and staining [27] the various protein bands were cut out and monitored for radioactivity. In both groups of animals 80% of the plasma radioactivity was located in the $\beta + \gamma$ globulin fractions and the remaining 20% was more or less evenly distributed between the albumins and α-globulins.

DISCUSSION

A search through the literature failed to reveal any details regarding the immediately fatal dose of inorganic or organic selenium for the sheep. It is obvious from our results that this lies in the order of 3—4 mg Se/kg for either form of the element, both of which are exceedingly toxic for the sheep. These findings are similar to those recorded by Rosenfeld and Beath [28] for small laboratory animals.

The symptoms of the acute intoxication produced by either inorganic or organic selenium in sheep are also similar to those described by Rosenfeld and Beath [28] for small laboratory animals, dogs and cats.

Chronic organic selenium intoxication has as its major effects in the sheep, serious disturbances in protein metabolism. These are reflected by abnormal wool growth, almost complete cessation of growth, emaciation and anaemia. The latter is probably a low grade haemolytic anaemia. This assumption is based on the fact that so far we have only observed the abnormal haemoglobin C in anaemias of this nature and in post-haemolytic anaemias [7, 29]. The main naturally occurring haemolytic syndromes in sheep in South Africa are geeldikkop and enzootic icterus [4, 6, 7]. We have reproduced in the present work one of the main features of these diseases, notably, the appearance of haemoglobin C.

Organic selenium has been shown to be incorporated extensively into the body proteins of small laboratory animals and man and is also known to be extensively re-utilized [30]. Seleno-proteins are also known to have a longer turnover time than ordinary proteins. This has been demonstrated for instance with Se 75-albumin in rats [30]. Selenomethionine is freely miscible with its sulphur counterpart in the protein precursor pool and has a higher utilization factor than the latter [30]. It is apparent from our data that the sheep is no different in this respect.

The high levels of activity found in the kidney and pancreas proteins are remarkable when compared with other body tissues as in Figure 1. This is probably due to a higher utilization rate of the sulphur analogues by these two tissues. The kidney has for long been known as an organ which accumulates more selenium than other body tissues [5, 28]. Since most of it's physiological activity is located in the cortex, our findings in this re-

gard are not surprising. It is known to contain appreciable amounts of a sulphur and cadmium-rich metal binding protein designated metallothionein [30]. Factors like this may be linked with accumulation of the two labels in cortical tissue.

Mitochondrial labelling is presumably due mainly to labelling of the enzymes in these structures. The soluble components of the cell sap include the enzymes of carbohydrate metabolism [32], some of which, e.g. aldolase and phosphorylase, have now been shown to incorporate the selenium labels during their synthesis. The supernatant fraction obtained from differential centrifigation of cell homogenates also contains the ribosomes, lysosomes and microsomes, all of which may have incorporated the label, as well as various metabolic intermediates such as S-adenosylamino acids and in this case, Se-adenosyl homologues. Our figures with regard to the distribution of label between supernatant and mitochondrial fractions of kidney homogenates are similar to those obtained by Bell and Wright [33] on sheep liver homogenates using an inorganic selenium label. These authors found a greater incorporation of selenite label into the supernatant and microsomal fraction than we did with organic selenium. Pertinent differences have been pointed out in the handling of selenium by rats and sheep [34].

The labelling of albumins and globulins by inorganic and organic Se-75 has been demonstrated by Imbach and Sternberg [34] and Sternberg and Imbach [35]. They have postulated plasma albumins as carriers of inorganic selenium and have shown that although these proteins are extensively labelled thirty minutes after injection, the globulin fractions take up most of the label six hours later.

The incorporation of Se-75 into haemoglobin following injection of labelled selenite was demonstrated by McConnell [36] in 1963. Penner [37, 38] then used Se-75 selenomethionine for studying erythrocyte turnover. The present paper seems to be the first report of the isolation of crystalline selenohaemoglobin and the demonstration of the fact that labelling of red blood sells is due to its formation.

An earlier report of the excretion of Se-75 labelled compounds in bile was that of Goidsenhoven, Denk, Pfleger and Knight [39], who demonstrated a portion of the activity in the biliary proteins and mentioned the possibility of the excretion of labelled taurine. Imbach and Sternberg [34] found no labelling of bile acids in rats following injection of Se-75 sodium selenite. This also seems to be the first report of the excretion of selenotaurine by sheep following administration of seleno-amino acids and of its isolation and identification in this medium. The formation of selenotaurine is presumptive evidence of the utilization of methionine for taurine synthesis by the ovine liver.

Taurine has recently been found in brain tissue [40]. The significance of its presence here is not known. It is possible that some of the activity found by us in brain tissue may be due to the presence of selenotaurine, since the total lipid extracts of the various organs mentioned earlier contained negligible amounts of the label.

An entero-hepatic circulation of selenium-labelled compounds has been demonstrated in rats by Imbach and Sternberg [41]. McConnell [36] has shown that dogs can retain selenium in their plasma proteins for as long as 310 days after injection of Se-75 selenite. The label was also present in haemoglobin, cytochrome C, myoglobin, myosin and muscle aldolase. The very small amounts of organic selenium excreted by sheep have been referred to. This becomes even less in intact animals when one considers the marked enterohepatic circulation of bile salts in this species [42]. Labelled biliary proteins are presumably digested and the amino acid label also largely reabsorbed along the length of the digestive tract.

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THE CHEMICAL PATHOLOGY OF OVINE ICTERIC STATES
3. ICTEROGENIN INDUCED CHOLESTASIS

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SUMMARY

The haematology and chemical pathology of icterogenin intoxication in the sheep is described in detail. The condition induced is a typical intrahepatic cholestasis accompanied by severe photosensitivity. The main effects of icterogenin are on membrane permeability, liver cell membranes being primarily affected. Kidney and muscle cell and erythrocyte membranes are affected to a lesser degree and under special circumstances. Some of the metabolic disturbances leading to the failure in membrane permeability are discussed.

INTRODUCTION

The discovery of the icterogenic triterpene acids, the early work on their toxicology and the elucidation of their chemical structures have been described elsewhere [1-5]. These compounds induce in experimental sheep a clinical syndrome of icterus and photosensitisation outwardly similar to geeldikkop. Icterogenin has been used extensively by us for the experimental production of intrahepatic cholestasis in the sheep [2] since it is readily prepared in sufficient amounts from *Lippia rehmanni*, Pears (Verbenaceae). Its activity in this respect is, however, far less than that of 22 β-angeloyloxy-oleanolic acid and 22 β-angeloyloxy-hedragolic acid and comparable to that of 22 β-angeloyloxy-24-hydroxyoleanolic acid and 22 β-angeloyloxy-24-oxo-oleanonic acid [3-5]. The first named compound occurs naturally in very small amounts together with icterogenin and rehmannic acid, the latter three substances are synthetically produced derivatives [3-6].

Icterogenic potency is due to the presence of a β-equatorially orientated hydroxyl group at C(3) or a hydroxyl at C(24) and a 22 β-angeloyl side chain on the triterpene molecule. Stereoisomer specificity is shown in respect of icterogenicity of these compounds since the epimers of two of these substances carrying α-axially orientated hydroxyls at C(3) have been shown to have no such effect on bile flow or bilirubin excretion. Removal or saturation of the angelic acid side-chain, substitution of the hydroxyl groups or replacement of these with a ketone function and esterification of the C(28) carboxyl group were followed by loss of activity. In the case of the latter instance loss of icterogenic potency was probably the result of a decrease in the solubility of the compound [3-5].

Some of the biochemical effects of icterogenin on the liver of the rabbit have recently been studied at the height of intoxication, when the effects on bile flow and bilirubin excretion were maximal [7, 8]. Some reduction in the ability of the liver to conjugate bilirubin following intoxication by icterogenin was evident in all the experimental animals. Uridine diphospho-glucose dehydrogenase was found to be either unaffected or increased at the end of the test period. The most noteworthy effects of the intoxication were found to be a marked decrease in the activity of succinic dehydrogenase and glyceraldehyde-phosphate dehydrogenase. Diaphorase, cytochrome C reductase and DPN-ase activities were not significantly affected although some reduction of ATP-ase activity was evident in most cases. It was tentatively concluded that icterogenin had little effect on mitochondrial respiratory chains, but appeared to exert a selective depressant effect on the activity of certain dehydrogenases [7]. Icterogenin was found to cause a fair to marked decrease in the amount of linolenic acid present particularly in the neutral lipids of the liver cell walls and associated structures. This was found to be associated with a rapid decline in bilirubin excretion [8].

The haematology and general chemical pathology of icterogenin intoxication in the sheep are described in this paper.

A. SINGLE ORAL DOSES OF ICTEROGENIN

Animals, Materials and Methods

Fifteen adult Merino wethers weighing

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between 19 — 25 kg were used in the work reported here. They were maintained on a diet of green lucerne, crushed maize and water all given *ad libitum*. Twelve of these sheep received single oral doses of icterogenin given at dosage levels varying from 100 mg — 600 mg/kg. The icterogenin was generally dissolved in 25 — 100 ml warm ethanol and immediately prior to dosing this was diluted with about 300 ml of water. Two animals received split oral doses of icterogenin. The dosing regimen concerned will be described later in this paper. The remaining animal received the compound by intravenous injection.

The animals were introduced into metabolism cages about two weeks before dosing. Bleeding for baseline values was performed daily for one week before administration of the compound and daily thereafter until subsidence of the clinical symptoms. Urine was collected by means of a bottle placed beneath the sump of the cage and faeces were collected in standard faeces bags. The animals were tested for photosensitivity as described later in this paper.

Haematological investigations were performed according to standard laboratory procedures. With the exceptions mentioned below, the methods used for the various blood and urine analyses and enzyme assays were the same as given in the first paper of this series [9]. Total plasma copper, plasma copper fractions, red cell copper, tissue and urine copper and liver iron were all determined accordingly to methods presented elsewhere [2]. Ceruloplasmin was determined by the method of Houchin [10], plasma ascorbate by the dichlorophenol — indophenol method cited by King and Wootton [11], and creatine phosphokinase by the method outlined in the Sigma technical bulletin No. 661 [12].

The icterogenin used in the early stages of this work was either supplied by former co-workers, Drs. P. R. Enslin and W. T. de Kock and Mr. L. A. P. Anderson, then all of the National Chemical Research Laboratories, Pretoria, or it was prepared from the freshly collected root bark of *Lippia rehmanni* (Pears), Verbenaceae, according to the procedures outlined by Anderson, de Kock and Enslin (1961) and Anderson (personal communications).

The Haematology and Chemical Pathology of Icterogenin Intoxication

The results described in this section were obtained from the sheep given single doses of icterogenin as shown in Table 1. The animals were each studied for a week after administration of the compound.

Table 1: DETAILS OF SINGLE DOSES OF ICTEROGENIN RECEIVED BY EXPERIMENTAL SHEEP DURING THIS WORK

Sheep No.	Dosage level mg/kg	Amount received (g)
7061	250	5.00
7054	280	7.00
16841	400	10.70
21513	600	12.00
19449	600	12.00
89091	100	2.36
1624	100	2.36
1726	100	1.45
88716	200	4.28
89551	200	3.90
1780	200	5.40
1780A	200	6.40

(a) *Haematology:* No changes were observed during the week following dosing in the red blood cell counts, white blood cell counts, packed cell volume, haemoglobin, sedimentation rate or absolute eosinophile counts in animals that had received doses of icterogenin of less than 200 mg/kg. Icterus and photosensitisation were apparent in all these animals at some time during the test period.

The animals which had received a dose larger than 200 mg/kg developed a hypocythaemic normocytic normochromic anaemia, as can be seen from the typical data presented in Table 2. Haematological determinations were made each day for a week after dosing but only the results on the day immediately following dosing, the third day and the final day of the experiment have been included in this table to indicate the trend.

Although the red blood cell count, packed cell volume and haemoglobin all decreased proportionately as the experiment proceeded in these animals, no corresponding decrease in total plasma protein levels was generally apparent. The decreases mentioned could thus not be attributed to haemodilution as a result of water retention.

No white blood cell dyscrasias were observed in any of the animals given single doses of icterogenin, nor did the erythrocyte sedimentation rate show any deviations from normal at any time.

(b) *Liver function:* Plasma total bilirubin levels commenced to rise from 24 — 48 hours after administration of icterogenin and gener-

Table 2: HAEMATOLOGY OF THE ANIMALS THAT RECEIVED DOSES OF ICTEROGENIN HIGHER THAN 200 mg/kg

Sheep No.	Day	RCC	PCV	Hb	WCC	MCH ($\mu\mu$g)	MCHC (%)	MCV (CuM)
7061	1	7.60	28.0	8.20	5.30	10.78	29.27	36.8
	3	7.53	28.0	7.24	6.40	9.61	25.85	37.2
	7	6.44	21.0	5.55	6.30	8.61	26.42	32.6
7054	1	10.19	25.0	8.06	4.75	7.90	32.24	24.5
	3	6.17	24.0	8.20	4.75	13.30	34.16	38.9
	7	6.07	22.0	6.27	4.30	8.86	28.5	31.11
16841	1	11.80	33.0	9.79	10.10	8.28	29.66	27.9
	3	10.40	30.0	9.79	11.60	9.41	32.63	28.8
	7	7.10	26.0	9.79	10.00	13.80	37.65	36.6
19449	1	8.50	27.0	6.12	7.80	7.20	22.66	31.8
	3	7.50	24.0	5.12	7.70	6.82	21.33	32.0
	7	5.30	22.0	4.87	4.90	9.18	22.13	41.5

(Day 1 refers to the day immediately after dosing and Day 7 to the last day of the experiment. RCC = red cell count (millions/cumm), PCV = packed cell volume (%), Hb = haemoglobin (g%), WCC = leukocyte count thousands/cumm, MCH – Mean corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin concentration, MCV = mean cell volume).
N.B. Normal values for the various determinations noted here have been given elsewhere [2].

ally clinical icterus was seen 48 — 72 hours after dosing. The figures presented in Table 3 are typical of the results obtained in this regard with various dosage levels. The *duration* of the block to bilirubin glucuronide excretion by the liver was related to the dosage employed as can be seen from those figures. The *severity* of bile pigment regurgitation and the *intensity* of the clinical icterus were related more to the duration of the effects of icterogenin than to the dosage level. This point is illustrated by the data obtained from sheep 89551 and 19449 and presented in Table 3. Sheep 88716, not featured in this table, developed the severest icterus seen in this series of experiments. This animal had received 200 mg of icterogenin per kg bodyweight. The plasma total bilirubin level in this case was 2.7 mg% 24 hours after dosing and then it rose steadily to reach a value of 22.3 mg% seven days after dosing, after which a slow return to normal over the following week was observed. At least two-thirds of the bile pigment circulating in the plasma of this animal during the experimental period was bilirubin glucuronide. It will be seen from Table 3 that, although in most cases the greater part of the bile pigment being returned to the plasma is the water-soluble glucuronide, appreciable amounts of bilirubin are also retained. This is particularly striking in the case of sheep 89091.

Table 3: PLASMA BILIRUBIN LEVELS IN CASES OF ICTEROGENIN INTOXICATION

Sheep No.	Dosage level	Days	Total bilirubin	Bilirubin glucuronide	Bilirubin
89091	100 mg/kg	1	0	0	0
		2	3.85	2.70	1.15
		3	2.95	1.35	1.60
		4	1.80	0.20	1.60
		5	1.10	0.90	0.20
		6	1.10	0.45	0.65
89551	200 mg/kg	1	1.10	0.65	0.45
		2	5.90	3.85	2.05
		3	7.00	4.55	2.45
		4	8.20	6.75	1.45
		5	2.00	1.35	0.65
		6	1.35	0.65	0.70
16841	400 mg/kg	1	0	0	0
		2	1.06	0.76	0.30
		3	3.30	2.20	1.10
		4	4.40	2.50	1.90
		5	3.40	2.00	1.40
		6	1.20	0.90	0.30
19449	600 mg/kg	1	0	0	0
		2	1.04	0.76	0.28
		3	2.20	1.06	1.14
		4	4.00	2.60	1.46
		5	9.40	5.90	3.50
		6	7.60	6.90	0.70

Bilirubinuria was always seen in icterogenin intoxication in the sheep, particularly at the height of the reaction. It was generally mild and bore little relation to the high plasma levels of bilirubin glucuronide which

may be found. The specific gravity of the urine in all our cases fell from predosing values of 1.015 — 1.045 to values of 1.010 — 1.015 when bilirubinuria occurred. This phenomenon had been noted earlier in experimental cases of common bile duct obstruction in the sheep.

Urobilinogen disappeared from the urine in cases where the hepatic block to bilirubin glucuronide was sustained for a number of days, e.g. sheep 88716, 1780A and 21513. The amount of bile acid salts excreted in the urine generally increased markedly within 36 hours of dosing, the excretion of these compounds following in general the excretion of bilirubin. Although blood levels of these compounds were not determined, it is obvious that their hepatic excretion was blocked simultaneously with that of bilirubin.

Porphyrin excretion by the liver was markedly impaired during icterogenin intoxication. Photosensitivity was seen about two to four days after dosing, but marked coproporphyrinuria appeared after about twenty four hours. The figures presented in Table 4 are typical results. Baseline levels for plasma phylloerythrin and the 24 hourly urinary excretion of coproporphyrin for the various control animals used were within the normal limits given elsewhere [2].

The data given in this table support the contention that there probably has to be a certain definite level of phylloerythrin in the blood before affected animals will become photosensitive.

The presence of photosensitivity was determined in these two particular cases by using a high-energy emission Xenon lamp as source of the activating rays and a set of cut-out filters as used by Riemerschmid and Quin [18] taped in place over depilated areas on the backs of the sheep. Only mild erythema was evoked on the second day of dosing in the areas covered by filters corresponding to the action spectrum of phylloerythrin [18]. No evidence was noted of discomfort or erythema after exposure of their depilated heads and ears to solar radiation of half-an-hour. On the third day after dosing marked erythema and subsequent oedema was produced by Xenon lamp—irradiation through these filters for a period of five minutes, while solar irradiation for half-an-hour produced the same effects. The animals were intensely photosensitive on the fourth day after dosing and both types of irradiation produced severe hyperaemia, pain and oedema in the exposed areas followed later by necrosis of the superficial layers of the skin. Neither animal was significantly photosensitive on the seventh and subsequent days.

Hypercoproporphyrinuria was present 24 hours after dosing icterogenin and lasted for over a week after dosing in the two cases featured in Table 4.

The almost immediate and precipitous fall in the levels of plasma iron observed in cases of common bile duct obstruction and the return to normal about three to five days later [9] was also seen in animals that had received doses of icterogenin of 400 — 600 mg/kg but not in those having received lower doses. In animals 16841, 21513 and 19449 plasma iron levels fell from predosing values of 140 — 250 mcg% to 77 — 80 mcg% twenty four hours after dosing, reaching 22 — 38 mcg% on the third day. Thereafter plasma iron levels slowly rose once more, regaining the predosing values on the sixth to seventh day. The reason for this peculiar phenome-

Table 4: PLASMA PHYLLOERYTHRIN LEVELS AND THE 24 HOURLY URINARY EXCRETION OF COPROPORPHYRIN IN CASES OF ICTEROGENIN INTOXICATION

Sheep No.	Porphyrin	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
16841	Plasma phyllo.	0.84	18	21	39	42	22	18	13
	Urine copro.	96	180	312	80	324	358	121	101
19449	Plasma phyllo.	0.78	12	17	25	28	32	30	27
	Urine copro.	82	135	14	36	89	76	64	59

Phyllo – phylloerythin, mcg%; Copro – coproporphyrin, mcg excreted/24 hours. Days = days after dosing).

non, which must be related to general impairment of biliary secretion, is unknown.

Liver iron levels were determined on specimens from sheep 7054 and 7061 seven days after administration of icterogenin. The values found were 14.8 mg% and 12.8 mg% (wet tissue) respectively, both within the normal limits. Kidney iron values were 19.0 mg% and 11.2 mg% (wet tissue) respectively. The value found in the case of sheep 7054 was somewhat higher than that found in the controls used throughout this work, but no particular significance is attached to this finding [2].

No changes of note in the various plasma protein fractions or the albumin: globulin ratio were observed in any of the sheep during the week following administration of icterogenin, except in the case of animal 21513. In this case a gradual and sustained fall in total plasma protein levels from 7.97 g% to 6.24 g% occurred during the week following dosing. This was associated with a similar decline in the albumin level from 3.98 to 3.28 g% and in the globulins from 3.99 to 2.96 g%. Haemoglobin levels in this animal fell from 15.2 to 9.93 g%. Since the slow fall affected both major protein fractions and the red blood cells, it was related most likely to water retention and haemodilution.

The thymol turbidity, thymol flocculation, zinc sulphate turbidity and colloidal gold flocculation tests showed no deviations at any time after dosing from the predosing (and "normal") values in any of the experimental animals.

The results of BSP clearance tests in these cases are represented by the data presented in Table 5. These results indicate an increasing inability to clear the dye from the blood stream during the first three to four days after intoxication and thereafter a return towards normal in this respect. These findings are in excellent accord with those for bilirubin glucuronide and porphyrins. In most cases normal clearances were found seven days after administration of the compound. Sheep 88716, which had extreme-

Table 5: THE RESULTS OF BROMSULPHALEIN (BSP) TESTS IN SHEEP POISONED WITH ICTEROGENIN

Sheep No.	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1780	7.10	—	—	67.10	—	—	20.0
1780A	—	79.0	—	—	—	—	5.3
1726	17.3	24.4	12.7	—	3.4	—	0
88716 (See also foot of table)	35.0	61.5	76.0	90.0	80.0	80.0	88.0
89091	6.2	44.8	56.0	9.2	0.8	0.5	0
89551	32.8	67.2	67.0	82.0	34.0	2.0	0
1624	34.4	60.3	58.4	59.4	41.8	1.2	0
7061	—	74.9	76.7	26.8	—	—	0
88716 Total bilirubin mg%	2.70	8.85	10.80	12.15	12.15	13.80	22.30

(The figures given represent the % of the test dose retained in the blood stream 30 minutes after injection. "Days" refer to days after dosing).

ly high levels of total bilirubin throughout the entire test period, was remarkable in that the same phenomenon was observed in the case of BSP. The total bilirubin levels found in this animal are given at the foot of Table 5 for comparison. It will be remembered that this case had received a dose of 200 mg/kg and responded most severely to icterogenin at this level of administration. These data illustrate how the elimination of several substances normally excreted in bile may be affected simultaneously and to the same degree. The concentration of bile pigment found in the plasma of sheep 88716 is almost identical to the figure which could be expected if the amount of bile pigment excreted during 24 hours in the bile were returned to the blood stream daily for seven days. This hypothetical figure has been calculated to be 19.74 mg% [9]. The figure found at the end of the seven day test period in animal 88716 was 22.3 mg%. It is apparent thus that in this case, at any rate, the biliary excretion of conjugated bilirubin remained completely blocked over the whole test period.

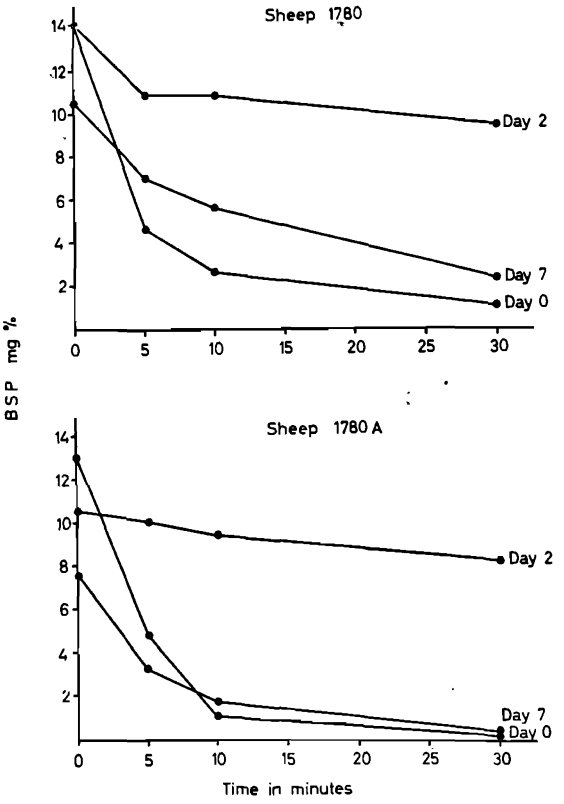

Fig.: BSP clearance in cases dosed with icterogenin (Day O = the day before dosing was commenced; Days 2 and 7 are days after dosing; BSP mg% – plasma levels of BSP; Time in minutes – minutes after injection).

The composite Figure shows the rate of clearance of injected BSP from the blood stream of two of the animals given icterogenin, compared with similar data obtained before intoxication. BSP concentrations were determined on samples of blood drawn 1, 5, 10 and 30 minutes after injection; concentration of BSP was then plotted against time. It is obvious from the shape of the curves obtained from tests made during the intoxication with icterogenin, that clearance of BSP from the blood stream by the liver is seriously impaired. Considering the data found for sheep 88716 discussed immediately above, and by analogy with the relevant findings in geeldikkop, it is likely that the excretion of BSP into the bile is also seriously impaired. A similar failure to clear the compound from the blood is seen in early enzootic icterus [2].

Throughout the experiments alkaline phosphatase activity was determined on plasma samples from the animals which had received doses of icterogenin of 200 mg/kg and higher. In no instance was any deviation found from the "normal" range of 5 — 25 units.

Levels of activity of glutamic oxalacetic (GOT), and glutamic pyruvic (GPT) transaminases, phosphohexose isomerase (PHI), aldolase (Ald), isocitric dehydrogenase (ICD) and lactic dehydrogenase (LD) in blood plasma were determined only in the case of sheep which had received doses of icterogenin of 400 mg/kg and higher, i.e. sheep 16841, 21513 and 19449. GOT, PHI and Ald plasma levels were elevated in all three cases within two days of dosing and values for the activity of these enzymes remained high during the whole test period. The magnitude of the increase in activity of these enzymes in the plasma of affected animals was directly related to the dosage level employed, eg. the highest recorded values for the plasma activity of each enzyme during the week following dosing were:— Sheep 16842 (400 mg/kg); GOT 315, PHI 313, Ald 54; Sheep 19449 (600 mg/kg); GOT 515, PHI 408, Ald 142; Sheep 21513 (600 mg/kg); GOT 587, PHI 235 and Ald 138. Maximum levels of plasma activity of these enzymes were found four or five days after dosing.

Plasma ICD levels remained unaltered during the week following dosing in all three cases. GPT levels remained similarly unal-

tered in animals 16841 and 19449, and plasma LD levels remained normal only in the sheep which had received the lower dose, *i.e.* 16841. Plasma levels of this enzyme rose in the case of sheep 19449 on the 5th and 6th day after dosing. This sudden rise in LD activity could be related to the severe photosensitisation reactions evoked by the solar and Xenon emission irradiations described earlier. Plasma LD rose in this case from 350 — 610 during the first four days after dosing to 1020 — 1360 on the following two days.

Sheep 21513 (600 mg/kg) was remarkable in that notable elevations of plasma activity of GOT, PHI, Ald and LD were present from 24 hours after dosing and persisted throughout the entire test period. LDH values rose from 850 on the day after dosing to 2310 three days later. This was the only sheep used during this work in which a marked and sustained rise in GPT levels was found, *viz.* 137 twenty-four hours after dosing to 177 four days later. The results of these plasma enzyme assays differed so from those obtained in the other two animals that we suspected the presence of muscle lesions in this case. Creatine phosphokinase (CPK) activity was determined on plasma samples which had been deep frozen for later mineral analyses. Values of 6—7 units (*i.e.* well within the normal range for sheep [13] were found in the samples from the first two days after dosing; in the subsequent samples markedly elevated values of 35 — 41 units were determined. The sheep was slaughtered at the end of the experiment and extensive focal muscle degeneration was found in the cadaver at autopsy examination.

(c) *The erythrocyte in icterogenin intoxication:* Red blood cell fragility remained unaltered throughout the entire experimental period at all the dosage levels employed. Erythrocyte copper levels rose from predosing values of 66 — 79 mcg% to values in the range 125 — 146 mcg% two to three days after dosing and remained in this range for the rest of the experimental period of one week in some of the cases. Such raised values for red blood cell copper were generally above the normal 80% range found for sheep but were still within the upper 98% limit.

(d) *Kidney function* Blood urea nitrogen, creatinine, uric acid and amino acid levels were studied and the usual routine urine analyses were performed before and during icterogenin intoxication. Urea nitrogen levels remained normal in the animals receiving less than 400 mg icterogenin per kg, except in the case of animal 1780, in which a rise occurred from the usual levels of 9.0 — 23.0 mg% to 29.0 and 31.0 mg% during the last two days of the experiment respectively. Elevations of the same order were observed during the entire week after dosing, in sheep 16841 (400 mg/kg) and 21513 (600 mg/kg). Sheep 19449 (600 mg/kg) also suffered the same very mild uraemic disturbances but on the seventh day after dosing blood levels of urea rose from 29.4 mg% to 82.8 mg%.

No alterations upon icterogenin administration were observed in the blood levels of any of the other non-protein nitrogenous compounds mentioned. It is interesting to note that although some of these sheep were allowed to develop extensive and severe lesions of photosensitisation involving the depilated head and back (eg. sheep 7054 and 7061) after solar irradiation, no elevations in their blood uric acid levels occurred. These ranged in the first animal from 1.2 — 1.8 mg% and in the second from 0.6 — 1.0 mg%.

Plasma magnesium levels remained within the range 1.20 — 3.2 mg% in all animals after dosing except in the case of sheep 7061 (250 mg%) when values of 5.6 — 6.0 mg% were found during the three days immediately following administration of the compound; thereafter normal values were obtained once more. Plasma inorganic phosphate levels similarly remained within normal limits in all the animals except those which had received 600 mg of icterogenin per kg. Values of 10.7 and 11.1 mg% were found in plasma samples from each sheep respectively on the seventh day after dosing.

Apart from the mild bilirubinuria and disappearance of urobilinogen from the urine (of some cases) after dosing, no other abnormalities were detected in the daily samples from any of the experimental animals.

(e) *Adrenal function:* This was assayed by determining plasma sodium, potassium, chloride, bicarbonate and performing absolute eosinophile counts [2]. Hyponatraemia associated with hypochloridaemia was observed in only two of the experimental animals after dosing icterogenin. Both phenomena appeared two days after dosing the compound and were present for a further four to five days. Hypokalaemia associated with hyperchloridaemia was seen towards the end of the experimental period in animal 19449 (600 mg/

kg). Plasma bicarbonate levels fell gradually in the same case from predosing levels of 24 — 26 meq/l to 16.0 meq/l at the end of the seven day experimental period. Absolute eosinophile counts remained within the normal limits in at least half the animals studied after giving icterogenin. The remainder underwent a reduction of the eosinophile count of variable magnitude, figures of 20 — 80 cells/cu mm being found during the seven day period after dosing icterogenin.

In general icterogenin intoxication was associated with a stress reaction of mild and variable nature. No definite pattern of electrolyte disturbances was found to be present, as is the case in diseases like geeldikkop[2].

Plasma calcium levels were studied in two of the sheep only, 7054 and 7061. No deviations from the normal range of 9—12 mg% were noted at any time after giving icterogenin.

Blood sugar and ascorbic acid levels were studied in cases 88716, 89091, 89551, 1624, 1726, 1780A, 1780, 7054 and 7061. Predosing blood sugar levels in these animals ranged from 33 — 55 mg%. A mild hyperglycaemic tendency was seen in sheep 1780 and 1780A, blood sugar levels ranging from 65 — 77 mg% being regular daily findings in both cases for the entire seven day period after dosing. This phenomenon was not observed in the other cases that received the same or higher doses. Plasma ascorbic acid levels remained unaltered in all these cases at the predosing levels of 0.44 — 0.70 mg%.

(f) *Copper metabolism:* Total plasma copper levels were studied in sheep 7061 (250 mg/kg), 7054 (280 mg/kg), 16841 (400 mg/kg), 21513 and 19449 (both 600 mg/kg). Total plasma copper levels ranged from 100 — 150 mcg% in these animals before giving icterogenin. A transient elevation in total plasma copper commencing two to three days after dosing and lasting for a further two to three days was seen in all the animals bar sheep 19449 in which no change in total plasma copper was observed. These elevated copper levels ranged from 200 — 250 mcg% in all instances except sheep 16841, in which plasma levels ranging from 165 — 174 mcg% were found. The various blood copper fractions were determined on samples from sheep 16841, 21513 and 19449. The results are presented in Table 6. The values for the fractions were normal, as given earlier[2,9]. Dosing with icterogenin was followed 24 hours later by a rise in the loosely bound copper fraction in two out of the three animals. This fraction increased as the intoxication proceeded. In sheep 21513 the ceruloplasmin fraction was markedly elevated instead. This latter fraction only rose above normal levels towards the end of the experimental period in the

Table 6: BLOOD COPPER FRACTIONS IN ANIMALS POISONED WITH ICTEROGENIN

Sheep No.	Fraction	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
16841	Total plasma Cu	146.0	124.6	165.0	152.0	164.3	174.0	168.0
	Loosely bound Cu	8.2	0	1.4	2.4	10.0	25.0	11.3
	Ceruloplasmin	10.3	6.0	5.1	7.6	19.6	19.9	15.1
	Red blood cell Cu	105.7	116.6	114.3	140.7	96.6	98.4	121.6
21513	Total plasma Cu	158.1	—	206.6	—	168.0	—	118.1
	Loosely bound Cu	0	—	0	—	0	—	11.0
	Ceruloplasmin	27.4	—	21.2	—	7.9	—	18.0
	Red blood cell Cu	79.4	—	145.8	—	182.2	—	147.8
19449	Total plasma Cu	139.7	104.8	126.7	116.7	117.9	133.3	141.7
	Loosely bound Cu	6.8	0	0	1.2	8.8	11.3	12.6
	Ceruloplasmin	6.6	5.7	4.5	5.7	12.2	18.3	15.7
	Red blood cell Cu	65.8	124.5	141.8	121.0	104.3	117.6	121.7

(Values are mcg %, except in the case of ceruloplasmin where they are mg %. "Days" are days after dosing icterogenin).

first two mentioned sheep. Blood copper fractions were found to be normal once more in all three cases ten days after dosing. Ceruloplasmin levels were down to 8.2 — 8.7 mg% and loosely bound copper ranged from 6.7 — 11.1 mg%.

Red blood cell copper contents were found to rise above the normal 80% level on the second or third day after dosing. Elevated levels persisted throughout the experimental period in sheep 21513 (in which the loosely bound plasma copper did not rise until near the end of the experiment, but ceruloplasmin levels had risen soon after dosing). In the other two animals elevation of this copper fraction was transient.

The liver and kidney copper content was determined on samples of these tissues from animals 7054 and 7061 taken at the end of the experimental period. Kidney copper levels were within normal limits[2], 0.83 and 0.42 mg% being the respective values found. Liver copper values were 45.0 mg% and 33.34 mg% respectively. Liver copper was thus increased in the former sheep only.

B. INJECTION AND SUCCESSIVE SMALL ORAL DOSES OF ICTEROGENIN

Animals, Materials and Methods

As will have been noticed many of the abnormal events which occur after dosing icterogenin, do so irrespective of the dosage level employed if this is above that sufficient to induce the intoxication. It seems that once *sufficient* icterogenin is given to induce the typical disturbances in biliary excretion, its effect is maximal from the start, the only variable being the duration of the disturbances, which are dosage dependent. The dose of 200 mg/kg seems to be the lowest oral dose which gives reproducible results in the sheep. Even at this level, the total dose of icterogenin which must be employed to induce intoxication in sheep is large and a considerable amount of precious material must be used. If a higher dose is used to obtain a prolonged effect (See Table 1, for instance) the amount of material which has to be used is even greater. We have preferred to use fully grown adult sheep for these studies, because of the volume of blood which must be drawn daily to permit all the determinations described in this paper and to facilitate the repeated performance of tests like BSP clearance which require frequent bleeding for sample collection.

One of the major difficulties inherent in dosing icterogenin is its insolubility. Small doses may be dissolved in small volumes of ethanol and dosed by stomach tube, or aqueous solutions of the sodium salt may be given the same way. Single doses of 10 — 12 g require considerable volumes of alcohol for solution, the dosing of which has its own particular well-known hazards. To keep the volumes as small as possible, the solutions must be given hot and a considerable amount of icterogenin will thus precipitate when the solution mixes with the rumen fluid. The same objections hold for the sodium salt of the compound.

It was decided therefore, to give the total dose of icterogenin in the form of small single daily doses orally or by injection and to see whether this method produced the required prolongation of the effects of the compound which had been obtained with the single large doses given earlier. Three sheep were used for these studies and the following dosing regimens were employed: Sheep 89291 received a total of 8.2 g of icterogenin given in the form of daily doses of 3, 2, 2 and 1.2 g of the compound each dissolved in 40 ml alcohol and given by stomach tube; sheep 1981 received 7 g of the compound given in daily doses of 3, 2 and 2g in alcohol as above; sheep 1541 received 4 daily doses of 0.25 g icterogenin given by intravenous injection. The solution for injection was prepared by dissolving 1 g of icterogenin in 22.6 ml of 96% ethanol. To this was added 17.4 ml of 0.1 N NaOH and after mixing, 80 ml of isotonic sodium chloride was added. A dose of this solution equivalent to 0.25 g of icterogenin was then given. It was found that this was the maximum dose which could be given safely by the intravenous route. Doses of 0.5 g, even if well diluted and injected very slowly, caused death within minutes after injection. Generally observed autopsy findings included cyanosis, pulmonary oedema and hyperaemia, subepicardial haemorrhages and haemorrhages into the liver substance, tumor hepatis, tumor lienis, oedema of the gallbladder and gastrointestinal tract and haemorrhages into the abomasum and small and large intestines. Death was attributed to shock, cardio-vascular collapse and generalised haemorrhagic diathesis.

RESULTS

Both ways of administering icterogenin produced the desired prolonged effect which resulted in the production of a clinically very severe icterus and photosensitisation comparable outwardly to severe early cases of geeldikkop. When the compound was dosed orally, clinical icterus appeared about 48 hours after the first dose was given and photosensitivity 24 hours later. Both symptoms persisted for 5 — 7 days after the last dose was given. When the compound was given by injection, severe regurgitation of bilirubin was apparent within 24 hours and photosensitivity appeared within 48 hours after giving the initial dose. The effects by this route, however, are far more transient than when icterogenin is given by mouth. Cessation of the daily injections of the compound resulted in a return to normal within 24 — 48 hours. This suggests that a considerable part of the prolonged action of orally given icterogenin is due to a slow and sustained rate of absorption from the digestive tract. The data pertaining to plasma bile pigment levels found in sheep 89281 and 1541 during the experiments concerned illustrate these points. These data appear in Table 7. The injection of icterogenin may cause considerable haemolysis as judged by the large amounts of bilirubin in the plasma of sheep 1541 immediately after the injections. The fall in packed cell volume and haemoglobin from predosing values of 38% and 9.42 g/100 ml to 25% and 7.12 g/100ml on day 4 and 20% and 7.12 g/100 ml on day 7 supports this statement. Examination of bloodsmears taken from this animal showed marked anisocytosis and a marked monocytosis and lymphocytosis on each of the seven days following the first injection. The monocytes were generally seen to be packed with phagocytosed red blood cells. Differential leukocyte counts done daily over this period revealed a relative neutrophilia as well. The following figures incorporate all the daily counts made on the seven days following the first injection:— neutrophiles, 25 — 32%; lymphocytes 20 —

Table 7: THE EFFECTS OF ICTEROGENIN ON PLASMA BILE PIGMENT LEVELS WHEN IT IS GIVEN BY SPLIT DAILY ORAL DOSES OR BY DAILY INJECTION OF SMALL DOSES

Sheep No.	Days	Dosing regimen	Total Bilirubin	Bilirubin glucuronide	Bilirubin
89281	0	3 g orally	0	0	0
	1	2 g "	3.21	2.14	1.07
	2	2 g "	4.29	2.86	1.43
	3	1.2 g "	8.57	6.07	2.50
	4	——	5.75	2.89	2.86
	5	——	10.00	6.43	3.57
	6	——	8.93	5.70	3.23
	7	——	7.15	2.86	4.29
	8	——	3.57	1.79	1.78
	9	——	3.22	1.79	1.43
	10	——			
1541	0	0.25 g injected	0	0	0
	1	0.25 g "	8.13	3.13	5.00
	2	0.25 g "	10.00	5.62	4.38
	3	0.25 g "	13.12	7.51	5.61
	4	——	8.75	3.75	5.00
	5	——	1.25	0.31	0.94
	6	——	0.65	0	0.65
	7	——	0.25	0.20	0.05

(Values are mg %. "Days" are days after commencement of the experiment).

60%; monocytes, 18 — 59%; eosinophiles 1 — 3% and basophiles 0 — 1%. These changes were not observed in the animals receiving split oral doses of icterogenin.

Total plasma proteins, albumin and globumin and globulin levels remained unaltered throughout the experimental period in the animal which had received icterogenin by injection. In the animals which had received the compound orally, total plasma protein levels rose about two days after the last dose was given, the rise being due to an increment in the total globulin fraction from predosing levels of 3.16 — 3.44 g% to 4.35 — 4.80 g%.

Thymol turbidity and flocculation tests and the zinc sulphate and colloidal gold flocculation tests yielded negative results throughout the experiment in all three cases and plasma alkaline phosphatase values remained in the predosing range of 5.0 — 13.3 units.

Plasma iron levels were not studied in the case of sheep 1541, but remained unaltered throughout the experiments in the animals which had received icterogenin per os. Total plasma copper levels remained within the predosing range of 100 — 155 mcg% in all three animals.

Blood urea nitrogen and creatinine levels remained unchanged in sheep 1541 at the predosing ranges of 14.7 — 18.4 mg% and 2.2 — 2.8 mg % respectively. In the case of sheep 89281 a marked uraemia developed two days after the last dose was given (i.e. day 5, indicated in Table 7). Urea levels rose from 11.6 — 18.3 mg % to 58.3 mg % at this time and finally to 73.3 mg % at the end of the experiment. Plasma creatinine levels varied in this animal form 3.4 — 4.4 mg % during the course of intoxication.

During the experiments routine urine examinations were performed on samples from all three cases. Bilirubinuria and bileaciduria were severe in the animals receiving icterogenin orally and negligible in sheep 1541, in spite of the high levels of conjugated pigment in the blood of this animal. Urobilinogen disappeared from the urine of all three animals soon after commencing the dosing regimen, the urine from all three subsequently remaining free from urobilinogen for the duration of the experiment. Once bilirubinuria appeared, urinary specific gravity values fell from 1.015 — 1.030 before dosing to 1.010 for as long as bilirubinuria persisted in the animals concerned. Sheep 89291 developed a marked albuminuria and haematuria concomitantly with the uraemia mentioned earlier.

No changes of note were observed in plasma magnesium or inorganic phosphate levels in any of the animals as the experiments proceeded. Calcium levels likewise remained within normal limits in all instances.

BSP clearance from the blood was affected in the same manner as described earlier but to a much severer degree in the animals receiving split oral doses of icterogenin. In animal 89281, for instance, 90.6% retention of the dye at the end of the half hour test period was observed after the second dose had been given. On day 7 indicated in Table 7, i.e. four days after the last dose had been given, 72.5% retention of the test dose was observed at the end of the half hour test period. Traces of BSP were found in the urine 12 hours after the first test. The BSP from this dose continued to be excreted in the urine *over the next three days.* The blood plasma contained large amounts of BSP during these three days, as judged by the intense purple colour developed on alkalinisation. By the time the second test was done, the plasma and urine were clear of the dye. The same phenomena were observed once more after this second test: appreciable amounts of BSP remained in the plasma for the following three days, the dye appearing in the urine at the same time.

Injection of the first small dose of icterogenin into sheep 1541 produced 31% BSP retention within 24 hours. After the third dose, 65% rentention was found at the end of the test period and the dye appeared in small amounts in the urine. Three days after the last injection, 15% of the injected BSP was still retained at the end of the test period.

The intravenous injections of icterogenin produced no significant changes in plasma electrolyte concentrations or in the absolute eosinophile counts during the entire test period in animal 1541. Oral dosing of the compound produced a typical Addisonian syndrome in sheep 89281 two days after the last dose of the compound had been given. This was manifested by a progressively worsen-

ing hyponatraemia, hypocloridaemia, hyperkalaemia, low plasma bicarbonate levels and a very low absolute eosinophile count. The values for the various blood constituents found five days after the last dose of icterogenin had been given, were: sodium, 118 meq/l; potassium, 8.2 meq/l; chlorides, 77.0 meq/l; bicarbonate, 13.9 meq/l; absolute eosinophile count, 20/cu. mm. Sheep 1981, which had received a lower total oral dose of icterogenin, suffered only a progressively worsening hyponatraemia and falling absolute eosinophile count as the intoxication progressed.

A hyperglycaemic tendency was observed in all three animals during the course of the intoxication. Blood sugar levels in sheep 89281 varied between 34 — 53 mg% during the four days before icterogenin was dosed. On the day after the last (fourth) dose had been given, the blood sugar level was 70 mg%, on the following day 65 mg% and 86 and 90 mg% on the next two days respectively. This tendency was not so pronounced in either sheep 1981 or 1541 (which had received the compound intravenously).

Apart from icterus, lesions of photosensitisation and severe bile pigmentation of the liver and kidneys, the only notable autopsy feature of uncomplicated icterogenin intoxication was an extremely severe gastro-intestinal stasis. This was seen particularly in animals receiving small daily doses of the compound for a number of days and was characterised by some atrophy of the gastro-intestinal tract and considerable desiccation of the contents of the forestomachs, caecum and colon. This gastro-intestinal stasis was not observed in the sheep which had received icterogenin in the form of repeated injections.

Microscopic examination of the liver and kidneys revealed only bile pigmentation and mild fatty infiltration of the cells of these organs. Cloudy swelling could be seen in some parenchymal cells of the liver and some of the kidney tubule cells. The lymphoid tissue of the body seemed to be unaffected by the doses of icterogenin which had been given.

GENERAL DISCUSSION

Seawright[14] studied the pathology of *Lantana camara* L. (Verbenaceae) intoxication in sheep and cattle, consequent upon dosing of dried powdered leaf of the plant, and lantadene A (= rehmannic acid). The latter is now known to have no icterogenic properties but preparations of it are usually contaminated with the highly potent icterogenic agent, 22 β-angeloyloxy-oleanolic acid[3]. Seawright[14] found the same absence of significant changes in the liver cells in preparations from his animals but noted kidney lesions in his cases of protracted illness of three weeks' duration. These lesions included massive fatty degeneration and necrosis of the tubular epithelium with occlusion by casts and consequent cystic dilation of the tubules. Such kidneys were associated with terminal uraemia, polyuria and albuminuria. Heikel, Knight, Rimington, Ritchie and Williams[15] found that icterogenin produced no discernible morphological changes in the liver of rabbits which could explain the disturbances in biliary function.

As can be seen from the data presented in this paper, the most obvious effect of icterogenin intoxication is that on the biliary excretion of bilirubin and BSP conjugates, bile acids and porphyrins. If sufficient icterogenin is given, the block in the transfer of these substances into the bile is sudden and complete and of variable duration. The effects of icterogenin, however, are more profound than just a block in biliary excretion of certain compounds. The studies with BSP show a failure on the part of the liver cells to clear the dye from the blood and, what is more, a decreased ability of the kidneys to eliminate the large amounts of the dye present in this fluid. This embarrassment of renal function extends to bilirubin glucuronides, bile acids and possibly phylloerythrin as well, as can be judged by the mildness of bilirubinuria and bileaciduria and the severity of photosensitisation once sufficient levels of porphyrin have been built up in the blood. The block in renal excretion is by no means as severe as that in biliary elimination.

Icterogenin appears to have a definite harmful effect on the erythrocytes of animals dosed with it. This is seen particularly in animals receiving single doses of more than 200 mg/kg and in those receiving repeated small doses of the compound. The evidence for this is the development of hypocythaemic normocytic normochromic anaemia in these cases, the signs of active phagocytosis of de-

generating cells by the markedly increased number of circulating monocytes and the presence of appreciable amounts of bilirubin in the plasma of those cases (although this latter finding may also be due to some interference of bilirubin conjugation). This action of icterogenin is most likely a direct one on the red blood cell membrane.

Evidence has been presented in this paper that, when given in high doses, icterogenin produces definite muscle lesions, which are manifested in the live animal be marked elevations in plasma GOT, PHI, Ald and CPK activity levels. Those changes are not observed with lower dosage levels, but the presence of a distinct hyperglycaemic tendency in many of the cases studied may not be without significance in this regard [2]. Elevations of the plasma enzyme levels mentioned are generally maximal about four days after the dose of icterogenin was given. When uraemia is seen, and it is more frequent in animals receiving high doses of icterogenin, it also appears some time after the compound was given. It seems as if the initial "biochemical" lesion, in the kidneys and muscle membranes at any rate, may progress to a more lethal one if the intoxication is sustained, such factors as anoxia and biliary nephrosis contributing towards the appearance of these secondary changes [2].

The presence of appreciable amounts of bilirubin as well as its glucuronide in the plasma of the experimental animals may indicate the existence of a haemolytic process or may be the result of some disturbance in conjugation as a result of a general decline in energy production in the liver cell as has been found in rabbits [7]. The labile nature of bilirubin glucuronide no doubt accounts for some of this bilirubin as well. Whitehouse, Dean and Halsall [16] have demonstrated that glycerrhetic, fusidic and some related triterpenoids are potent uncouplers of oxidative phosphorylation. The first mentioned compound approaches 2, 4-dinitrophenol in this regard. Uncoupling activity is dependent on the presence of 3-hydroxy, 11-oxo and 30-carboxylic acid groups. Inversion of the configuration at C-18 (D/E ring junction) or replacement of the 11-oxo-12-ene system in ring C by a 9(11), 12-diene system abolishes uncoupling activity. We have demonstrated that although icterogenin appears to have no direct effect on various member enzymes of the mitochondrial respiratory chain, its overall effects are basically similar to those found by Whitehouse *et al* [16]. with glycerrhetic acid.

The biliary excretion of copper is affected in the same way by icterogenin as that of other components of bile. This effect is seen as an increase in liver copper levels, a rapid and sustained increase in the loosely bound copper fraction of plasma which occurs soon after dosing, an increase in red blood cell copper two or three days later and an increase in plasma ceruloplasmin some days after the compound was dosed. These effects have been observed in common bile duct obstruction in the sheep and the role of the ovine red blood cell in the emergency transport of copper has been mentioned [9,17].

The time lag of 24 — 72 hours in the appearance of many of the typical biochemical effects of icterogenin is undoubtedly due to a slow but continuous rate of absorption of the compound from the digestive tract. Large doses prolong the duration of these effects, injected small doses bring about the effects much earlier and in such cases the effects are of short duration.

The action of icterogenin on the various cell membrances, when given in single doses, brings about a syndrome of icterus and photosensitivity, which, if the exposure of the animal to solar radiation is kept minimal, does not appear to constitute a severe stress. Repeated administration of the compound however, may induce a typical Addisonian collapse in electrolyte balance.

It is apparent from these studies and others cited in the text that the main effects of icterogenin are on membrane permeability. The most powerful effects are on the liver cell membranes; those of kidney and muscle cells and erythrocytes are affected to a lesser degree and under special circumstances.

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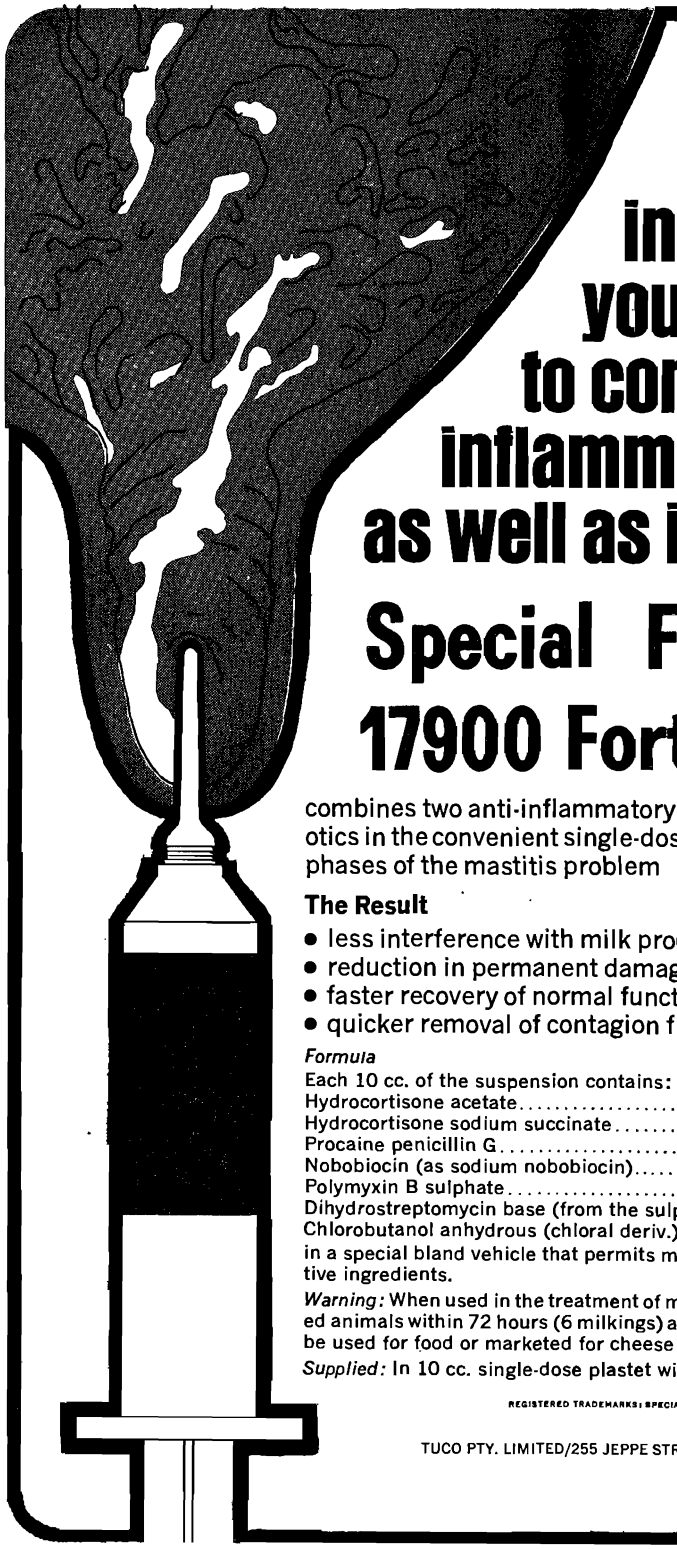

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Jl S. Afr. vet. med. Ass. 39 (3): 51-52 1968

A NOTE ON THE GAMMA-GLOBULIN CONTENT OF THE SERUM OF NEWBORN CALVES

I. S. WARD-COX*

The absence of γ-globulin in mammalian newborn is a wellknown phenomenon. Recently McCoy et al [1] reported the presence of traces of γ-globullin in calf serum immediately after birth. In this laboratory, the sera of six newborn calves were subjected to immunoelectrophoresis immediately after birth and serum from one calf was similarly examined on each of the first four days of life. The results are illustrated, using the enumerations according to Crowle [2].

On the first day (strip 14 of figure) the albumin, α and β zones are well-developed, the major constituents being albumin and β_1-transferrin. α_1 A- and α_1 B-globulins appear in the curvature of the albumin together with the anodic tip of the α_2 M- and α_2-glycoprotein cutting through the antigen wells. A fainter zone, X, appears at the cathodic extremity of the β_1-transferrin zone. The lack of γ-globulin is evident.

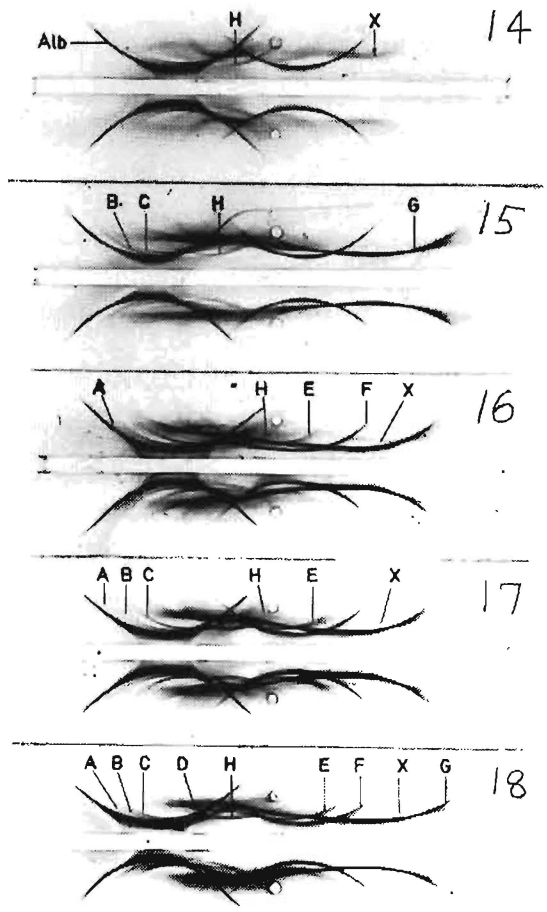
On the second day (strip 15 of figure) the γ-globulin arc is well-developed into the antigen well and then vaguely into the albumin area. Beginnings of the α_1C appear alongside the α_1 B-globulin.

On the third day (strip 16 of figure) the γ-globulin clearly extends into the albumin region and a β-globulin, presumably β_1-lipoprotein, has appeared alongside the β_1 -transferrin arc.

On the fourth day (strip 17 of figure) the α_1C arc has moved anodically to lie near the α_1 A and α_1 B arcs and the β_1-lipoprotein arc has broken away from the γ-globulin to lie near the β_1-transferrin. The α_2 M arc has become indistinct due to the presence of γ-globulin. At 96 hours old the pattern resembles that of the adult (Strip 18).

The zone X is seen to undergo progressive positional changes until it lies parallel to the γ-globulin arc, becoming fainter as it extends progressively towards the cathodic end of the gel. In strip 14, this as yet un-

Constitution of newborn calf serum by immunoelectrophoresis.



Strip 14 At birth.
Strip 15 Twenty-four hours old.
Strip 16 Forty-eight hours old.
Strip 17 Seventy-two hours old.
Strip 18 Ninety-six hours old.
A. α_1A -globulin. B. α_1B -globulin. C. α_1C -globulin. D. α_2M -globulin. E. β_1 -lipoprotein. F β_1 -transferrin. G. γ -globulin.
H. β_1 -glycoprotein. X. Unidentified zone.

* Veterinary Research Institute, P.O. Onderstepoort.

identified fraction could represent the γ-globulin as found by McCoy *et al*[1], although the difference in molecular weight and immunological characteristics reveals that it is not true γ-globulin. As the immunoelectrophoretic picture of adult sera is devoid of this zone, it would appear to be of a foetal nature.

ACKNOWLEDGEMENTS

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BOOK REVIEW

VETERINARY PARASITOLOGY

GEOFFREY LAPAGE

Second Edition, Oliver & Boyd, Edinburgh & London, 1968. pp XLVIII + 1132, Figs. 502. Price £8.8.0.

This very comprehensive textbook is divided into three sections each comprising a book on its own, namely helminthology, entomology and protozoology.

A wealth of information is presented in a systematic manner in each section, stress being laid on the recognition of the parasitic species dealt with, its life history and effects upon the host. Numerous illustrations in support of the text form a very useful addition and the diagrams illustrating life histories in the helminthological and protozoological sections serve a very useful purpose in clarifying these cycles in the mind of the student.

In the section dealing with the phylum Arthropoda the introduction is perhaps more fitting to a textbook on entomology than one on parasitology as practically all the orders, many of which contain entirely non-parasitic species are discussed to the possible detriment of a more detailed discussion of important species within, for example, the order Diptera. The nematoceran genus *Culicoides* which has assumed great importance in many parts of the world is only very briefly discussed and that in relation to Britain only. In like manner the discussion of the non-British genus *Glossina* contains several inaccuracies indicating a rather cursory perusal of the very extensive literature on this group.

The order Acarina and in particular the ticks is dealt with for the most part in relation to the British and European species little or no account being taken of the species responsible for major economic losses in other parts of the world. In the section on protozoology, however, a number of the more important species are listed in relation to the protozoon parasites transmitted by them. An unfortunate omission in this section is the genus *Besnoitia* which has received considerable attention in recent times.

The two appendices, one by T. E. Gibson on the chemotherapy of helminth infections and the other by W. N. Beesley on the application of insecticides and acaricides to the control of arthropod infestations provide excellent supplementary information to that given in the text. So far as this is possible in this era of rapid changes they deal with the latest therapeutic agents available at the time of going to press.

A comprehensive bibliography and index complete what must be regarded as a work covering a very wide field and one which with a few imperfections forms an extremely useful addition in a single volume to present scientific literature.

R. M. du T.

Jl S. Afr. vet. med. Ass. 39 (3): 55-59 1968

TICK INFESTATION OF LIVESTOCK IN NATAL
The Rôle Played by Goats as Reservoirs of the Economically Important Cattle Ticks

MAUREEN K. BAKER AND F. B. W. DUCASSE*

SUMMARY

The incidence of the economically important cattle ticks in Natal and their predilection sites on goats is given. The important rôle played by goats in maintaining tick infestation on stock farms is stressed as also is the importance of including goats in tick control programmes. Control measures based on predilection sites, seasonal variation and known feeding periods are suggested.

INTRODUCTION

During 1965/1966 an intensive tick survey was undertaken in Natal to determine the degree of tick infestation of cattle and the seasonal variation of the important cattle ticks [1]. Part of the survey included the possible rôle played by goats as harbourers of the more important cattle ticks. Although ticks have been recorded from goats by numerous workers [2, 3, 4] no records can be traced of any systematic study of tick infestation of goats.

In this paper the incidence of the various tick species found on goats is recorded as an indication of the importance of goats as alternate or intermediate hosts of cattle ticks.

PROCEDURE

A. Field Collections

Monthly tick collections were made from a goat, randomly selected, on five farms at four different altitude levels in Natal:—

(i) Coastal zone, between O-100 feet. T/A 85.
(ii) Thornveld zone, between 1000-3000 feet. T/A 224 & D. D. Ranch.
(iii) Mistbelt zone between 3000-5000 feet. T/A 56.
(iv) Highveld zone over 5000 feet. T/A 118.

With the exception of D.D. Ranch, all the farms are in Bantu areas where goats are more prevalent.

The tick predilection sites were determined by dividing the goat's body into sixteen collection sites, utilising the same anatomical areas as those for calves (Part 1) [1] with the exception that the dewlap was combined with the neck, and the tail brush with the tail.

Each site was first coarsely combed and then carefully gone over by hand, and the remaining ticks removed by means of forceps. The fine nit-comb used so successfully on the calves, proved to be inadequate on the goat, because its coarser hair.

Apart from the above differences, collections from goats were executed as described for calves [1].

B. Dipping

In accordance with Government regulations, the cattle on all farms, with the exception of the two calves set aside for hand deticking and with the exception of the cattle on T/A 224 and D. D. Ranch, were plunge-dipped in a O.16% arsenical dipwash, at weekly intervals, weather permitting, during summer and fortnightly during the winter months of early June to mid-October. At T/A 224 no stock had been dipped for the past six years. At D. D. Ranch cattle were conscientiously sprayed in an effective spray race at weekly intervals, using a proprietary organophosphate-toxaphene mixture, except for the two selected calves for hand deticking.

No goats were ever dipped on any farm during the period of the survey.

C. Laboratory Examination

The technique of cleaning, sorting, identifying and recording was the same for goats as that described for calves [1].

RESULTS

The total numbers of all ticks collected from the various collection sites on goats are given in Table 1.

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Table 1: TOTAL NUMBER OF TICKS COLLECTED ON DIFFERENT PARTS OF THE BODY OF GOATS

Collection Sites	B. decoloratus	B. microplus	A. hebraeum			R. appendiculatus			R. evertsi		R. tricuspis	R. simus
	All stages	All stages	Adults	Nymphae	Larvae	Adults	Nymphae	Larvae	Adults	Immatures	Adults	Adults
Muzzle	6 1.9%	9 3.4%		1 0.5%	11 1.4%	5 2.8%	147 6.7%	211 10.7%		2 0.1%		
Periorbital zone	14 4.4%	0			26 3.3%	2 1.1%	206 9.4%	159 8.1%				
Head	18 5.7%	16 6.0%			1 0.1%	6 3.4%	67 3.1%	103 5.2%				
Pinna	160 50.8%	40 15.0%			100 12.6%	46 25.7%	1423 65.1%	771 39.1%		94 2.4%		
Ear passage	2 0.6%	20 7.5%			5 0.6%	1 0.6%	75 3.4%	24 1.2%		3766 97.3%		
Poll	16 5.1%	7 2.6%			5 0.6%	113 63.1%	11 0.5%					
Neck	3 1.0%	7 2.6%				2 1.1%	6 0.3%	37 1.9%		1 0.02%		
Axilla	4 1.3%	28 10.5%			6 0.8%			12 0.6%		3 0.1%		
Sternum	9 2.9%	22 8.3%	3 11.5%		9 1.1%			70 3.6%		1 0.02%		
Belly and groin	24 7.6%	56 21.1%	19 73.1%	1 0.5%	20 2.5%		14 0.6%	167 8.5%		4 0.1%		
Lower perineum	1 0.3%	3 1.1%						1 0.05%	2 1.1%			
Upper perineum	2 0.6%	4 1.5%		1 0.5%	1 0.1%	1 0.6%	16 0.7%	4 0.2%	181 98.4%			
Tail		27 10.2%										
Feet	38 12.1%	5 1.9%	4 16.4%	205 94.0%	502 63.3%		210 9.6%	166 8.4%	1 0.5%	1 0.02%	2 —	
Legs	18 5.7%	16 6.0%		10 4.6%	107 13.5%		10 0.5%	218 11.1%			1 —	2 —
Body		6 2.3%				3 1.7%	1 0.04%	28 1.4%				
TOTAL	315 100.0%	266 100.0%	26 100.0%	218 100.1%	793 99.9%	179 100.1%	2186 99.94%	1971 100.05%	184 100.0%	3872 100.06%	3 —	2 —

Table 2: COMPARATIVE TICK YIELDS OF A GOAT AND THE MEAN OF TWO CALVES FROM CONCURRENT COLLECTIONS

FARM	T/A 118		T/A 56		T/A 224		D.D. RANCH		T/A 85	
Species	Goat	Calf	Goat	Calf	Goat	Calf	Goat	Calf	Goat	Calf
B. decoloratus	8	376	64	10,060	238	3,333	5	598	—	—
B. microplus	—	—	—	—	—	—	—	—	266	16,346
A. hebraeum										
Adults	—	—	0	—	26	156	0	—	0	0
Nymphae	—	—	0	—	218	392	0	1	0	1
Larvae	—	—	0	—	790	645	0	—	3	54
R. appendiculatus										
Adults	0	—	47	152	77	184	54	40	1	15
Nymphae	0	1	1,504	1,011	196	304	446	34	40	48
Larvae	0	2	978	1,290	242	1,063	722	327	29	85
R. evertsi										
Adults	5	10	76	158	44	81	27	45	32	37
Immatures	276	54	983	2,351	1,468	1,457	611	1,066	534	1,627
Number of collections	9	9	9	9	10	10	11	7	9	9

The comparative tick yields obtained from goats and the average yield from two calves on each of the five farms under review are recorded in Table 2. In most instances, the infestation on goats rose proportionately with the increase of infestation on the calves. At D. D. Ranch, the burden of *Rhipicephalus appendiculatus* (all stages) was greater on goats than on calves, thus incriminating the goat as an important reservoir for this species on this farm.

Infestations by Individual Tick Species

Boophilus decoloratus (Koch 1844)

As this is a one host tick, all references to the blue tick include all stages of the life cycle. Of the total of 315 specimens collected, there were 107 adults, 112 nymphae and 96 larvae, indicating that *B. decoloratus* will complete its development on goats. This species was found on all four farms above 1000 feet. At T/A 118, in the nine collections made, only eight boophilids were collected in keeping with the low degree of infestation of calves (refer Table 2). The greatest number collected from one goat at one collection at the peak of activity was 73 at T/A 224. The predilection site on goats is apparently the pinna which harboured 51 percent of the total. The greatest tick activity occurred in November and persisted until the end of April. Odd specimens, however, were collected during the remaining months of the year.

Boophilus microplus (Canestrini 1888)

This species was prevalent on the coastal farm of T/A 85 on both cattle and goats. The total number collected was 266, of which 200 were adults, 30 nymphae and 216 larvae. The greatest single collection at the peak of activity yielded 131 ticks of all stages. The predilection site for this species was the belly and groin (21%), followed by the pinna (15%). The period of greatest activity was from November until the end of March.

Amblyomma hebraeum (Koch 1844)

This species was found on only two farms, namely T/A 85 and T/A 224. On the former only three larvae were collected (an equally limited number were collected from calves); the bulk of the ticks were collected on the thornveld farm of T/A 224. No *A. hebraeum* were collected from the thornveld farm, D.D. Ranch: they had been eradicated apparently.

Even though relatively few adults were collected, one can yet conclude that the belly and groin is the predilection site. Adult activity occurred during the months of December until approximately mid-February.

During their respective peak activity periods, a total of 60 nymphae and 375 larvae were collected from one goat at one collection.

The preferred site of attachment for nymphae was indisputably the feet (94%), followed by legs (5%). Nymphae were active from March until mid-September.

Larval predilection sites were feet (63%), legs (14%) and pinna (13%). Larval activity was confined largely to the period from the beginning of February until mid-May. From Table 2 it can be seen that, per animal, a greater number of *A. hebraeum* larvae were collected from goats than from cattle.

Rhipicehalus appendiculatus (Neuman 1901)

This species was found on all four farms below 5000 feet. Above this altitude, at T/A 118, no specimens were found. These findings closely correspond with those for the calves. (Refer Table 2).

Contrary to expectation, the predilection site for the adult, was the poll (63%), followed by the pinna (26%). The greatest single collection yielded 33 adult ticks. The main adult activity occurred from the beginning of January until the end of April.

The bulk of the nymphae were found on the pinna (65%), followed by feet (10%), periorbital zone (9%) and muzzle (7%). A total of 495 nymphae was collected in a single collection from one goat during the peak activity period from mid-March until the end of August.

Larvae were found to be most numerous on the pinna (39%), followed by legs (11%), muzzle (11%), feet (8%) and periorbital zone (8%). During the peak activity period, a total of 463 larvae was recorded at one collection. Larvae were most active from the beginning of February until approximately mid-July.

Rhipicephalus evertsi (Neuman 1897)

This species was encountered on all five farms. The incidence above 5000 feet was relatively low, being five adults and 276 immatures over the entire collecting period, low numbers also being recorded on calves at this altitude. As this is a two host tick, the larval and nymphal stages are referred to as immatures.

The adult stages were found almost exclusively on the upper perineum (98%). A total of 20 adults was collected from one goat at one collecteion during the peak activity period which extended from mid-January until the end of May.

The predilection site for immatures was the ear passage (97%), the pinna (2%) possibly carrying the overflow from the ear passage. The highest single collection of immatures was 488 during the peak activity, which started in early November and receded towards the end of June. Reduced numbers were consistently found throughout the remaining months of the year on all the farms below 5000 feet.

R. simus and *R. tricuspis* were found in negligible numbers.

DISCUSSION

If one considers the egg-laying potential of one female ixodid[5] e.g. *B. decoloratus* (1,000 — 2,500), *A. hebraeum* (up to 18,500), *R. appendiculatus* (300 — 5,700) and *R. evertsi* (5,000 — 7,000), it becomes evident that goats can play an important rôle in maintaining the economically important cattle tick populations, hence any dipping scheme for tick control in cattle, to be fully effective, must, of necessity include goats.

Boophilus spp.

From Table 2 it would appear that goats do not play an appreciable rôle in maintaining boophilids. Where either *B. decoloratus* or *B. microplus* is a problem on cattle, however, the same dipping programme should be applied to goats as to cattle in order to obtain optimum control. As the pinna in the case of both *Boophilus* spp. and the belly and groin in the case of *B. microplus* were the preferred sites of attachment and as both these species are one host ticks, completing their life cycle on the host in 21 — 23 days[5], dipping and hand-dressing of the ears at three-weekly intervals during their peak activity periods should, however, offer an effective alternative control measure.

A. hebraeum

This tick was found only on the Bantu-owned thornveld farm of T/A 224. Prior to the start of this survey, considerable numbers of cattle and goats died from heartwater on the European-owned thornveld farm, D.D. Ranch. As a result of conscientious and consistent dipping of cattle and goats prior to the start of the survey, this tick appears to have been almost entirely eradicated on this farm: only one *A. hebraeum* nymph was collected from a calf and none from the goats during the entire survey period.

Whilst Theiler[2] records that immatures occur mainly on birds, goats can apparently play an important rôle as intermediate hosts for both nymphae and larvae. The adult stages were encountered in relatively low numbers.

As the immature stages were largely confined to the limbs, and larvae engorge in 4 — 7 days and nymphae in 4 — 20 days (Gertrude Theiler — personal communication 1965), the use of a walk-through through at weekly intervals during the respective peak activity periods might prove to be an effective means of controlling this tick by disrupting the life cycle at the immature stages.

R. appendiculatus

All stages of this tick, particularly the immatures, will apparently attach and feed on goats. An attack against any stage of the life cycle, utilising the given predilection sites and respective activity periods, should theoretically, control this species effectively. As all stages are found on the pinna, thorough hand-dressing at weekly intervals during these activity periods, utilising a selected acaricide in an oily or greasy medium, should result in effective control.

R. evertsi

Both adults and immatures were collected in relatively high numbers from goats, thus incriminating the goat as a suitable alternate host for the species.

Strategic hand-dressing, *i.e.* hand-dressing only during the peak activity periods of either the upper perineum in the case of adults, or the ear passage in the case of immatures, should theoretically effectively control this tick. As immatures engorge in 10 — 15 days and the adults in 6 — 10 days (Gertrud Theiler — personal communication 1965), fortnightly hand-dressing of the ear passage possibly offers a more economic means of control than does weekly hand-dressing of the upper perineum against the adults.

Some discrepancies occurred in the seasonal variation of the same tick species e.g. *R. appendiculatus* on goats as compared to cattle. Possibly such slight differences in either the extent and/or the period of activity could be attributed to the differences in grazing habits of the two hosts, goats as browsers being more prone to enter scrub-vegetation.

It is thus seen that goats can be definitely incriminated as harbourers of significant numbers of *A. hebraeum*, *R. appendiculatus*, *R. evertsi* and, to a lesser extent, of *Boophilus* spp., and as reservoirs for reinfestation of cattle. To be effective any tick control or eradication programme directed against these species must thus include goats. As only negligible numbers of any one of the aforementioned tick species are found on the body or neck regions of goats, local spraying, hand-dressing of the ears or upper perineum and/or the use of a walk-through trough possibly offers an adequate means of controlling ticks on goats.

ACKNOWLEDGEMENTS

The Chief, Veterinary Services, is thanked for permission to publish this paper. We wish to thank Dr. Gertrud Theiler of Onderstepoort for her unstinting help and encouragement given for the duration of the Natal tick survey. Our thanks, too, go to all the laboratory and field staff involved in the laborious task of collecting and sorting of ticks.

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A SIGNIFICANT NEW BREEDING SITE OF *CULICOIDES PALLIDIPENNIS* CARTER, INGRAM AND MACFIE (DIPTERA: CERATOPOGONIDAE)

E. M. NEVILL*

Culicoides pallidipennis, the transmitter of bluetongue virus in sheep [1], is the most abundant of all *Culicoides* spp. captured in a suction-light trap at Onderstepoort. On 2nd March, 1967 a record catch of 503,200 *Culicoides* was made in one night by a single trap, 97.4 per cent being *C. pallidipennis*. In spite of these numbers, repeated attempts by several workers have failed to reveal a breeding site where this midge occurs in any numbers [2].

Recent evidence indicated that *C. pallidipennis* requires a more terrestrial breeding place than other species since its pupae are unable to float on water and consequently drown [2]. This pupal behaviour corresponds with that of *C. brevitarsis* Kieffer in Australia, which Cannon and Reye found breeding in dry cow pats [3]. Dry cow pats were therefore gathered at Onderstepoort during October 1967 and kept at 21°C in laboratory emergence cages where they yielded *C. pallidipennis* adults within 24 hours. Subsequent collections have consistently yielded adult *C. pallidipennis*, one pat giving rise to up to 124 midges. The term "dry cow pats" refers to pats with a very hard crust, a dry sponge-like centre, and a moist lower layer about an inch thick in direct contact with damp soil underneath. Frequently fungi are found growing in this moist layer and producing many small mushroom-like fruiting bodies around the edge of the pat.

More recently, pats about three days old with a soft wet light-green interior yielded *C. pallidipennis* after 17 days, while pats about seven days old produced *C. pallidipennis* three or more days after collection. The latter had a dry top layer forming a crust while the lower layers had a very moist dark greenish-brown appearance.

These observations show that *C. pallidipennis* oviposits in dung which is only a few days old. Development of the larvae proceeds concurrently with the desiccation of the pat so that by the time the pupal stage is reached only the lowest layer retains a little moisture, a situation most favourable for this water-shy stage.

Du Toit [4] has shown that cattle play an extremely important role as summer reservoirs of bluetongue, the virus being isolated in one experiment from each member of a group of 25 cattle at Onderstepoort by the 17th January, 1957. He also concluded that *C. pallidipennis* feeds more readily on cattle than on sheep, and this has been substantiated by precipitin tests on *Culicoides* spp. blood-meals (Nevill and Anderson — unpublished data). Since *C. pallidipennis* has now been shown to breed readily in cow dung, the relationship between cattle and this vector must be considered ideal for the establishment and maintenance of bluetongue in an area where cattle and *C. pallidipennis* both occur.

ACKNOWLEDGEMENTS

Dr. Anna Verster is thanked for her criticism of the manuscript.

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*Gibbons, W.J. (1951). Vet. Med., *46*:397.

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THE ANTHELMINTIC EFFICACY OF TETRAMISOLE HYDROCHLORIDE AND PYRANTEL TARTRATE AGAINST THE WIREWORM, *LIBYOSTRONGYLUS DOUGLASSI* OF OSTRICHES.

D. K. Shone*, R. K. Reinecke** and D. Saayman*

SUMMARY

1. Tetramisole hydrochloride at 67.5 mg/kg is 88.8% effective against immature and 99.7% effective against adult *Libyostrongylus douglassi* of the ostrich.
2. A dose of up to 408 mg/kg tetramisole hydrochloride had no adverse effects on young birds but at 529 mg/kg was lethal to both chicks and adult ostriches.
3. The efficacy of pyrantel tartrate was erratic. At 50 — 60 mg/kg it was 72.3% effective against immatures and 50.2% effective against adult worms. At 100 mg/kg it fell to 43.6% against immature, but rose to 77.3% against adult worms.
4. Pyrantel tartrate at 100 mg/kg caused the death of three out of four chicks.
5. The macroscopic lesions of the disease "vrotmaag" caused by *L. douglassi* are described as well as some of the other pathological lesions observed at autopsy.

INTRODUCTION

The only true farming with the ostrich, *Struthio camelus,* in the world is found in the Little Karoo and Eastern Province regions of the Cape Province of South Africa, with the town of Oudtshoorn as the marketing centre.

During the ostrich feather boom, ostriches were farmed over the greater part of the Eastern Province, and many famous breeders were in this area, such as Oscar Evans of Bedford, George White of Port Beaufort and the Honourable Arthur Douglass of Grahamstown. Most of the feathers were auctioned in the Feather Market Hall, in Port Elizabeth.

In 1913 there were 757,000 ostriches in the Cape. The feather market slumped in 1914 and many farmers became insolvent or abandoned ostrich farming; hundreds of thousands of birds were slaughtered and many others were turned loose to roam wild. By 1916 the number of ostriches had been reduced to 379,000 [1].

The slump in ostrich feathers lasted from 1915 to 1945, until virtually the only ostrich farmers were to be found in the Little Karoo. The number of ostriches also dwindled until a nadir was reached in 1960 when the stock census gave the number as 31,662 [2]. Since 1960 the numbers have increased and the latest available figure (1966) is 63,085 [3].

The dry climate of the Little Karoo and Eastern Cape is well suited to the ostrich, whose natural habitat is the arid and semi-desert regions of the African continent. In the Little Karoo the majority of birds are maintained on lucern pastures which are irrigated by flooding. The remaining birds are grazed on the natural veld. Apart from brief periods, some of the lucerne pastures have had ostriches on them for close on 100 years. Usually no drinking water is provided, the birds apparently obtain sufficient moisture from the succulent lucern to meet their needs.

Since 1945 the financial return from ostrich farming has improved and it is once again an economic farming enterprise. The wing plumes are used in the fashions trade, the body feathers turned into dusters, the skin used for the manufacture of hand bags, shoes and other leather goods, and the meat sold locally or processed. Top quality plumes have fetched as much as R52.00 per lb. in recent years.

Libyostrongylus douglassi (Cobbold, 1882) was named in honour of the Honourable Arthur Douglas, of Heatherton Towers, Gra-

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hamstown, who collected the first specimens of the worm. Theiler and Robertson[4] studied the bionomics and described the morphology of the larvae and the adults. Eggs were found to remain viable for as long as two years and the infective larvae survived on grassed soil for up to 17 months. The prepatent period is 36 — 39 days. The *in vivo* and *in vitro* anthelmintic activity of a number of compounds was investigated but none was found to be effective in the treatment of infested birds.

The mortality rate of the ostrich chicks up to the age of eight months is high. No figures have been compiled for these losses but from discussions with farmers, we estimate the number to exceed 50 per cent of chicks hatched. Similarly no statistical data is available regarding the aetiology of these deaths, but it has been assumed that a major cause of these mortalities is "vrotmaag"*, a disease caused by the wireworm, *L. douglassi.*

The proventriculus of every ostrich which dies or is slaughtered, is found to be heavily infested with wireworm. Robertson[5] recovered from 3 to 7 million, ± 5,000, wireworm from each of five ostriches.

The advent of new potent anthelmintics, with in some cases a systemic mode of action, gave stimulus to this work with the hope that one or more of these compounds would be found to be effective against *L. douglassi* and provide a cure for "vrotmaag", a disease which has plagued ostriches for so many years.

This work was undertaken as four separate trials but for the sake of clarity, the anthelmintic efficacy trials are described separately and the data from the toxicity studies combined.

MATERIALS AND METHODS

Trial 1

Ostrich chicks: Twelve ostrich chicks approximately two months of age were purchased from a farmer in the Oudtshoorn district and transported to Cape Town where they were kept on Kikuyu grass during the period of the trial. No ostriches had previously been kept on this ground.

Anthelmintic: Four ostriches were used as untreated controls; four were treated *per os* at a dose of 45 mg tetramisole hydrochloride** per kg bodyweight; three were treated at 90 mg/kg, and one at 135 mg/kg. Doses were based on exact bodyweight and the ostriches were slaughtered 42 hours after treatment.

Worm recovery: The ostriches were slaughtered by decapitation, the proventriculus removed and Shone's waterbath[6,7] was used for the recovery of the worms. The ingesta of the proventriculus was left in the traps for two hours and the glandular portion and mucous membrane of the proventriculus, for three hours. The mucous membrane and glands were subsequently digested in pepsin/HCl for three hours. After incubation at 40°C in the waterbath, filtrates were poured into buckets, iodine was added to kill the worms and the suspension washed through sieves (apertures 53 microns). The residues on the surface of the sieves were washed into labelled jars and preserved with formalin. The worms present in at least three one-tenth aliquots were counted. Total counts were made when less than 500 worms were present in a specimen.

The parasitic stages of *L. douglassi* were identified according to the description of Theiler and Robertson[7].

Trial 2

Ostrich chicks: Thirty-four ostrich chicks of three to four months of age were obtained from a farm in the Oudtshoorn district and transported to the neighbouring district of George, where they grazed for the duration of the trial on pastures which had never previously held ostriches.

Anthelmintics Each ostrich was weighed and dosed orally as follows:-

Tetramisole hydrochloride — Five ostriches at 47 mg/kg and five at 67.5 mg/kg.

Pyrantel tartrate — The powder was placed in capsules and dosed to two ostriches at 50 mg/kg and a further two at 100 mg/kg. The following ostriches were dosed with a solution of pyrantel tartrate in polyethylene glycol*:- One at 50, one at 60, two at 100 and two at 200 mg/kg respectively.

Apart from those deaths due to overdosage, all treated birds were killed three or four days after treatment. Undosed control birds were killed in the interim.

Worm recovery: The method used differed from that of the first trial in that the mucous membrane and glandular portion of the

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* Ripercol — trademark of Janssen Pharmaceutica, Belgium.

* Banminth — trademark of Pfizer Laboratories (Pty.) Ltd.

proventriculus were digested without first inducing migration and sieves used had apertures of 37 microns instead of apertures of 53 microns.

Toxicity trials

Tetramisole: There were two separate trials. The one on the adult ostriches was conducted in the Pretoria area and the tetramisole solution was dosed into the oesophagus through a length of rubber tubing. The trial on the ostrich chicks was conducted at George and the chicks were from the same source as those used in the efficacy trial. The tetramisole solution was administered with a dosing gun.

Pyrantel tartrate: Some of the chicks in the anthelmintic trial at George died(see below)

RESULTS

1. *Anthelmintic efficacy:* Detailed results are presented in Tables 1 and 2. In Trial 2,

Table 1: ANTHELMINTIC EFFICACY OF TETRAMISOLE AGAINST **L. DOUGLASSI** — Trial 1

Dose	Number of ostriches	Bodyweight/kg	NUMBER OF WORMS RECOVERED		
			3rd Stage	4th Stage	Adults
Controls	4	Mean 5.8 Range 5.5 — 6.4	98 23 — 266	4,688 4,020 — 7,473	31,986 27,819 — 42,890
45 mg/kg	4	Mean 5.3 Range 4.5 — 6.8 Average reduction	2 0 — 7 98.0%	406 184 — 623 91.3%	6,464 293 — 14,210 79.8%
90 mg/kg	3	Mean 5.9 Range 5.7 — 6.4 Average reduction	1 0 — 3 99.0%	678 451 — 904 85.5%	380 307 — 450 98.8%
135 mg/kg	1	Average reduction	100%	98.7%	98.8%

Table 2: ANTHELMINTIC EFFICACY OF TETRAMISOLE HYDROCHLORIDE AND PYRANTEL TARTRATE AGAINST **L. DOUGLASSI** — Trial 2

Compound	Dose	Number of ostriches	Bodyweight/kg	NUMBER OF WORMS RECOVERED	
				4th Stage	Adults
Controls	—	9	Mean Not recorded Range Not recorded	905 363 — 1,270	15,082 6,893 — 32,697
Tetramisole hydrochloride	47 mg/kg	5	Mean 12.7 Range 9.1 — 15.9 Average reduction	147 48 — 239 83.8%	67 31 — 126 99.6%
	67.5 mg/kg	5	Mean 10.4 Range 6.8 — 13.6 Average reduction	101 48 — 157 88.8%	41 15 — 99 99.7%
Pyrantel tartrate	50 mg/kg 60 mg/kg	3 1	Mean 10.6 Range 7.5 — 12.5 Average reduction	251 203 — 327 72.3%	7,513 3,412 — 10,221 50.2%
	100 mg/kg	1	12.5 Average reduction	510 43.6%	3,417 77.3%

the number of 3rd stage parasitic larvae recovered was negligible and has been omitted.

These results are summarised below. The anthelmintic efficacy is expressed as the percentage reduction of the mean number of worms recovered from the treated ostriches compared with the mean number of worms recovered from untreated ostriches.

	Percentage Reduction Stage of Development		
	3rd Stage	4th Stage	Adults*
Tetramisole hydrochloride			
45 mg/kg Trial 1	98.0	91.3	79.8
47 mg/kg Trial 2	—	83.8	99.6
67.5 mg/kg Trial 2	—	88.8	99.7
90.0 mg/kg Trial 1	99.0	85.5	98.8
135.0 mg/kg Trial 1	100.0	98.7	98.8
Pyrantel tartrate			
50 & 60 mg/kg Trial 2	—	72.3	50.2
**100 mg/kg Trial 2	—	43.6	77.3

2. *Recovery of the worms:* In Trial 1, a total of 142,661 worms were recovered from the proventriculus wall, and only 4,424 or 3% of the total, from the ingesta of the four control birds. In Trial 2, a total of 141,327 worms was recovered from the proventriculus wall of nine birds and 2,563, or less than 2% of the total, from the ingesta. Careful removal and discarding of the ingesta, therefore, will have no significant effect on the number of worms recovered at autopsies. The thick mucus covering the mucous membrane of the proventriculus of infested birds, within and under which many of the worms are to be found, interferes with the migration of the helminths through the nylon mesh into the trap in the waterbath. Numerous worms were observed to be trapped in the mucus after three hours incubation. In Trial 1, only 5,199 of 18,888, 3rd and 4th stage larvae had migrated, while only 24,050 of 123,773 adults had migrated. Subsequent digestion of the proventriculus released these worms.

3. *Macroscopic lesions observed at autopsy* The ostrich has no crop and the oesophagous opens directly into the proventriculus. The proventriculus has a glandular portion and numerous immature and adult worms penetrate into these glands as well as into the mucous membrane. Numerous worms are also present on the surface of the mucous membrane of the proventiculus.

In infested ostriches the glands of the proventriculus are swollen, brick red in colour and a thick tenacious mucus is secreted. The mucous membrane is covered with a necrotic membrane, grey in colour.

After successful therapy with tetramisole, the glands of the proventriculus were reduced in size, the necrotic membrane was absent, the mucous membrane assumed a far healthier appearance and the thick tenacious mucus disappeared. Lesions indirectly attributable to the wireworm infestations were also observed. The damage to the wall of the proventriculus caused by the worms appeared to make it much more susceptible to damage by penetrating objects and in several cases seed heads, awns and other objects were found embedded in the wall.

Ulceration of the wall of the proventriculus was observed in several ostriches and in one the wall was perforated.

In several birds, small greyish-yellow necrotic foci were observed disseminated throughout the liver parenchyma. It is postulated that these are probably caused by bacteria which had gained entrance to the body through lesions in the proventriculus wall and had become trapped in the liver.

Toxicity Studies

Tetramisole hydrochloride: Details of the quantities of tetramisole hydrochloride administered to ostriches of various ages and the sequelae, are presented in Table 3. It will be noted that none of the birds, which were dosed at the rate of 408 mg/kg or less, showed any symptoms.

* The fifth stage is clasified with the sexually mature helminths as adults.

** Only one bird dosed at 100 mg/kg survived. The remainder of the ostriches dosed with pyrantel tartrate at 100 mg/kg and 200 mg/kg died from the effect of the drug.

Table 3: TOXICITY STUDIES ON OSTRICHES DOSED WITH TETRAMISOLE HYDROCHLORIDE

Ostrich	Body-weight/kg	Dose mg/kg	Outcome
3—4 month chick	13.6	136(1)	no effect
3—4 month chick	9.1	136	" "
Adult cock	81.8	185	" "
Adult hen	62.3	185	" "
Adult hen	72.7	185	" "
3—4 month chick	11.4	270	" "
3—4 month chick	11.4	270	" "
3—4 month chick	13.6	408	" "
3—4 month chick	13.6	408	" "
Adult cock	67.3	529	died
3—4 month chick	13.6	557(1)	died

(1) Same ostrich, second dose administered 4 days after the first.

The total number of ostriches is small but based on these findings the safety index of tetramisole hydrochloride is not less than six, if the therapeutic dose is taken as 66 mg/kg. *Pyrantel tartrate:* The ostriches dosed at the rate of 200 mg/kg and 3 of the 4 dosed at the rate of 100 mg/kg died within an hour of administration. Symptoms observed were: staggering, sternal recumbence and severe opisthotonos with the neck eventually forming a complete circle. The symptoms are probably not pathognomonic for pyrantel tartrate poisoning, as old photographs of ostriches poisoned with carbolic acid show birds in similar attitudes.

No macroscopic lesions were observed at autopsy.

DISCUSSION

Ever since the wireworm, *Libyostrongylus douglassi,* was decribed by Cobbold in 1882 and the recognition of the rôle played by this helminth as the cause of the disease "vrotmaag" of ostriches, attempts have been made by both scientific workers and farmers to find a cure, but to date apparently only tetramisole possesses the requirements of efficacy and safety to fill this role. Robertson [5], and Theiler and Robertson [4] failed to find an effective drug. It is known that other anthelmintics, including phenothiazine and the organo-phosphates, have been tried but were apparently ineffective, for the results were never published. The efficacy of pyrantel-tartrate varies; moreover it is too toxic to be advocated for general use.

Theiler and Robertson [4] described a method of rearing ostriches free of wireworm by maintaining them either on concrete floors or by regular removal of the faeces from the pens. This method is not practical for the ostrich farmer today, and the feather slump precluded its use at that time.

The immense burdens of wireworms invariably found in all dead ostriches has in the past served to blanket the possibility of other disease entities. The discovery that tetramisole is highly effective against *L. douglassi* should enable investigations to be pursued into the aetiology of other diseases of ostriches which undoubtedly occur.

The massive infestations of wireworm which have built up over the past years and the resistance of the eggs and 3rd stage infective larvae against desiccation means that the task of reducing the infestations to reasonable numbers will be no easy task.

Improved feeding and animal husbandry practices such as the pregrazing of pastures with non-susceptible cattle and sheep, combined with regular treatment with tetramisole hydrochloride, will undoubtedly make the task easier.

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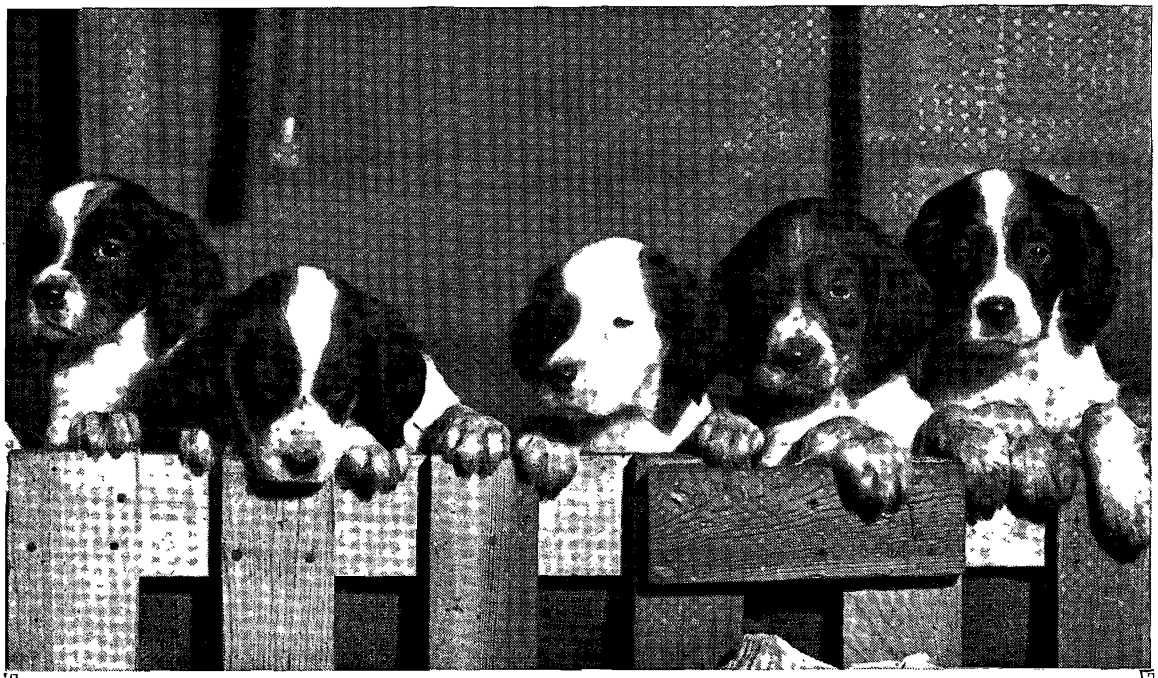

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BLASTOMYCOTIC MASTITIS IN SOUTH AFRICA

W. H. GIESECKE*, ELLEN E. NEL** AND L. W. VAN DEN HEEVER***

SUMMARY

Attention is drawn to the existence of blastomycotic mastitis in South African dairy herds. Twenty different yeasts were isolated from cases of mastitis in three herds. The yeasts were isolated in pure culture and concurrently with common bacterial mastitogens. Strains of *Staphylococcus aureus* isolated from yeast-infected quarters exhibited a higher incidence of antibiotic resistance than those obtained from other quarters in the same herds.

Data relevant to the problem, based on clinical, bacteriological, and mycological examinations, are tabulated and the literature is reviewed.

INTRODUCTION

The discovery of various antibiotic substances [1] inevitably led to their extensive use in mastitis therapy [2-4]. Subsequently certain significant changes were reported in the relative frequency of the various micro-organisms causing mastitis in herds under regular control [5-19]. Reports indicate that the incidence and importance of *Streptococcus agalactiae* as a major mastitogenic agent seem to be diminishing in favour of other streptococci, staphylococci and other species [9, 10, 15, 19-46]. Generally, it appears that although an increased realisation of the importance of mastitis has resulted in a steadily increasing variety of mastitogenic agents being detected [36, 47-51], the elimination of antibiotic sensitive species originally responsible for most cases of the disease has resulted in an increase in the incidence of mastitis caused by partially or completely resistant microbial agents [14, 52]. If such development can take place where herds are under professional mastitis control, these tendencies are likely to be intensified in dairy herds where protracted and intensive, but completely irrational and professionally uncontrolled mastitis thereapy is applied, and the incidence of drug resistant mastitogenic micro-organisms will be correspondingly high.

In South Africa most mastitis remedies are freely available to stock owners and conditions appear to be favourable for the development of drug resistance and the increase of mastitis due to antibiotic refractory micro-organisms such as yeasts and yeast-like organisms (YLO's). This view is supported in part by a review of the existing literature on the subject. An investigation into the incidence and type of this form of mastitis was instituted, and the data obtained are presented and discussed.

LITERATURE

Even before the antibiotic era, yeasts and YLO's were demonstrated in milk sediment — by injection of laboratory animals — in the mammary gland, and as a cause of mastitis [53-58]. Subsequently, an increasing number of mastitis cases caused by a variety of yeasts and YLO's have been reported [59-127]. A summary of reported clinical and experimentally produced cases is furnished in Table 1.

Prior to the intensive and widespread use of antibiotics, yeasts and YLO's were considered to be of minor importance as mastitogens. The increasing frequency with which these organisms have been isolated from abnormal mammary secretions in recent years may be associated with certain side effects which have been observed in both medical and veterinary fields after administration of various antibiotics. These have been listed as:

1. local tissue reactions and/or sensitization and/or predisposition [82, 83, 128-132];
2. reduction or elimination of sensitive organisms followed by an increase in the resistance of surviving flora [133, 134], the dis-

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Table 1: SUMMARY OF YEASTS AND FUNGI RELATED TO BOVINE MASTITIS

SPECIES AND LITERATURE REFERENCES
Cryptococcus neoformans [59 - 71]
Candida pelliculosa [59, 72]
C. tropicalis [69, 70, 72 - 83]
C. albicans
C. catenulata [76, 89]
C. guilliermondii [69, 70, 76, 79, 84, 90, 91 - 94]
C. krusei [70, 76, 78, 83, 84, 87, 89 - 94]
C. mesenterica [70, 76, 95]
C. mycoderma [76, 96]
C. pulcherrima [70, 76, 90, 94, 97]
C. rugosa [69, 70, 73, 76, 88]
C. solani [76,85,89]
C. norvegensis/zeylanoides [69, 70, 73, 76, 84, 94]
C. parapsilosis [69, 70, 73, 74, 78, 82, 84, 88, 94, 99, 100, 101]
C. pseudotropicalis [70, 82, 88, 98, 99, 101]
C. humicola [82, 89]
C. utilis [89]
C. brumptii [89]
C. tenuis [70, 78, 79]
C. curvata [89]
C. lusitenae [70]
C. robusta [70]
C. membranaefaciens [85]
Pichia farinosa [69, 70, 76, 84, 102]
Pichia membranaefaciens [70]
Trichosporon granulosum [103]
T. pullulans [89]
T. cutaneum [69, 70, 73, 89, 103, 104]
Saccharomyces fragilis [69, 70, 72, 78, 82, 85, 90, 105, 106]
S. marxianus [78]
S. chevalieri [70]
Hansenula anomala [69, 70, 76, 85]
H. angusta [76]
Torulopsis candida [70, 76, 82, 89]
T. famata [76, 89, 94]
T. sake [94]
T. glabrata [83, 89, 94]
T. aeria [89]
T. inconspicua [89]
T. pseudaeria [89]
T. globosa [70]
Geotrichium candidum [107]
Prototheca zopfii [108]
Rhodotorula mucilaginosa [89, 94]
R. glutinis [82]
Sporobolomyces salmonicalor [89]
S. roseus [76]
Aspergillus fumigatus [109]
Nocardia asteroides [110 - 124]

turbance of microbial equilibrium resulting in increase of the less sensitive survivors [89, 135-146];

3. a decrease of the natural individual defence machanism of the host after antibiotic administration [128, 147-166] or deleterious effect on defence mechanisms (phagocytosis and antibody production [167]);

4. the creation of hypovitaminosis by prolonged administration of antibiotics, particularly regarding Vitamin A [85, 152, 153];

5. directly promoting the growth of yeasts eliciting blastomycotic disease [59, 64, 66, 73, 77, 78, 80, 82, 85, 89, 94, 101, 102, 105, 106, 152, 170-184].

It appears, however, that locally administered antibiotic mastitis remedies cannot be held solely responsible for the increasing appearance of blastomycotic forms of mastitis, and factors such as an increased exposure to mechanical and chemical trauma by improper milking techniques, disinfectants and various udder infusions should not be neglected. As microbial inhabitants of the *ductus papillaris* cannot be removed prior to intramammary administration of remedies therapy by udder infusion may lead to super infection [185]. This possibility is accentuated by the prevalence of micro-organisms which survive prolonged and excessive mastitis treatment or overdisinfection of the teats with selective and highly concentrated disinfectants.

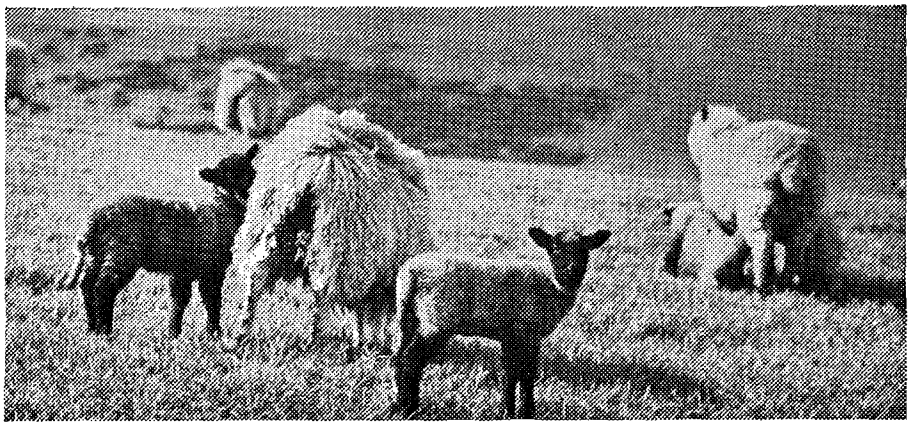
Many yeasts and YLO's, including the most pathogenic one *Cryptococcus neoformans* are ubiquitous in the environment of cattle and in the natural tracts of the animals [91, 93, 101, 184, 186-208].

Among the various species described, *Hansenula anomala, H. angusta, Candida humicola, C. mesenterica, C. rugosa* and *C. catenulata* are generally not considered pathogenic [66, 73, 82, 85, 91]. Some of these, as well as *Candida curvata* and *Trichosporon pullulans* seem to be tolerated in the mammary gland and are then excreted within a short time after having entered it without having caused any harm. Other organisms such as *Candida krusei, C. tropicalis, C. parapsilosis* and *C. pseudotropicalis* are able to establish themselves in the epithelial crypts, spreading from there into the glandular tissue after antibiotic infusions [89, 209, 210]. Where apparently "harmless" organisms have been described as mastitogenic, the inherent or acquired susceptibility (under medication) of the udder appears to be implicit. Considerable variations in susceptibility have been recorded in experiments with various yeasts. Infusion of cultures of *Candida albicans, C. solani, C. krusei, C. membranaefaciens, Hansenula anomala, Pichia farinosa, Torulopsis candida, Geotrichium candidum* and *Rhodutorula mucilaginosa* into the udder caused no symptoms [66, 72]. On the other hand varying degrees of mastitis have been produced by *Cryptococcus neoformans, Candida tropicalis, C. pelliculosa, C. krusei, C. parapsilosis, C. pseudotropicalis, C. albicans* and *Saccharomyces fragilis* [66, 72, 75, 82, 89, 103, 106, 211, 212].

Establishment of infection, usually via the *ductus papillaris,* may be aided by certain characteristics of the oganisms such as growth at body temperatures of mammals, anaerobic carbohydrate metabolism, formation of pseudomycelia, resistance of vegetative forms against heat, acids, and enzymes, utilization of the nitrogen in some antibiotics, and pellicle formation on surfaces of dairy equipment or milkers hands [210, 213, 218]. Micro-organisms, whose optimal temperature and nutrient requirements in the laboratory differ considerably from the normal temperature in the udder and the constituents available in milk, may find suitable and compensating conditions in the diseased udder which enable them to become mastitogenic.

Mastitis caused by yeasts and YLO's has been reported as a primary infection [57, 60, 65, 66, 70, 75, 78, 79, 87, 94, 101, 103, 105, 106, 108, 121, 200, 211, 219-221]. and as secondary pathogens following the use of contaminated therapeutic substances or equipment when administering mastitis remedies [59, 60, 64, 77, 80, 87, 91, 94, 95, 101, 102, 105, 106, 122, 173, 182, 211, 219-223].

The diagnosis of mastitis caused by yeasts and YLO's is not always readily accomplished. Only mastitis caused by *Cryptococcus neoformans* exhibits a distinct symptomatology. As blastomycotic mastitis is uncommon, clinical diagnosis is unlikely, although in some cases it may be suspected, particularly if there is a history of previous antibiotic medication. Furthermore, diagnostic difficulties are increased where yeasts have been introduced into the udder in the course of treatment originally directed against other micro-organisms. In such cases, the clinical



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symptoms of the secondary yeast mastitis and those of the original form of mastitis are usually inextricable. The laboratory investigation of such cases becomes incomplete unless special media, capable of supporting the growth of yeasts, are used, as the slow growth of yeasts on the usual media such as blood-agar and over-growth by the more common bacterial agents readily lead to inaccurate conclusions. In our experience, the efficiency of the rather difficult and tedious microscopic demonstration of yeasts in the secretion from the less obvious cases of yeast mastitis is increased if preceded by successful cultural demonstration.

The problem of efficient therapy of blastomycotic mastitis still requires to be solved when viewed in the light of *in vitro* and *in vivo* tests [60, 63-65, 77, 82, 85, 90, 96, 181, 212, 219, 224-231].

Mastitis caused by yeasts and YLO's is of particular importance not only from the clinical point of view but also because of public health considerations, as some organisms may survive pasteurisation and many of them have been described in systemic or localised mycotic disease in both man and animals [76, 213, 214, 216, 232-252].

MATERIAL AND METHODS

In the course of investigating the mastitis problems of certain herds three herds were encountered which presented rather similar histories. Mastitis, in its usual clinical form, had occurred periodically for some years but the herd owners had managed to deal with it with apparent success, by administration of various registered remedies. Later, treatment became less successful despite eventual use of practically all the available remedies. Both acute and chronic "incurable" cases resulted in loss of quarters, lowered milk production, culled cows and formidable drug accounts. Only at this stage was veterinary advice sought. This report represents the results of examination of the herds (A, B & C) concerned.

The quarters, teats and supramammary lymph-nodes of all the cows in the three herds were subjected to detailed clinical examination. Milk sampling was carried out after disinfecting the udder and vigorously swabbing the teat tips and particularly the teat orifices with a pledget of cotton wool moistened with 70% alcohol. After discarding the first three jets of milk, foremilk quarter samples were aseptically taken into clean sterile, McCartney bottles held at an angle to prevent the entry of extraneous matter. Laboratory examination consisted of plating milk sediment onto tryptose-blood-agar [253], Sabouraud-agar [253] and Wickerham-stock-culture-medium [254]. Cultures of yeast-like organisms on blood tryptose agar after 48 hours incubation at 37°C, and on selective media after seven days at 30°C, were picked off for preliminary microscopic identification before transfer onto Bacto-yeast-morphology-agar [253] for storage pending final identification.

Leukocyte counts were established from Prescott-Breed smears of whole milk, and microbiological-cytological examination on smears of milk sediment was executed. Antibiotic sensitivity of bacterial isolates was assessed by means of high level sensitivity discs on tryptose-blood-agar.

For the purpose of evaluation of results, udders or quarters were classified according to bacteriological-cytological standards as follows:

(a) *Healthy:* secretion free of mastitogenic organisms and containing up to 250,000 leucokytes/ml.

(b) *Aseptic diseased* (possible covert infections remaining undetected): secretion free of mastitogenic organisms and containing over 300,000 leukocytes/ml.

(c) *Suspicious* (chronic cases, or sample possibly contaminated): secretion containing mastitogenic organisms and as many as 250,000 leukocytes/ml.

(d) *Slightly diseased* (chronic cases, or possibly aseptic diseased but sample contaminated): secretion containing mastitogenic organisms and 300,000 — 500,000 leukocytes/ml.

(e) *Severely diseased:* secretion containing mastitogenic organisms and over 500,000 leukocytes/ml.

The yeasts were identified according to the standard methods of Lodder and Kreger van Rij (1952) [214], Seeliger (1956) [255], van der

Table 2(A): DISTRIBUTION OF RESULTS OF LABORATORY EXAMINATION OF QUARTER MILK SAMPLES FROM DRY COWS IN THREE PROBLEM HERDS

Herd	Examined		Healthy		Diseased	
	U.	Q.	U.	Q.	U.	Q.
A	27	103	9	66	18	37
B	6	22	2	11	4	11
C	11	44	6	28	5	16
Total	44	169	17	105	27	64
Percentage	100	100	38.6	62.1	61.4	37.9

U. = Udders; Q. = Quarters

Table 2(B): DISTRIBUTION OF RESULTS OF LABORATORY EXAMINATION OF QUARTER MILK SAMPLES FROM LACTATING COWS IN THREE PROBLEM HERDS

Herd	Examined		Healthy		Aseptic Diseased		Suspicious		Slightly Diseased		Severely Diseased	
	U.	Q.	U.	Q.	U.	Q.	U.	Q.	U.	Q.	U.	Q.
A	83	326	19	129	13	83	7	19	5	14	39	81
B	26	102	2	15	1	28	—	5	—	4	23	50
C	51	199	3	36	3	36	10	51	6	17	29	59
Total	160	627	24	180	17	147	17	75	11	35	91	190
Percentage	100	100	15	28.7	10.6	23.4	10.6	11.09	6.9	5.5	56.9	30.5

U. = Udders; Q. = Quarters

Walt (1962) [256], and Kreger van Rij (1964) [257] by the *Microbiological Research Group* of the South African Council for Scientific and Industrial Research.

RESULTS

Ignoring 20 "blind" quarters in 12 lactating and four dry pregnant cows, the state of udder health of all the cows in the three herds concerned is summarized in Tables 2 (A) and 2(B).

A classification of the micro-organisms grown in pure or mixed cultures after primary inoculation with the secretion of dry quarters and the milk sediment of lactating quarters, is given in Tables 3(A) and 3(B).

Table 3(A): MICRO-ORGANISMS ISOLATED FROM DISEASED UDDERS AND QUARTERS OF DRY COWS IN THREE PROBLEM HERDS. (See Table 2(A))

Micro-organisms isolated	Diseased	
	U.	Q.
Staphylococcus aureus	7	17
Streptococcus agalactiae	7	24
Streptococcus dysgalactiae	2	7
Corynebacterium pyogenes	2	3
S. aureus + Sc. agalactiae	7	10
S. aureus + Sc. dysgalactiae	1	2
S. aureus + C. pyogenes	1	1

U. = Udders; Q. = Quarters

Table 3(B): MICRO-ORGANISMS ISOLATED FROM SUSPICIOUS OR DISEASED UDDERS/QUARTERS OF LACTATING COWS IN THREE PROBLEM HERDS. (See Table 2(B))

Micro-organisms isolated	STATUS					
	Suspicious		Slightly diseased		Severely diseased	
	U.	Q.	U.	Q.	U.	Q.
Staphylococcus aureus	10	48	5	23	25	70
Streptococcus agalactiae		2		4	4	25
Streptococcus dysgalactiae	3	8				
Streptococcus uberis	2	5				
Corynebacterium pyogenes						1
Corynebacterium bovis	2	5		1	1	1
Yeast-like-organisms (Y.L.O.)				2	6	33
S. aureus + Y.L.O.		6	4	3	26	29
S. aureus + Sc. agalactiae			1	1	12	9
S. aureus + Sc. agalactiae + Y.L.O.			1	1	8	3
S. agalactiae + Y.L.O.					2	11
Sc. dysgalactiae + Y.L.O.		1				3
Sc. uberis + Y.L.O.						1
S. aureus + Sc. dysgalactiae + Y.L.O.					3	1
S. aureus + Sc. uberis + Y.L.O.					1	
S. aureus + Sc. uberis					1	2
S. aureus + Sc. agalactiae + C. pyogenes					2	
Sc. agalactiae + C. pyogenes						1

U. = Udders; Q. = Quarters

Table 4: DRUG SENSITIVITY OF **STAPHYLOCUCCUS AUREUS** AND STREPTOCOCCUS ISOLATES FROM YEAST-FREE SEVERELY DISEASED QUARTERS

Therapeutic substance	**S. aureus** (95)			Streptococci (30)		
	sensitive	partly sensitive	resistant	sensitive	partly sensitive	resistant
Chlortetracycline (50 mcg)	93.5%	2.8%	3.7%	98.2%	1.8%	—
Chloramphenicol (50 mcg)	98.1%	1.4%	0.5%	98.5%	1.5%	—
Furazolidone (100 mcg)	91.9%	6.8%	1.3%	69.1%	17.5%	13.4%
Penicillin (5 units)	51.2%	6.5%	42.3%	91.0%	2.3%	6.7%
Streptomycin (25 mcg)	68.4%	4.1%	27.5%	12.3%	9.1%	78.6%
Oxytetracycline (50 mcg)	86.3%	8.9%	4.8%	99.1%	0.9%	—

Together with the antibiograms for *S. aureus* and mastitis streptococci isolates as shown in Table 4, the results reflect the efficiency of mastitis control and therapy as performed by the dairy farmers themselves. The number of mixed infections and drug resistant micro-organisms found in these herds furnish some indication of therapeutic difficulties which result from such medication.

Therapeutic problems arise particularly in cases of mastitis caused by yeasts and yeast-like organisms either in pure culture or mixed infections. These micro-organisms were isolated from suspicious and slightly diseased and in considerable numbers from severely diseased lactating quarters.

Specific identification of yeasts was only undertaken in a limited number of isolates which were all selected from severely diseased quarters of lactating cows. The identifications are detailed in Table 5.

Table 5: YEASTS ISOLATED FROM MILK SAMPLES FROM 77 SEVERELY DISEASED QUARTERS OF COWS IN THREE PROBLEM HERDS; DETAILS OF RELATIVE DATA

Species	Number of isolates	Cell content/ml milk from yeast infected quarters (in thousands)		PRIMARY CULTURES														Microscopy: Yeast cells visible in milk sediment:						HERD		
		Mean	Range	Pure	Mixed with bacteria:							Mixed with other yeasts:						Pure cultures			Mixed cultures					
					S. aureus	Sc. agalactiae	Sc. dysgalactiae	Sc. uberis	S. aureus + Sc. agalactiae	S. aureus + Sc. dysgalactiae	S. aureus + Sc. uberis	Cryptococcus albidus	Candida tropicalis	Rholotorula mucilaginosa	Rhodotorula glutinis	Rhodotorula graminis	Kloeckera apiculata	+	±	−	+	±	−	A	B	C
Cryptococcus albidus	17		1650 -uc	4	7	2	2			1			1	2	1	1	1	4	—	—	5	3	5		1	16
Candida tropicalis	8		1400 -uc	—	3	2	1		1		1	1						—	—	—	1	1	6	6		2
C. albicans	2	1150 ± 200		1	1													1	—	—	—	1	1		2	
C. guilliermondii	5	1950 ± 200		2	2	1												2	—	—	—	1	2			5
C. krusei	7		1700 -uc	6	1													2	—	4	—	—	1	7		
C. rugosa	2	uc		—	2													—	—	—	—	—	2		2	
C. norvegensis	1	900		1														—	—	1	—	—	—			1
C. zeylanoides	4	4100 ± 3500		—		3	1											—	—	—	1	—	3			4
C. parapsilosis	10		1400 -uc	2	5		1	1		1								2	—	—	2	—	6		9	1
C. utilis	1	3350		—	1													—	—	—	—	—	1	1		
C. lusitaniae	4	1880 ± 200		2	2													2	—	—	—	—	2	2		1
Candida sp.*	1	4150		1														1	—	—	—	—	—			1
Trichosporon beigelii	3	1700 ± 450		1	2													—	—	1	1	—	1	1	1	1
Hansenula anomala	2	uc		—	1				1									—	—	—	1	1	—			2
Prototheca sp.	1	2750		—					1									—	—	—	1	—	—			1
Rhodotorula mucilaginosa	5	1900 ± 550		1	3	1						2						—	—	1	—	1	3			5
R. glutinis	1	1750		—	1													—	—	—	1	—	—			1
R. graminis	1	uc		—								1						—	—	—	1	—	—			1
Kloeckera apiculata	1	3350		—		1												—	—	—	—	—	1			1
Aureobasidium pullulans	1	1050		—		1												—	—	—	—	—	1			1

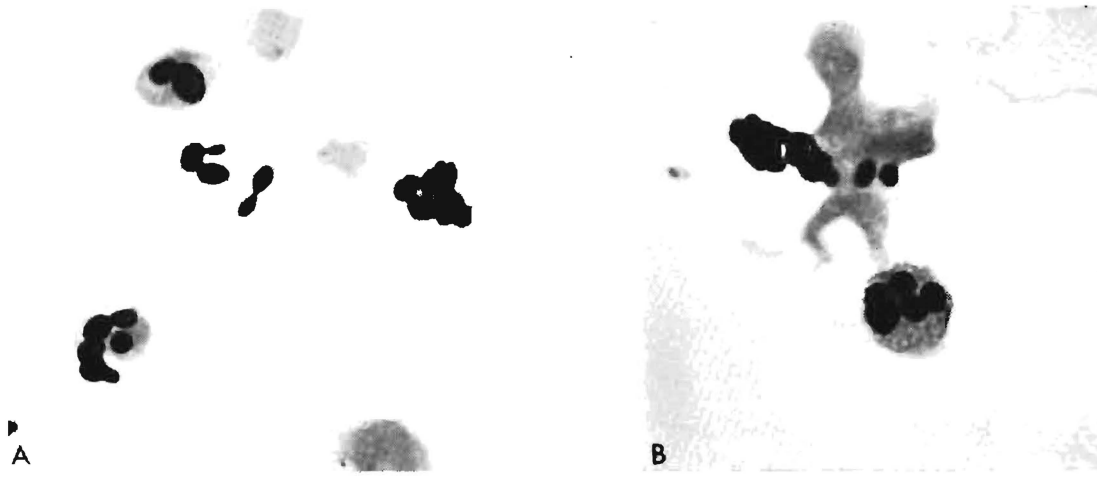
* Will be described elsewhere.

uc: innumerable

Yeasts were isolated from 77 quarters as shown above, whereas 15 out of a total of 92 severely diseased quarters yielded pure or mixed infections of *S. aureus, Sc. agalactiae* and *Sc. dysgalactiae.*

The results given in Tables 3(B) and 5 indicate that yeasts associated with mastitis were frequently isolated in pure culture, they were more often associated in mixed infections with either bacteria, particularly *S. aureus*, or other yeasts. Furthermore, it is apparent that the different quarters of a cow may yield the same yeast in pure or mixed culture, or, on the other hand, that different yeasts species may be present in each of the four quarters. Regarding the yeasts on a particular farm, it can be seen from Table 5 that *Candida tropicalis* and *C. krusei were* dominant on farm A, *C. parapsilosis occurred* frequently on farm B, and *Cryptococcus albidus, Candida guilliermondii, C. zeylanoides* and *Rhodotorula mucilaginosa* were most prevalent on farm C, other species appearing less frequently. The close association of the yeasts to mastitis was confirmed microscopically in 60.8 per cent where yeasts were isolated in pure culture and in 25.0 per cent where the yeasts were mixed with bacteria.

The microscopic visibility of yeasts in milk is apparently dependent not so much on the numbers of organisms present in the milk but more on the microscopic appearance of the sediment smear and the staining method. In sediment slides stained with



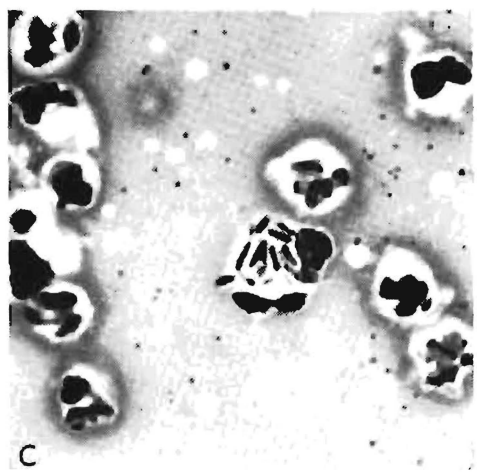


Fig. 1: Microphoto of milk sediment from quarter infected with yeasts (**Candida guilliermondii**):-

A. Typical appearance of yeast cells (PAS Stain)

B. Yeasts clearly phagocytosed by granulocyte (PAS stain)

C. Yeast cells closely associated with polymorphonuclear leukocytes (Giemsa stain)

Giemsa, the presence of many densely clumped somatic cells rendered demonstration of yeast cells almost impossible, or the structures observed could not be identified as yeast cells with certainty. In 10 mycologically positive cases with innumerable somatic cells in the sediment smears, yeasts could be confirmed microscopically in only two, a further two being suspicious. In 60 cases presenting lower cytological densities, the diagnosis could be confirmed microscopically in 26 instances and three were clearly suspicious. Periodic acid Schiff staining renders the yeasts visible and leukocytes rather inconspicuous. (See Figure).

In view of the numerous occasions on which yeasts and *S. aureus* and/or streptococci were isolated in mixed culture, it was desirable to obtain information concerning the drug sensitivity of such mixed infections. The results of antibiograms executed on strains of *S. aureus* and mastitis streptococci which were isolated simultaneously with yeasts are summarized in Table 6.

Comparison of this data with that of Table 4 indicates clearly that the number of resistant strains of *S. aureus* isolated from quarters with concomitant yeasts infections is significantly higher than is the case with *S. aureus* isolates from quarters free of yeast infections. Mastitis streptococci did not show any altered resistance pattern. The prognosis for successful therapy of such mixed *S. aureus* — yeast infections of the mammary gland would thus appear to be rather poor.

CONCLUSIONS

As shown in Table 3(B) yeasts were isolated from milk samples having normal, slightly increased or distinctly increased somatic cell counts. As contamination of milk samples with various micro-organisms may easily occur and successive confirmatory examinations were not undertaken, specific identification was restricted to those yeasts isolated from distinctly diseased quarters, where the risk of incorrect diagnoses has been shown to be considerably lower [258]. The identification of the yeasts yielded a variety of species, the majority of which have previously been described as being mastitogenic under certain conditions. These conditions do occur in many South African dairy herds and thus create a potential source of mastitogens which are able to complicate the existing difficulties inherent in the uncontrolled haphazardous approach to mastitis. This applies particularly to mastitis caused by drug resistant *S. aureus* and to streptococci rendered inaccessible by pathologic changes in the udder. Such forms of mastitis are freguently exposed to prolonged, excessive and repeated antibacterial therapy, thus facilitating the establishment of single or mixed yeast infections in the quarter. Certain types of yeasts were found to prevail in diseased quarters of the cows on particular farms.

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Table 6: DRUG SENSITIVITY OF **STAPHYLOCOCCUS AUREUS** AND MASTITIS STREPTOCOCCUS ISOLATES FROM SEVERELY DISEASED QUARTERS WITH CONCOMITANT YEAST INFECTIONS

Therapeutic substance	S. aureus (36)			Streptococci (20)		
	sensitive	partly sensitive	resistant	sensitive	partly sensitive	resistant
Chlortetracycline (50 mcg)	86.1%	—	13.9%	100%	—	—
Chloramphenicol (50 mcg)	88.9%	11.1%	—	100%	—	—
Furazolidone (100 mcg)	52.8%	—	47.2%	65%	20%	15%
Penicillin (5 units)	5.6%	—	94.4%	80%	15%	5%
Streptomycin (25 mcg)	36.1%	5.6%	58.3%	5%	15%	80%
Oxytetracycline (50 mcg)	77.8%	19.4%	2.8%	100%	—	—

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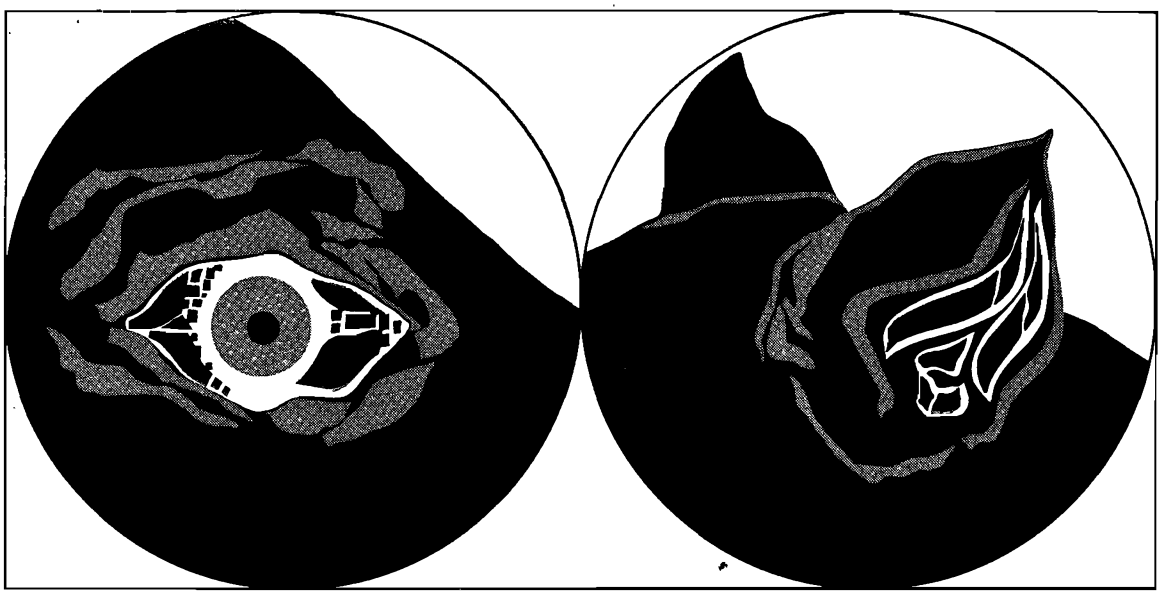

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THE TREATMENT OF "LUMPY WOOL", *DERMATOPHILUS CONGOLENSIS* INFECTION IN MERINO SHEEP WITH STREPTOMYCIN AND PENICILLIN

D. J. LE ROUX*

SUMMARY

A single intramuscular injection of 70,000 i.u. penicillin and 70 mg streptomycin cured 86.3% and brought about a marked improvement in 3.6% of Merino sheep, chronically infected with *Dermatophilus congolensis*. Spontaneous recoveries occurred in 13.5% and marked improvement in 1.5% of sheep from the same flocks, which were given a placebo of sterile distilled water.

INTRODUCTION

Extensive outbreaks of lumpy wool are commonly experienced in Merino flocks in the higher rainfall areas of South Africa. Reports from sheep farmers indicate that this disease is a regular seasonal problem which may have a high morbidity rate particularly amongst lambs up to a year of age. The disease is also known as "harde lammers" — literally hard lambs, when young lambs are affected. The causal agent of "lumpy wool" and other actinomycotic infections of the skin of various herbivorous animals, is now accepted as being *Dermatophilus congolensis*. Roberts [1] has recently reviewed the literature and described the conditions under which the disease occurs in Australia.

Roberts and Graham [3] and Roberts [2] investigated the efficacy of eight antibiotic preparations against lumpy wool and reported that a single intramuscular injection of 50 mg streptomycin and 50,000 i.u. penicillin per kg cured 95% of chronic cases, while a combination of 70 mg streptomycin and 70,000 i.u. penicillin per kg gave a 100% cure. Penicillin was ineffective against *D. congolensis*, but the combination of penicillin and streptomycin had a synergic effect. It was decided to evaluate efficacy of this treatment under South African conditions.

MATERIALS AND METHODS

Sheep: The trial was conducted in the Elliot District of the Eastern Cape Province, where severe outbreaks of lumpy wool occur. A total of 265 chronically affected sheep, the majority of which were year old lambs, were available out of four flocks. Each sheep was examined clinically before treatment. The activity of the lesions was established by the fact that the crusts or scabs were adherent to the skin. These cases dated back to an outbreak some 6 of 7 months previously, when the primary infection had occurred. As a result of the chronic lesions some of the sheep were emaciated and showed marked discomfort when handled.

Therapy: The sheep were identified with ear tags and randomly allocated into two groups of approximately equal numbers on each of the four farms. The animals in one group received a single intramuscular injection of a combination of 70,000 i.u. penicillin and 70 mg streptomycin per kg and those in the other, a placebo of an equivalent volume of sterile distilled water, also intramuscularly.

Evaluation of result of therapy: The sheep were re-examined 4 and 8 weeks after treatment without knowing the treatment group to which each belonged. The response to treatment was judged according to the following classification.

Cured — all crusts or scabs lifted, i.e. clear of the skin.

Improved — a few of the crusts or scabs still attached to the skin but most lifted clear of the skin.

No effect — all or most of the crusts or scabs still attached to the skin.

RESULTS AND DISCUSSION

The results are presented in the Table. A spontaneous cure or improvement took place in 15% of the sheep which received the placebo (controls), while 90% of the treated sheep were cured or improved.

It is clear that this treatment can be a

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Table 1: THE RESULTS OF TREATING SHEEP CHRONICALLY INFECTED WITH LUMPY WOOL USING A COMBINATION OF PENICILLIN AND STREPTOMYCIN

Treatment	Sheep	Cured	Improved	No effect	Totals
Penicillin/ Streptomycin	Number	120	5	14	139
	Percentage	86.3	3.6	10.1	
Control (Sterile distilled water)	Number	17	2	107	126
	Percentage	13.5	1.5	85.0	

valuable aid in combating a disease for which at present no other practical control measure or treatment exists.

Roberts and Graham[3] and Roberts[2] considered that all topical applications for the treatment of *D. congolensis* infections were rendered ineffective by the thick, adherent crusts or scabs which cover the lesions and the depth to which the hyphae penetrate the wool follicles. Roberts[1] pointed out that most published reports on the use of topical agents described the prior removal of the crusts, which suggested that spontaneous recovery had already commenced since the scabs were already partially detached; furthermore, no distinction was usually made between acute and chronic infections, nor of the rate of spontaneous recovery in untreated controls.

According to Roberts[1] treatment with penicillin and streptomycin is indicated only in cases of chronic infections, and is unnecessary in acute infections; it is also unlikely to influence the course of the disease in cases of continuous re-infection, unless given as a long series of injections; it is therefore important to distinguish between chronic infections and the persisting condition resulting from continous re-infections; chronic infections are distinguished from re-infections by the steady build up of scab over a period of months; treatment should be delayed as long as possible to permit the maximum number of cases to heal spontaneously, but should be given at least 8 weeks before shearing so that the shears can pass under the lifted crusts or scabs.

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BOOK REVIEW

VETERINARY PATHOLOGY

HILTON T. SMITH AND T. F. JONES

Third Edition, Lea & Febiger, Philadelphia, U.S.A., 1966. Pp 1192, 839 illustrations. Price R16.20.

In its third edition this well-known textbook of Pathology, which has become a standard text for the teaching of Pathology at the Faculty of Veterinary Science at the University of Pretoria, has been revised and augmented by the addition of new chapters on genetically determined diseases and diseases due to organisms of uncertain classification.

In several chapters, the references have been rearranged by placing them in juxtaposition to the text rather than at the end of the chapter. A large number of new references and new illustrations have been added.

Both general and special pathology are included in one volume. It is no small achievement to cover such a wide field adequately in one volume. This is made possible by being concise without omitting essential detail.

The only criticism that one can offer is the fact that some of the infectious diseases occurring in Africa are not adequately dealt with. East Coast fever serves as an example. The information is very skimpy and not opto-date. The only reference is to the work of Steck 1926. It is still claimed that the disease cannot result in a carrier state.

The book is very well prepared and beautifully illustrated. Both publishers and authors deserve every credit for an excellent book. It gives me great pleasure in recommending this book to everyone interested in Pathology.

It will certainly play a major rôle in demonstrating the practical value of pathology to both students and practitioners.

J.D.S.

Jl S. Afr. vet. med. Ass. 39 (3): 91-99 1968

BRACKEN POISONING IN CATTLE IN THE NATAL MIDLANDS

R. C. TUSTIN*, T. F. ADELAAR* AND C. M. MELDAL-JOHNSEN**

SUMMARY

An outbreak of bracken *(Pteridium aquilinum)* poisoning in cattle in the Estcourt district of Natal is described. This is the first recorded outbreak in South Africa although several others have been known to occur. The condition is, however, rare in this country. The symptomatology, macro- and micropathology and the results of haematological examinations are presented.

Fifty-five cattle were affected, 28 fatally. Therapy which comprised single or multiple blood transfusions and intravenous administration of protamine sulphate was highly successful.

INTRODUCTION

Although bracken *(Pteridium aquilinum* (L.) Kuhn) is a very common plant with a wide distribution in South Africa, cases of poisoning in farm animals are rare. This is probably due to the fact that the plant is not very palatable and that large quantities must be consumed over a period of some weeks before symptoms of poisoning occur. Unpublished cases of suspected poisoning have occurred in the Ixopo [1], Knysna [2] and Eastern Province [3] areas.

Natural cases of bracken poisoning have been recorded in cattle, sheep [4, 5], pigs [6] and horses [7, 8]. Fresh and dried fronds as well as the rhizomes are toxic [9]. Various toxic fractions are present in the plant. These are a cyanogenetic glycoside — present in very small quantities [10, 11] — which does not appear to play any rôle in the pathogenesis of the poisoning, thiaminase [8, 12-15], and an unidentified fraction which depresses the function of red bone marrow with consequent granulocytic leukopaenia and thrombocytopaenia [16-19].

The disease syndrome in cases of bracken poisoning varies according to the species of animal concerned. In monogastric animals such as rats, pigs and horses, the effect of this plant is due to thiamine destruction in the stomach and gut by the enzyme, thiaminase, and the resultant syndrome is characteristic of thiamine deficiency which responds well to therapy with this vitamin [8, 14]. The symptoms seen in horses are: progressive staggering and inco-ordination, positioning of the feet and legs in unnatural stances, muscular tremors, somnolence and finally recumbency followed by convulsive seizures and death [8].

Bracken poisoning in cattle is characterized by leukopaenia, especially of the granulocytes, and thrombocytopaenia both of which are due to decreased formation resulting from damage to myeloid tissue, increased capillary fragility, widespread haemorrhages, prolonged bleeding time, defective clot retraction and pyrexia. The depressed bone marrow activity is not due to thiaminase, but to an agent with radiomimetic properties which can be extracted from the plant by suitable solvents [14, 16] but which has not yet been identified, although a depression in urinary and faecal excretion of thiamine is observed when cattle are fed a bracken hay diet [14]. Therapeutic administration of thiamine, vitamin B12 and other vitamins to cattle suffering from the disease has proved unsuccessful.

According to Evans, Evans and Hughes [16] outbreaks of bracken poisoning in cattle are often sporadic and morbidity is usually low, but mortality high. The ecological conditions usually conducive to outbreaks in cattle are those causing scarcity of pasture herbage, e.g. adverse climatic conditions in late spring, such as drought or cold weather, or the overstocking of restricted areas. Bracken grows vigorously and hungry animals will eat it, thereby, it is suspected, acquiring a taste for it. They state that the incidence of the disease (in Britain) is highest in the late sum-

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mer. Apparently, under circumstances where the grass is lush, cattle develop a craving for fibrous material which they satisfy with bracken if nothing else is available. It is considered wise to put down hay as an alternative form of roughage. Relatively large quantities of the plant have to be consumed by cattle over a long period of time before poisoning occurs, and experimentally it has been produced in heifers 32 to 86 days after the commencement of the feeding of bracken [19]. That the toxic principle is cumulative, was demonstrated by Evans *et al* [16], who stated that, in their experience, animals have been known to succumb with typical symptoms up to eight weeks after any possible access to the plant.

Naftalin and Cushnie [18, 19] consider that concomitant with the rise in temperature of poisoned animals there is a bacterial invasion of the blood and that bacterial infarction of the liver, lungs and kidney may result. The bacteraemia is associated with complete, or almost complete, disappearance of polymorphs from the peripheral blood. Evans *et al* [16, 24], on the other hand did not find any close correlation between positive blood cultures and the presence of fever.

The hypothesis that bovine enzootic haematuria is possibly related to bracken poisoning has been reviewed by Munday [20]. Rosenberger and Heeschen [21] fed bracken to cattle over long periods and described lesions in the mucosa of the urinary bladder which closely resembled those seen in cases of bovine enzootic haematuria. Georgiev, Vrigasov, Antonov and Dimitrov [22] prepared an extract from the urine of cattle fed hay from districts in which haematuria occurs which, when introduced into the urinary bladder of dogs, produced changes of the mucosa similar to haemangioma. Applications of this extract to the skin of white mice produced papilloma-like excrescences. Evans and Mason [23] further investigated this carcinogenic effect of bracken by incorporating dried milled bracken into the died of rats which was supplemented with vitamin B_1 in order to counteract the thiaminase activity. Many of the rats died from multiple tumours of the small intestine which were identified as adenocarcinomas.

Sheep appear to be more resistant to the toxic factor responsible for myeloid depression and show slight depression in blood elements when fed a comparative quantity of fronds or rhizomes which proved fatal to cattle, but Evans *et al* [14] have produced symptoms of thiamine deficiency in adult sheep fed a diet supplemented with bracken rhizomes. They were unable to explain this remarkable species difference in susceptibility of sheep and cattle to the bone marrow depresant, although they had evidence that the crucial factor is dosage. Parker and McCrea [5] on the other hand have described outbreaks of poisoning among sheep in Yorkshire where the animals had little alternative choice of feed and where a haemorrhagic syndrome very similar to that observed in cattle with leukopaenia and reduced blood platelet count was seen.

During November 1967 an outbreak of bracken poisoning in cattle on a farm in the foothills of the Drakensberg in the Estcourt district of Natal was investigated; the results of the investigation forms the basis of this report.

HISTORY

The farm on which the bracken poisoning occurred is 2,600 acres in extent and carried at the time about 700 head of Drakensberger cattle of all age groups, or approximately 3.7 acres per beast. The nature of the veld is typical for that part of the country, being sourveld which provides nutritious grazing during spring and early summer and then becomes fibrous, unpalatable and of low nutritional value for the rest of the year. During winter the cows and calves are moved to another farm; the young stock and oxen remaining on the farm being fed a supplementary ration.

Bracken fern is a very common plant in this region, many farms being infested with it. It is the general practice of farmers in the area to burn the majority, if not all, of the veld on their farms every August irrespective of whether rain has or has not fallen. On the farm concerned most of the veld was burnt on 1st August, thus also removing the old bracken fronds of the previous season's growth. After burning the veld grass and bracken grew well until towards the end of September, when an unseasonably heavy frost on two subsequent nights destroyed all the rapidly growing young grass and bracken, the latter being at this stage about 25 cm high. This cold spell was followed by warm weather which stimulated new shoots of bracken again to grow at a faster rate than the grass.

The camp in which most of the cases of poisoning occurred is 600 acres in extent and parts of it are heavily infested with bracken. A total of 183 cattle from one to three years of age, which, prior to this date, had been grazing on old cultivated lands and on old veld grass, were moved into this camp. They ate the bracken freely. The first mortality occurred 51 days after admission to the camp, and the farmer suspected snake bite because of the numerous haemorrhages present in the carcase, although no poisonous snakes occur on the farm. On the next day two of the cattle were noticed to be ill, one of which died after 24 hours and the other after 48 hours. Subsequently two or three of the animals in the camp were noticed to be ill every day, and several of them died. A practicing veterinarian and subsequently one of us (C.M.M-J.) agreed on the diagnosis of bracken poisoning; at this stage seven cattle had died and 12 were ill. Cattle, including cows with calves at foot, in other camps on the farm were also affected. With the exception of one case which fell ill 12 days after the outbreak had apparently ceased, no new cases occurred 21 days after the first mortality.

A total of 28 animals died of bracken poisoning during the outbreak and 27 recovered following treatment with protamine sulphate and blood transfusions. Affected cattle were kept in a small camp near the homestead for observation and treatment. The remainder of the 183 cattle in the original camp were moved elsewhere seven days after the first death.

Six autopsies were performed on animals which had died or been slaughtered *in extremis*. Organ specimens were taken from some of these animals for histological examination and were preserved in 10% formalin. Bone marrow smears were made from the rib-bones of one slaughtered animal. Blood specimens from four cases (heparin being used as an anticoagulant), and urine specimens from two cases were collected for laboratory examination.

SYMPTOMATOLOGY

Affected animals lost condition, appeared depressed and could relatively easily be caught and handled in the open. If not too severely affected, they still grazed to a limited extent. Some appeared to be weak, especially in the hind limbs and were inclined to fall. Secretion of saliva, which became more copious when they were driven, resulted in frothing at the mouth. They were liable to collapse and die if driven some distance. The faeces of many cases contained blood clots, and some animals suffered from a diarrhoea. In several cases the farmer noticed persistent skin haemorrhage from the puncture wounds of hypodermic needles. One animal bled from an old brand scar, another showed haemorrhage into the anterior chamber of the eye and another conjunctival petechiation. A bloody discharge was present from the anus and nostrils of several cases. A fever was present in all affected animals, the highest recorded temperature being 108°F.

In many untreated cases deaths ensued within 48 hours of the first symptoms being noticed. Various forms of ineffective treatment prolonged life up to one week.

POST MORTEM EXAMINATION

The following is a composite picture of the autopsy findings observed in the six cases, the most outstanding lesions being haemorrhages of varying sizes.

The subcutis and intermuscular connective tissue contained numerous haemorrhages which varied in size from petechiae to diffuse extravasations (suggillations), whereas small haemorrhages only were in the tongue and diaphragm. Mild ascites, hydrothorax and hydropericardium were present, and in one case the peritoneal cavity contained approximately 4.5 litres of blood. The parietal and visceral peritoneum was studded with petechiae, ecchymoses and suggillations which occurred particularly over the rumen, gall bladder, spleen and caecum. A large subperitoneal haematoma containing clotted blood and approximately 90 by 45 cm in size was encountered in the abdominal wall of one animal.

The perilaryngeal and peripharyngeal tissues and mucosae of the larynx and trachea contained haemorrhages. An oedema of the lungs was seen in several carcases, and in one animal an area of consolidation in the right apical lobe was present.

Bracken fronds were observed in some rumens. Submucosal oedema of the folds of the abomasum with mucosal petechiation and small pinhead-sized erosions were also noted. The small intestine appeared relatively unaffected; submucosal oedema and scattered mucosal petechiae being present in only one case. The contents of the caecum and colon

in the majority of animals were either blood-stained or contained blood clots. An oedema of the wall of the colon was present in one, but mucosal haemorrhages of the large intestine — varying in size from petechiae to suggillations — occurred in all cases. Erosions and irregular ulcers up to 2 cm in diameter were also present in the colon and caecum. In the livers of several animals relatively numerous discrete yellow foci of necrosis up to 2 mm in diameter were surrounded by a narrow zone of hyperaemia and were irregularly distributed throughout the substance. Very numerous discrete yellowish-white areas about 1—2 mm in diameter were noticed in the liver parenchyma of one case, and difficulty was experienced in interpreting whether this was a *post mortem* or *ante mortem* change. Petechiae and ecchymoses were present in the wall of the gall bladder.

Large perirenal haematomas, either unilateral or bilateral in distribution were observed in four of the animals, the largest of these being the size of a football. In the kidneys of two cases haemorrhagic cortical infarcts occurred. Petectiae and ecchymoses in the renal pelvis and external surface and mucosa of the bladder were occasionally encountered. The urine was blood-stained in one animal.

Numerous subepi- and subendocardial haemorrhages from petechiae to suggillations were present in all cases. The myocardium in some had a few irregular indistinct patches which appeared paler in colour than normal, and the skeletal musculature, particularly of the large muscle groups of the hind limbs, also appeared lighter in colour. Many lymph nodes, particularly the retropharyngeal, bronchial, mediastinal, renal and periportal, were swollen, oedematous and haemorrhagic. The fatty bone marrow of the femur was normal.

HISTOPATHOLOGICAL EXAMINATION

The histological examination of specimens from four of the animals autopsied, in general, confirmed the macroscopical findings.

The foci of necrosis in the liver were irregular in shape and size, some being larger than a liver lobule. The majority were surrounded by a rather sparse reaction zone consisting of leukocytes, most of which were round cells, although in some a few neutrophiles were also present. In one animal som of the foci of necrosis were associated with haemorrhages, and in others colonies of short rod-shaped bacteria were present. In the liver of one animal there were focal areas of fatty infiltration which were mainly centrilobular in distribution. Rather rare hyaline droplet degeneration was noted in odd hepatocytes of another case. The numerous yellowish-white foci seen grossly in the liver of one case proved to be areas of *post mortem* decomposition.

In the spleen of one animal which was slaughtered for post mortem examination, relatively numerous discrete foci of coagulative necrosis about 1mm in diameter were scattered throughout the parenchyma, and in some of these, colonies of bacteria were detected.

In sections from various skeletal muscles of three cases and the tongue musculature of two cases hyaline degeneration affecting short segments of muscle fibres was present to a varying degree. The tongue musculature of two cases also showed similar lesions. In the myocardium of two of the three animals where this tissue was examined, hyaline degeneration of segments of muscle fibres was also observed. Small haemorrhages also occurred in the interstitium.

Haemorrhagic infarcts were seen in kidney sections from two cases. In one, the infarcts were associated with subcapsular haemorrhages. Interstitial oedema and haemorrhages were also present in one kidney.

In three of the four animals the lymph-nodes were oedematous and in two of these cases the germinal centres were atrophic. In a lymph node from one animal haemorrhages and a localized fibrinous inflammatory reaction beneath the capsule of the organ were seen. Relatively numerous colonies of bacteria were present in the exudate, which contained few leukocytes.

Ulcers from the large intestine from two cases were examined histologically. The ulcers extended into the submucosa and were filled with a fibrinous material which contained bacteria, erythrocytes and some leukocytes. In addition, haemorrhages were present in all layers of the wall in the vicinity of the ulcers, and one showed a superficial colitis with infiltration of neutrophiles.

In the sections of the lung which had macroscopically shown consolidation, an early fibrinous brondhopneumonia was present. Relatively numerous colonies of bacteria were

observed in the exudate but infiltrating leukocytes were sparse.

EXAMINATION OF SMEARS

Examination of bone marrow smears from a one year old ox which had been ill for six days before it was killed for autopsy, revealed the presence of a panmyelophthisis (reduction in the formation of granulocytes and erythrocytes, and their precursors) which was manifested by immaturity of the neutrophile myelocytes, some of which show the presence of "toxic granulations", absence of eosinophile and basophile myelocytes; anisocytosis of erythrocytes and leptochromasia of nuclei in general. No megakaryocytes were observed. (This may or may not be significant.) The differential leukocyte count of a blood smear from this case showed the presence of 60 per cent erythroblasts, lymphocytes and/or pronormoblasts (difficulty was experienced in differentiating these cells) and 40 per cent neutrophiles or their precursors. All the neutrophiles were in the young stage (three-lobed or younger) and "toxic granulations" were present in many of them. Many erythrocytes were anisocytotic; Joly bodies occurred in rare red blood cells and punctate basophilia was present in some. Few normoblasts were observed. Blood platelets were very scarce, varied in size and shape and contained few granules. No clumps of thrombocytes were seen. Unfortunately, erythrocyte and leukocyte counts of this animal's blood were not performed.

Blood smears were examined from six other affected cattle. In some, evidence of mild anaemia such as slight anisocytosis and poikylocytosis, and an apparent leukopaenia, especially of the granulocytic series and thrombocytopaenia were noticed.

EXAMINATION OF BLOOD

The blood of four cattle in various stages of the disease was examined. The analyses done and results obtained are presented in the Table. Chemical tests using routine techniques for transaminase (SGOT and SGPT), bilirubin, creatinine, blood urea nitrogen, glucose, total plasma proteins, phosphate, chloride and bicarbonate were also performed on the blood of five animals but the result did not reveal any definite deviation trends and are therefore not presented. The plasma of one animal, however, did contain increased transaminase (SGOT, 296 King Units and SGPT, 79 King Units).

The most significant conclusions to be drawn from these results are the leukopaenia in three of the cases (1, 2 and 4) and the apparent absence of any meaningful pathological trend as far as the erythrocytes are concerned. It is evident that, although a neutropaenia was mainly responsible for the leukopaenia, in at least two of the animals (1 and 2) there was also a decrease in the number of circulating lymphocytes.

EXAMINATION OF URINE

Urine samples of two animals collected at autopsy were examined; only the presence of occult blood could be determined.

TREATMENT

Initially, the majority of animals suffering symptoms were treated with antibiotics, Bu-Coag* (a systemic haemostatic agent) and vitamin A. This treatment did appear to prolong the lives of the animals but did not save them. Twenty days after the first animal had died and when the total number of deaths had amounted to 25, treatment with blood transfusions and injections of protamine sulphate** was instituted. These were given to all affected animals at that time and to newly detected cases thereafter with the exception of one animal, and, in all, 30 cattle were so treated.

Each affected animal was given a single injection of 10 ml of protamine sulphate by the intravenous route in order to counteract the anticoagulant effect of heparin.

The blood for transfusion was obtained from the local abattoir and the anticoagulant used was sodium citrate. The treated animals each received a transfusion of 4.5 l of blood; nine received a second transfusion of 2.25 l and one was given a third transfusion of 2.25 l. Three of the animals died following treatment; one of shock immediately after the second transfusion, one of bracken poisoning and the other of pleuritis. The latter animal had received three blood transfusions.

Several of the animals treated in this manner did not regain their appetites and were given supportive treatment consisting of an oral ruminatoric and injections of vitamin B complex which stimulated them to commence eating. After two to three weeks

* Bu-Coag V, Burns (S.A. Cyanamid)

** Protamine sulphate 1% (Evans).

Table: BLOOD ANALYSES PERFORMED AND THE RESULTS OBTAINED

Bovine No.	Sedimentation mm/hr	Haematocrit %	Haemoglobin g%	Erythrocyte count $\times 10^6$/ml.	Mean cell haemoglobin concentration %	Mean cell volume μ^3	Mean cell haemoglobin $\mu\mu g$	Leukocyte count $\times 10^3$/ml.	Leukocyte Differential Count %				
									Neutrophiles	Lymphocytes	Eosinophiles	Monocytes	Basophiles
1	4	28	7.5	8.3	28	33.7	9.3	2.9	8	86	4	1	1
2	4	35	10.25	6.9	14.9	50.7	29.3	1.1	—**	—**	—**	—**	—**
3	6	35	9	8.0	11.25	43.7	25.7	13.1	18	66	8	7	1
4	3	38	10.5	6.4	16.4	59.4	27.7	6.9	20	70	5	4	1
Normal*	0—5	30—40	10—12	7—8	26—34	40—60	14.4–18.6	9	26	61	4	7	0

* Normal figures given are those considered average for South African conditions

** Only 2 neutrophiles and 10 lymphocytes were observed in the blood smear of this animal.

they were all allowed to return to their normal camps.

DISCUSSION

The diagnosis of bracken poisoning in the cattle was based on the history, the autopsy and histological findings, haematology, the presence of the plant in large quantities on the farm and the evidence that it had been eaten. No other factors were determined which might have been responsible for a haemorrhagic diathesis.

No cases of bracken poisoning have been reported previously from this area over a considerable period of time, and apparently none had occurred on neighbouring farms, although the plant is also prevalent on them. The reason for the occurrence of this sudden and unprecedented outbreak must be sought in the systems of veld and animal management practised on the farm and in the inclement climate conditions which prevailed some weeks prior to the first cases of poisoning. Bracken grows at a faster rate than grass, and the cattle, which had been accustomed to a bulky diet before being moved to this camp, were probably induced to eat it in order to satisfy a need for roughage. Dayton [25] mentions that the palatability of the plant is poor except after frost when it may be eaten by stock. In addition, a shortage of forage probably arose, causing the cattle to graze less selectively than normally, and, although the rate of stocking of 3.7 acres per beast does not appear to be too high for rotationally grazed, high-rainfall sourveld for that time of the year, low temperatures and rainfall possibly aggravated this feed shortage. A possible contributory factor is the fact that cattle once used to bracken, acquire a taste for it [16].

One of us (C.M.M-J.) has had experience of bracken poisoning in cattle in New Zealand where the method of "breaking in" new country infected with bracken is to burn the vegetation and then seed the land after treating it with a superphosphate fertilizer. When the grass comes up large numbers of cattle are moved onto the land. Needless to say, the bracken comes up first and the cattle eat large quantities of it. This procedure is carried out extensively with no untoward results until suddenly 20 to 50 cattle die from bracken poisoning. Subsequently no deaths may occur for many years.

The most significant pathological changes observed in the cattle were the fever, haemorrhagic diathesis, ulceration of the large intestine, hepatic and splenic infarction associated with bacterial embolism, haemorrhagic infarction of the kidneys and leukopaenia. The leukopaenia was manifested chiefly by a neutropaenia but a decrease in the number of circulating lymphocytes was also partly responsible. The latter is substantiated by the atrophy of the germinal centres of some lymph nodes examined histologically. Naftalin *et al* [19] state that in their cases of bracken poisoning there was terminally a complete abscence of polymorphonuclear leukocytes and a severe reduction in the number of lymphocytes. Although no bacteria were seen in association with the renal infarction, it is assumed that bacterial emboli were responsible for them as they were in the liver and spleen. Leukocytic reaction zones around necrotic foci were not as dense as would have been expected and comprised mainly round cells: this fits in with the general picture of leukopaenia.

The pathogenesis of the disease has been elucidated by Naftalin et al [19], who state that the essential lesion is bone marrow damage with resultant diminution in the numbers of circulating platelets and granulocytes. The haemorrhagic purpura is the result of the thrombocytopaenia and the bacteraemia is associated with the neutropaenia and development of fever. If, after the onset of the bacteraemia, the animal lives some days, bacterial infarction of the liver, lung and kidney may take place. Haemorrhage becomes more severe after the onset of bacteraemia, and the animal dies from severe internal haemorrhage. The bacterial invasion is considered to take place through the gut by the following machanism: haemorrhage into the mucosa or submucosa is followed by ulceration; bacteria multiply on the surface or in the necrotic tissue, and then, singly or in clumps, enter the mesenteric and portal vessels, and are carried to the liver where they may lodge, or pass into the general circulation. In contrast to the marked fall in numbers of platelets and leukocytes, the number of erythrocytes remains within normal limits until the severe terminal bleeding, although examination of the bone marrow at death showed widespread destruction of the erythropoetic, as well as the leukopoietic tissue. This was attributed to the fact that the circulating life of a red blood cell (in man) is estimated to be about 120 days and that of a mature leukocyte nine

days. Kaneko[26] determined the normal survival time of a bovine erythrocyte to be 160 days. Adam *et al*[27] have estimated the mean lifespan of a human thrombocyte to vary between 8.5-17 days.

Evans and Howell[28] have reported that heparin blood levels increased in an experimentally induced case of bracken poisoning in a calf. According to them this suggested a release of heparin into the circulation in abnormal amounts due to an involvement or destruction of mast cells. Since these cells also store histamine, it was reasonable to suppose that this substance might also be present in increased quantities in a biologically active form, and they postulated that an increase in free heparin and histamine would help explain the prolonged clotting time and increased capillary fragility known to accompany bracken poisoning. They successfully treated calves suffering from bracken poisoning with the following combined therapy: (1) Toluidine blue, intravenous (antiheparin); (2) "Anthisan", intramuscular (antihistamine); "Predsolan" intramuscular (hydrocortisone); batyl alcohol, intravenous (bone marrow stimulant) and "Distavone", intramuscular (precautionary antibiotic therapy indicated by leukopenia). Evans *et al*[29] have also treated experimental cases with DL-batyl alcohol and antibiotics with success provided the circulating leukocytes and thrombocytes were not below 2,000 and 50,000 to 100,000 per c.mm respectively, and in a trial of this therapy in field cases they obtained about 80 per cent recovery rate. They also state that the administration of large doses of vitamin B complex, of antibiotic therapy alone and of small-scale blood transfusions had been unsuccessful, Large-scale blood transfusions (a gallon a day), in cases too severe for batyl alcohol to be sufficient were also not effective.

The increase in the heparin levels in the blood reported by Evans *et al*[28] prompted the therapeutic use of protamine sulphate in the outbreak under review. This, together with the administration of blood transfusions, the rationale of which was based on the thrombocytopaenia, leukopaenia and incipient anaemia, gave apparently outstanding results; 27 of 30 cattle so treated recovered. The success of this form of therapy may have been due to the facts that those animals which contracted the disease were at least one year old, recovery in young calves from acute bracken poisoning being rare[28], that they showed evidence of the disease towards the latter half of the outbreak and had perhaps not developed such an acute disease as those which died initially, and that the animals in the early stages of the disease were selected and removed from the bracken-infected pastures. On the other hand, the last animal to develop bracken poisoning some days after the apparent end of the outbreak was not treated and died of the acute disease.

Ruminatorics and appetite-stimulating drugs (vitamin B complex) administered to those animals which had apparently recovered from the acute disease but which had not yet commenced eating normally, seemed to have a beneficial result.

Bovine enzootic haematuria has not been definitely diagnosed in South Africa but its presence has been suspected in the Tzaneen district, in which bracken grows prolifically. The hypothesis that a relationship exists between this condition and the ingestion of bracken over long periods bears further consideration.

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CASE REPORT : 'SIAMESE TWINS'

G. P. Retief*

SUMMARY

A case of bovine 'Siamese twins' is reported where two perfectly formed bodies were attached to a common head.

HISTORY

A four-year-old Friesland cow had been in labour for ten hours when the owner had spent four hours trying to deliver the calf. Four hooves were presenting but his attempts to repel one set of limbs and extract the other, met with no success.

EXAMINATION

Four emphysematous and dry hind legs were presenting. The contused and swollen vaginal passage as well as the gross distention of the foetuses made examination cranial to the buttocks and sacral region impossible.

TREATMENT

Lignocaine (10 ml of a 2% sol.) was injected into the epidural space and one gallon of liquid paraffin instilled into the uterus by means of a rubber tube and funnel.

Attempts to repel one set of limbs while extracting the other failed. It was noted that the four limbs seemed to move together whether repelling or extracting was tried. This was thought to be caused by impaction of the two grossly distended bodies.

A wire foetotome was then employed for total foetotomy of both calves up to the mid-cervical region. Only at this stage could it be established that the two necks were attached to a single head, and these were delivered and dissected.

GROSS ANATOMY

The head was slightly enlarged, especially caudally, but looked normal otherwise, except that apart from the two ears in the normal position, there were two extra pinnae attached to the skin above the nuchal crest on a line with the normal ears. They were about half normal size, and joined at the anterior border of the conchae about 5 cm from the attachment to the skin. Only one annular cartilage could be found; it was loosely attached to the skin. The whole ectopic appendage could be moved freely with the skin. No *Mm scutulares* and only rudimentary *Mm. scutulo-auriculares* could be found.

The cranium, brain, nasal and buccal cavities were all normal up to a line drawn transversely through all tissues from the nuchal crest dorsally to the angle of the mandible ventrally. All structures caudal to this line were either duplicated or altered.

The pharynx opened into two oesophagi which continued in the usual way down both necks. There were two *aditi larynges* and two *epiglottides*. The one was in the usual position with the other about 2 cm behind it. The two larynges with two complete sets of laryngeal cartilages opened into two trac-

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heae which split and continued down both necks in the usual fashion. Most of the neck muscles were attached to their usual attachments on the skull but there were, of course, two muscles to each attachment — a certain amount of fusion having occurred between the duplicated muscles.

Certain bones in the basilar part of the cranium, however, were duplicated *(vide infra)* and only one muscle was attached to these.

The necks bifurcated only about 10 cm caudal to the nuschal crest and about 20 cm caudal to the angle of the mandible.

After dissection, and removal of the soft tissues, the skull was boiled in a solution of sodium hydroxide. Examination of the skull revealed the following:—

There were two *foramina magna* side by side, their medial borders being about 5 cm apart. There were two pairs of occipital condyles and two pairs of paramastoid processes but only one pair of *bullae tympanica*. A certain amount of duplication of the squamous and basilar parts of the occipital bone had taken place to accommodate the two foramina magna.

There was but one cerebellum. The medulla oblongata was slightly larger in diameter than usual and bifucated into two spinal cords which passed through the two *foramina magna* and down the spinal columns of each calf. The atlas of each calf articulated with the two pairs of occipital condyles previously described.

The calves were perfectly formed from the necks down and were full term. The 'right' calf was slightly smaller than the 'left'.

DISCUSSION

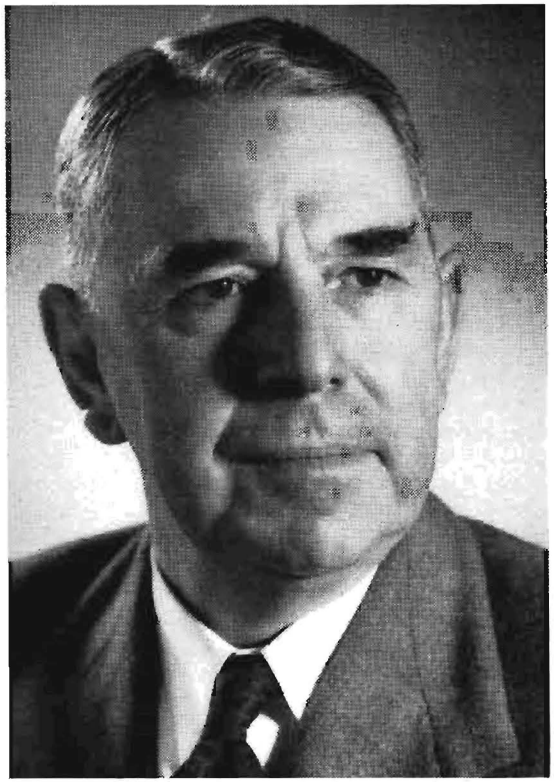
It seems a pity that veterinary assistance was sought at such a late stage when foetal death, emphysema and putrefaction had already set in. Had the calf still been alive a most interesting set of 'twins' could have been delivered by caesarian section. No reference to such 'twins' could be found in the literature, although two-headed calves are not unusual.

In Memoriam

JAN GEORG VAN DER WATH

Op 15 Mei 1968 het die veeartsenykundige professie in Suid-Afrika een van sy mees vooraanstaande lede verloor, nl. dr. Jan Georg van der Wath. 'n Paar maande voordat hy 61 jaar oud sou wees, het hy 'n slagoffer geword van die tragiese Boeing-ramp by Windhoek waarvan die besonderhede algemeen bekend is.

Nadat dr. van der Wath aan die einde van 1934 as veearts kwalifiseer, werk hy in



die laboratorium te Allerton. In 1936 word hy aangestel as navorser in die Afdeling Fisiologie te Onderstepoort, waar hy saam met dr. Quin baanbrekerswerk verrig op die mikrofauna en flora van die verteringstelsel van herkoms. Hy word aangestel al lektor in Fisiologie en ontvang in 1942 die D.V.Sc.-graad met 'n proefskrif getitel: "Studies on the alimentary tract of the Merino sheep with special reference to the rôle of the microfauna and flora".

Kort daarna besluit dr van der Wath om te gaan boer, maar verloor nooit sy belangstelling in die akademiese lewe en navorsing nie, soos duidelik blyk uit die poste wat hy later beklee het. Hy word gekies tot lid van die Raad van die Universiteit van Pretoria en in 1960 tot Voorsitter van die S.A. Wolraad en ook tot Voorsitter van die Internasionale Wolsekretariaat vir die betrokke jaar.

In 1962 word hy Voorsitter van die Raad van Beheer van die S.A. Woltekstielnavorsingsinstituut, 'n pos wat hy beklee het totdat hierdie Instituut ingelyf is by die W.N.N.R. in 1966. Hy word ook Voorsitter van die Veesiektenavorsingsfonds en in 1965 Voorsitter van die S.A. Wolkommissie.

Dr van der Wath het 'n diep insig gehad in alle sake wat die wolbedryf raak en kon met besondere visie leiding verskaf in al die organisasies waarin hy betrokke was. Hy was besonder geïnteresseerd in woltekstielnavorsing. Die sterk, wetenskaplik gefundeerde standpunt wat hy altyd ingeneem het, het bewondering afgedwing op nasionale en internasionale vlak.

As Direkteur van verskeie belangrike finansiële organisasies het hy ook sy bydrae gelewer tot die ekonomiese vooruitgang van die Republiek.

Omdat die Universiteit van Port Elizabeth, wat intiem by opleiding in Tekstieltegnologie betrokke is, aan dr van der Wath erkenning wou gee vir sy leidende rol in die wolbedryf as 'n geheel, is aan hom kort voor sy dood 'n ere-doktorsgraad toegeken.

In sy latere lewe was dr van der Wath só besig met sake van die wolbedryf, dat hy nie veel tyd kon vind om aan professionele aktiwiteite te wy nie, maar hy het nooit die kans laat verby gaan om die belange van die veearts op die mees positiewe wyse te bevorder nie. Hy het steeds navorsing en opleiding op sy hart gedra en gesorg dat belangegroepe geldelik bydra vir dié doel.

Die veeartsenykundige professie betreur sy heengaan en wil die diepste meegevoel betuig met mev. van der Wath en haar kinders.

MICHIEL BERG

Met die onlangse heengaan van dr. Michiel Bergh sal dit nie onvanpas wees om hulde te bring aan iemand wat alom bemind was nie, nie slegs deur sy kollegas nie, maar deur almal wat met hom in aanraking gekom het.

Michiel Bergh was een van die eerste groep studente wat in 1924 die eindeksamen vir die B.V.Sc. graad te Onderstepoort afgelê het.

Hy was aanvanklik as Staatsveearts op Middelburg, Transvaal aangestel en was daaropvolgens op Piet Retief, Louis Trichardt, Bethlehem en Johannesburg gevestig.

Met sy vriendelikheid en gemoedelikheid kon Mike 'n mens ure aan een onverveeld hou. Tipies van sy gemoedelikheid is die volgende staaltjie uit sy loopbaan. Toe hy op Piet Retief gevestig was, was daar nog Ooskuskoors in die gebied en die maatreëls ter bekamping daarvan moes treng uitgevoer word. Op 'n keer het die plaasbestuurder van die toenmalige Minister van Landbou versium om 'n paar verse onder toesig te dip, waarop dr. Bergh sonder meer die bestuurder ooreenkomstig die Veesiektewet aangekla het. Binne 'n dag of wat was daar 'n dringende navraag van hoofkantoor oor die voorval. Dr. Bergh se verklaring was kort en bondig: die Minister en sy bestuurder is burgers van die land en gevolglik moet hulle die landswette gehoorsaam; indien hy 'n uitsondering in die geval sou maak sou boere, wat self onder die streng regulasies gebuk gaan, gou 'n vinger na hom wys, en hy vrees die boere meer!

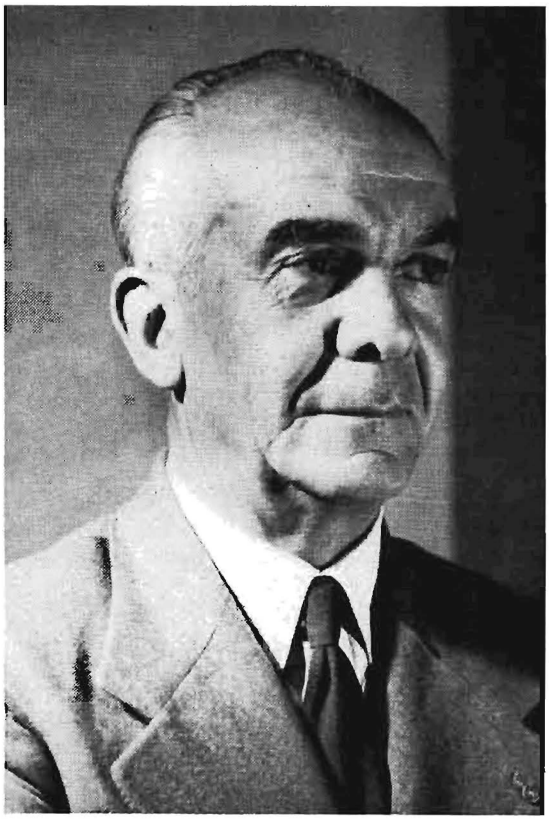
Op Piet Retief het Mike in die huwelik getree met mej. van Dyk. Ons innige simpatie gaan uit na mev. Bergh en hulle twee seuns in hulle droewige verlies.

PETRUS JOHANN DU TOIT

The death of one of South Africa's distinguished scientists Dr P. J. du Toit in Pretoria on 13th November 1967 is a great loss to the veterinary profession.

"P.J.", as he was affectionately known, was born in Somerset Strand on 16th March 1888. He matriculated in 1904 at the Paarl Boys High School and obtained a B.A. degree at Victoria College, Stellenbosch in 1907, shortly thereafter proceeding to Halle, Berlin and Zurich where he acquired the degrees of Ph.D. Zoology (Zurich) and Dr. Med. Vet. (Berlin) both *cum laude*.

During World War I he was unable to leave Germany but utilised his time very profitably by producing, in collaboration with Prof. Knuth, an outstanding book on tropical medicine which for many years served as a standard work.



Returning to South Africa in 1918 as Senior Research Officer at Onderstepoort, he succeeded the late Sir Arnold Theiler as Director of the institute and Dean of the Faculty of Veterinary Science in 1927, positions he held for 20 years. He became professor of Tropical Diseases at the age of 32 years, indicating his outstanding ability in this field.

His many contributions to veterinary research in South Africa are contained in over 130 scientific publications. He also played an extremely active part in the scientific field. He became a member of the S.A. Association for the Advancement of Science in 1920 and was elected sectional president in 1928 and

president in 1932. He helped to found the S.A. Akademie vir Wetenskap en Kuns in 1929 and was awarded the Havenga Prys (Geneeskunde) in 1947. An active member of the S.A. Biological Society, he received the Senior Capt. Scott medal for outstanding scientific achievement in 1929.

He was a member of the Council for Scientific and Industrial Research from 1945, became vice-president on retiring from Onderstepoort in 1948 and succeeded Dr. Schonland as president in 1950. As Chairman of the Historical Monuments Commission since 1947 he contributed a great deal to the preservation of historic and cultural antiquities.

During his extremely active career awards in the form of medals and honorary degrees were showered upon him from both overseas and local universities and institutions in recognition of his outstanding contributions to scientific endeavour. As far back as 1925 he received the Tropical Medicine Medal of the Bernard Nocht Institute of Hamburg, the medal of the German Society of Natural Sciences, Dusseldorf in 1926, the medal of the British Association (of which he was president) at its meeting in Bristol in 1930. In that year he received the medal of the Microbiological Society in Paris, as also an award from the International Poultry Association in London. Amongst the many medals and awards presented in memory of our collegue. are also to be found those of the British Association for the Advancement of Science (Cape Town, 1934), the Veterinary Congress in Switzerland (1938) and the German Academy of National Sciences, Stuttgart (1938). As a member of Rotary International his badge of office denotes his active participation.

Honorary degrees were conferred upon him by the Universities of Cape Town (1943), Rhodes (1948), Witwatersrand (1949), Glasgow (1951), the Orange Free State (1966) and the Free University of Berlin (1966). In 1951 he was elected a Fellow of the Royal Society, London.

Dr. du Toit was extremely interested in scientific and technical co-operation in Africa and was a foundation member of the Scientific Council for Africa South of the Sahara and its secretariat, C.C.T.A. established in 1950. In 1949 he organised and presided at the Regional Scientific Conference for Africa in Johannesburg which was the forerunner of C.S.A. established the following year. He acted in a diversity of positions in an active capacity one of which was his Chancellorship of Rhodes University, Grahamstown from which he retired in 1965.

As chairman of scientific gatherings he possessed the faculty of summing up involved situations by extracting the salient facts in a few words and so frequently pouring oil on troubled waters; as raconteur he was unexcelled.

Dr du Toit is survived by a son and two daughters. The Association wishes to express its sympathy in their loss and felicitate them on a man who served science, and particularly veterinary science, so eminently.

HARVEY SPURGEON PURCHASE

The death of Dr. Harvey Purchase on the 5th June, 1968, has deprived the profession of a good friend and worthy colleague. His passing is mourned by all who knew him.

He was born in 1906 in Fort Jameson, Northern Rhodesia and received his schooling in England. After matriculation he entered the Royal Veterinary College and Birkbeck College, London and obtained the Membership Di-

ploma of the R.C.V.S. and the degree of B.Sc. of the University of London in 1928. The Fellowship diploma was granted in 1931 (Thesis, 'Some Diseases of Domestication and their Prevention') and a Ph.D. degree of the University of London in 1938 (Thesis on Bovine Pleuropneumonia based on results obtained in the field).

He spent a year at the Ministry of Agriculture's Laboratory at Weybridge and then, after the award of a Colonial Fellowship, a year at the Molteno Institute at Cambridge, where he studied tropical diseases.

In 1931, Harvey returned to Northern Rhodesia as a Government Veterinary Officer, the first Rhodesia-born veterinarian to hold such a post. It is not generally known that Harvey had a knowledge of several Africal languages and, in his youth, was an fluent in Chinyanja as in English.

In 1938 he was transferred to Kenya, where, first as a Veterinary Research Officer and later as Chief of the Laboratory at Kabete, he devoted himself to problems concerning the livestock of East Africa. In 1948 he was a member of an F.A.O. Commission to Siam and China, sent to control rinderpest in those countries; his vast experience of the disease in Africa, both in the field and in the laboratory, was of inestimable value to the Commission.

For his valuable services to the Colonies, he was awarded an O.B.E. in 1952.

In 1952, at the comparatively early age of 46 years he resigned from the Colonial Service and joined the firm of Cooper and Nephews, South Africa, as their Technical Manager; later he was appointed to the Board of Directors. Harvey's wide experience of animal disease in Africa was of great value to his new employers, not only to the South African company but also to the whole group of Cooper and Wellcome companies throughout the world.

Because Harvey was a friendly person, he was well liked and because his work brought him into contact with many members of the profession in South Africa he was well known. A kindly man, he went out of his way to be helpful, forgave easily and was a very poor hater.

He was a devoted husband to Vera, his wife, a London girl whose first home in Africa was a house in the wilds of the Northern Rhodesian bush. He was equally devoted to his sons, both graduates in Veterinary Science of the University of Pretoria; Graham is a virologist in the U.S.A. and Iain a toxicologist at the C.S.I.R., Pretoria.

To all three, the profession offers its sincere sympathy.

ANTHONY SOUTH CANHAM

Anthony Canham, better known to his numerous friends and collegues as Archie or Tony, passed away after a brief illness at Port Edward, Natal on 6th March 1968.

Born in Lincoln, England in 1898, he came to South Africa at an early age, and was educated at the South African College School in Cape Town. After matriculating he returned to England where he entered the Royal Veterinary College and qualified as M.R.C.V.S. in 1920.

On returning to South Africa he was appointed as lecturer in veterinary science at the Potchefstroom College of Agriculture.

Here he also carried out studies on the blood of cattle, for which the Royal College of Veterinary Surgeons awarded him a Fellowship in 1926.

In 1927 he assumed duty as Government Veterinary Officer in Bloemfontein, and in 1931 he was transferred to the Allerton Veterinary Laboratory in Pietermaritzburg. Two years later Dr Canham was appointed Research Officer at Onderstepoort, and soon after that he was promoted to the post of Senior Veterinary Officer of the Transvaal.

He, however, never appeared to be completely happy with the purely administrative side of veterinary service, and relinquished that post in 1937 in order to return to research work at Onderstepoort. Here he devoted himself mainly to problems connected with the diagnosis of tuberculosis, and in 1942 obtained the degree of D.V.Sc. from the University of Pretoria for a thesis on "The Tuberculin Test in Guineapigs and Cattle: The Allergic Response of Animals to Extracts of Non-Pathogenic Acid-Fast Bacteria."

During his term at Onderstepoort Dr Canham also held the lectureship in Animal Management in the Faculty of Veterinary Science.

In 1943 he returned to the Allerton laboratory as its director. He served in this capacity for fifteen years, rendering valuable services to the agricultural community of Natal through his investigations and researches into the animal diseases of that province, and by raising the diagnostic service of the laboratory to a high level of efficiency.

He retired in 1958 and with his wife settled in Port Edward.

Tony's friends will remember him as a a very keen sportsman and enthusiastic fisherman throughout his life. He showed great proficiency in whatever game he played — first as a rugby wing threequarter for his school and for the Royal Veterinary College, and thereafter as a tennis player and bowler. In an unostentatious manner and unbeknown to most people, he did a great amount of charitable work. He was also a wellknown figure in Rotary and a past president of Pietermaritzburg Rotary.

He leaves a wife and two sons, to whom we extend our sincere sympathy.

BOOK REVIEW

HELMINTHS, ARTHROPODS AND PROTOZOA OF DOMESTICATED ANIMALS (MONNIG'S VETERINARY HELMINTHOLOGY AND ENTOMOLOGY 6TH EDITION)

E. J. L. SOULSBY

Baillière, Tindall & Cassel, London, 1968. Pp XIX + 824, Figs. 289, Plates XXVIII. Price 84c.

Each section has been reviewed by different workers and their remarks are presented in the same order in which these sections appear in the book.

Helminths

The classification of the trematodes and cestodes is similar to that of the previous volume but the nematodes, with the exception of the Spiruroidea, follow the classification of Skrjabin. In this connection the author has not always been consistent e.g. he retains the genus *Strongylus* for *S. edentatus* and *S. vulgaris,* whereas Skrjabin elevates them to the genera *Alfortia* and *Delafondia* respectively. The life cycles are extremely well done and are up to date, and *Fasciola hepatica* particularly is presented in extreme detail while the hookworms could have been given more detailed descriptions. The pathogenesis and symptoms are by and large lucidly presented and adequate for veterinary students, while in the author's own field of immunology the presentation is excellent and extremely succinct.

There are a few major defects. Readers will search in vain for any reference to the clinical pathology of the common gastro-intestinal parasites of ruminants which are of major importance to the livestock industry. This is in spite of the numerous excellent publications in the last decade on this important aspect. A surprising omission is Gordon's classical work on epidemiology of these worms. Their ecology also is only briefly discussed. These shortcomings have led to recommendations for prophylaxis which are similar in almost every respect to the first edition.

There is no reference to Horak's extensive studies on the host-parasite relationships of *Paramphistomum microbothrium,* which is doubtless due to this parasite being confined to Africa and the Middle East.

As in all previous additions, Fig. 56 which is *Taenia saginata* has been incorrectly labelled *Taenia solium. Hydatid* cysts in cattle have been stated to be 90 per cent sterile which is contrary to the monograph published by Verster. We are not aware that Ortlepp recovered *Echinococcus cameroni* from the lion.

As explained in the introduction anthelmintics have been reduced to a minimum but one minor error should be noted *viz.* that methyridine is highly effective against *Trichuris* app. while thiabendazole is inactive against this parasite in sheep.

These remarks should in no way detract from this, the best, edition of the book that has ever been published and as far as the section on helminths is concerned, it is of a very high standard indeed.

Arthropods

The introductory remarks regarding the Phylum have been slightly abbreviated but are adequate to introduce the subject to veterinary students. Imms' (1948) classification has been adopted and the order in which the subdivisions of the class Insecta is dealt with, commencing with the Apterygota and followed by the Pterygota, comprises a more realistic approach to the subject and makes for greater clarity.

Most of the original illustrations are retained but many are rearranged to conform with the discussion of the particular species and a few excellent line drawings amongst the Acarina are added.

To permit the inclusion of the section dealing with the Protozoa some contraction

of the subject matter of the section covering the Arthropoda was necessary, but this has in no way detracted from the valuable and informative nature of the book.

Together with systematically arranged references to literature dealing with the various topics discussed this new edition can be highly resommended for the use of the veterinary student as also the general biologist.

Protozoa

The addition of a section of parasitic protozoa to the 6th edition of Mönnig's book has added considerably to its value. Prof. Soulsby, who is solely responsible for this section, has certainly succeeded in giving his readers a largely accurate and up to date account of this important branch of parasitology.

A time-honoured classification has been followed, which may not suit the modern taxonomist but is workable and less likely to confuse the person more concerned with the pathogenic effects of these parasites. The morphology and life cycle of the parasite concerned, the pathogenesis and clinical manifestations of the disease it produces, and the immunology, treatment and control thereof are outlined under the appropriate systematic headings. The subject matter has been dealt with in a concise manner. Yet new discoveries, changes in concepts and basic facts have been woven into the text so expertly that it will not fail to maintain the interest of both the under- and postgraduate student.

It is, however, its very conciseness with regard to diseases like theileriosis, besnoitiosis and anaplasmosis which detracts somewhat from its value for South African veterinary students, for instance, who are expected to know more about these important diseases. A few misinterpretations, such as ascribing cases of human piroplasmosis to *Babesia bovis* instead of *B. divergens* infection, and perpetuation of the incorrect definition of the crithidial developmental stage of trypanosomes, have also been included; very few South African veterinarians who have been engaged in the control of *T. parva* (East Coast fever) and *T. lawrencei* (Corridor disease) infections would agree with Soulby's contention that they are different strains of the same parasite. The discovery that *Encephalitozoon* is actually a *Nosema* (Cnidosporidia) has also apparently escaped the author's attention. The quality of the illustrations is rather variable and there are important omissions such as the schizonts of *Theileria*. These criticisms are, however, of minor importance compared to the overall soundness of this section of the book.

R.K.R.

BOOK REVIEW

TIERÄRZTLICHE AUGENHEILKUNDE

GYULA KOMAR UND LASZLO SZUTTER

Translated into German by Adám Faragó

Verlag Paul Parey, Berlin & Hamburg, 1968. Pp 334, Figs. 151, 25 colour plates. Price DM 78.

It is a sobering thought that, until not so very many years ago, the best book on veterinary ophthalmology was published at the end of the last century. During the preceding two decades several works on veterinary ophthalmology have appeared but there was evidence that the language barrier stood in the way of doing justice to work published in other languages.

The present book was written in Hungarian and translated into German, thus making it accessible to more readers. The senior author is emeritus professor while the junior author is responsible for many contemporary articles on ophthalmology.

As is proper in a book of this sort, the detailed anatomy of the eye and adnexa is given first. Examination of the eye occupies 53 pages and a discussion of optics is included. After dealing with the refraction errors, the remaining more than 120 pages are given to pathology of the eye — the aetiology, symptomatology, complications, prognosis and treatment. The information presented is generally very complete. Occasionally some looked for information is not found, e.g. there

are methods of operation for entropion not mentioned, although deserving of description. The discussion of the type of chronic superficial keratitis which is almost confined to German Shepherds is sketchy. In a book entirely devoted to ophtalmology, detail at specialist level can be expected with justification. A fuller presentation of the indications, technique and difficulties associated with cornea transplantation, therefore, would have been in order.

These critical remarks should not obscure the fact that this book is a valuable reference work. It is suggested (if only the price will not rocket out of reach) that its value would be enhanced by a larger number of colour plates particularly of the ocular fundus. No verbal description can hope to take the place of such illustrations.

The book is written in an easy style. The text is attractively presented and the illustrations are clear. The quality of the paper is excellent. Taken as a whole the book is a welcome addition to our veterinary literature. C.F.B.H.

BEELDBERIG

ELEKTRONMIKROFOTO VAN 'n **COWDRIA RUMINANTIUM**-KOLONIE: 'n Nuwe beeld van die welbekende organisme wat hartwater by herkouers veroorsaak.

J. G. Pienaar, Dept. Pat., Veeartsenykundige Navorsingsinst., Pk. Onderstepoort.

FEATURE PAGE

ELECTRON MICROPHOTOGRAPH OF A COLONY OF **COWDRIA RUMINANTIUM**: A new view of the well-known parasite causing heartwater in ruminants.

J. G. Pienaar, Dept. Path., Vet. Res. Inst., P.O. Onderstepoort.

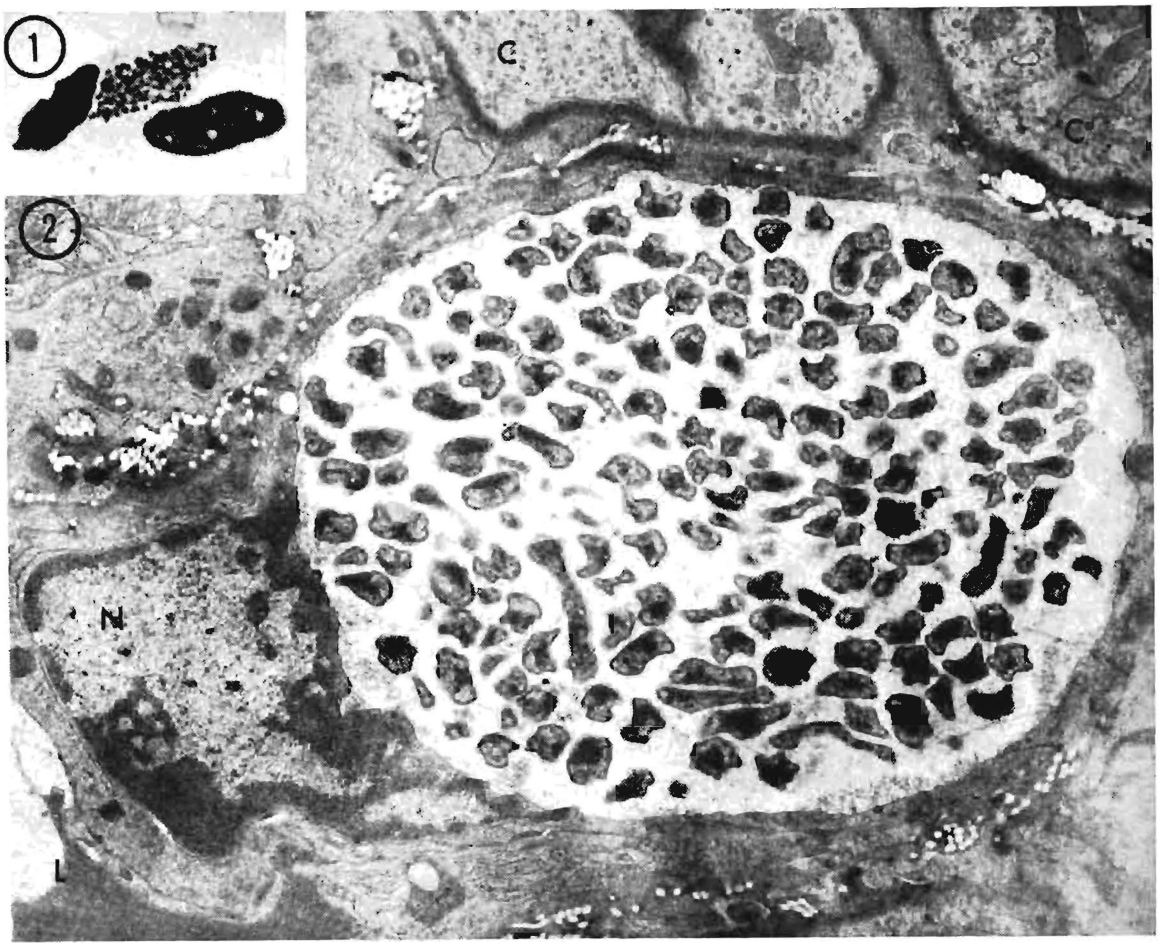


Fig. 1: 'n Kolonie van **Cowdria ruminantium** tussen die kerne van twee endoteelselle in 'n smeer van die hippocampus; Giemsa-kleuring, olieimmersielens, ligmikroskoop.

Fig. 2: 'n Soortgelyke kolonie in die sitoplasma van 'n vergrootte endoteelsel in die **plexus choroidalis**; elektronmikroskoop, x 12,000.

L = bloedvatholte; N = kern van besette sel. Die kolonie is deur 'n dun lagie sitoplasma van die endoteelsel omring. C = epiteelselle van die **plexus chroidalis.**

Fig. 1: A colony of **Cowdria ruminantium** lying between the nuclei of two endothelial cells in a hippocampus smear; Giemsa stain, oil immersion lens, light microscope.

Fig. 2: A similar colony in the cytoplasm of a bulging endothelial cell in the choroid plexus; electron microscope, x 12,000.

L = lumen of bloodvessel; N = nucleus of parasitized cell. The colony is surrounded by a narrow band of cytoplasm of the endothelial cell. C = epithelial cells of choroid plexus.