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# The Occurrence of Congenital Porphyrinuria (Pink Tooth) in Cattle in South Africa (Swaziland).

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## INTRODUCTION.

THIS communication deals with a grade short horn herd, in which some 13 cases of what has been diagnosed as congenital porphyrinuria occured after the introduction of a certain short horn bull as sire. The bull himself does not show any symptoms of the condition. In addition to the occurrence of the condition, the symptomatology and haematology of four clinical cases, as well as the pathology of two other cases will be discussed. The nature of the pigments present in the blood, urine, faeces (one case) and various organs (2 cases) will be discussed independently by Rimington elsewhere in this journal from the chemical point of view.

Congenital porphyrinuria is an abnormality in pigment metabolism, which rarely occurs in man and some animals. Günther (1925) devotes an entire chapter to the discussion of the whole condition under the heading "Haematoporphyrie". The abnormality is very extensively reviewed, particularly as it occurs in man. He points out that haematoporphyrinuria can only be regarded as being present when abnormal quantities of porphyrins are indentified in the urine, as traces occur normally in urine. Even when in various diseases, porphyrins are present in the urine somewhat in excess of the normal amounts, one cannot speak of a haematoporphyrinuria, and their presence in urine and faeces in certain organ diseases, is of no diagnostic significance from a clinical point of view. When abnormal amounts of porphyrins are present, it indicates a constitutional anomaly in pigment metabolism. Such individuals have a tendency towards nervous irritability, sleeplessness and neurosis.

Clinically Günther recognises two forms of the condition: (1) Acute haematoporphyrinuria, which comprises (*a*) the idiopathic form, and (*b*) the toxic form; (2) congenital haematoporphyrinuria.

(1*a*) *Acute idiopathic haematoporphyrinuria.*—Although a hereditary tendency is not well marked in the acute idiopathic cases, the case of Barker and Estes, quoted by Günther is nevertheless significant, as four sisters developed symptoms of the condition at more or less the same age (18 to 22 years). There would seem to be evidence that the mother and grandmother (of these four sisters) on the mothers side, had attacks of vomition, colic, obstipation and discolouration of the urine. Nervous disorders seem to occur in families of affected individuals. The symptoms include: intestinal disturbances—spasm of portions of the small intestine, atony of other portions of gastro-intestinal canal, with severe constipation; nervous disturbances—sleeplessness, paralysis, etc., markedly increased excretion of what is described as haematoporphyrin in urine and faeces, at the time of the acute attack, but the subsidence of the excretion of these substances during the intervals between attacks; pigmentation of the skin; no well defined changes are regularly present in the blood, photosensitization is absent. Intervals between attacks vary from weeks, months, to one to two years and more. Pigmentation of the bones is not a characteristic of this form of the condition. The cause is not known.

(1*b*) *Acute toxic haematoporphyrinuria.*—These acute toxic cases are differentiated from the idiopathic form in that the use of substances such as sulphonal and trianol bring on the attacks which are usually fatal, ending in ascending paralysis. Günther would seem to suggest that the insomnia which leads to the abuse of these narcotics may indicate a constitutional abnormality in which the use of the drugs mentioned would result in an acute attack of haematoporphyrinuria. The use of sulphonal favours attacks especially in women.

2. *Congenital haematoporphyrinuria.*—Whereas in the acute form 92 per cent. of the reported cases were females, the congenital form seems to occur more frequently in males (78 per cent.). Cases have repeatedly been found in more than one member of the same family and the presence of a hereditary factor is suggested, but not yet definitely established. In one case (also Gray's 1926 case) the parents were related. The symptoms are: (1) Photosensitization, especially during spring, producing lesions of exposed parts of the skin (Hydroa Aestivale), particularly of the ears, nose, cheeks, eyelids, upper lip, fingers, the back of the hand, nails. These lesions may produce very marked disfiguration and deformity. It is remarkable that lesions do not occur on the chin and the mouth region. There is pigmentation of the skin, hypertrichosis, frequently with excessive growth of hair under the arms, eyebrows, beard from the chin and sometimes of the pubic hairs. The hair of the head may be coarse.

(2) The excretion of porphyrins in urine and faeces. The urine is of reddish brown colour. The brownish discolouration is due to the temporary presence of large amounts of urobilin and urofuscin. In one case (Fischer and Schumm) quoted by Günther, the crude porphyrins determined quantitatively were excreted in unchanged amounts for a period of three years. In some cases porphyrins are

present in the serum. Schumm, quoted by Günther, found amongst others, uroporphyrin, whilst Fischer also quoted by Günther, believes that Coproporphyrin is present in the serum. Sweat and saliva are free from porphyrius. The teeth may be yellowish white, the roots reddish brown.

In some cases [Mackey and Garrod (1922) and (1926) and Ashby (1926)] the temporary and permanent teeth are pink. There is dark brown discolouration of the bones whilst tendons and ligaments remain unpigmented. In some cases there is a severe anaemia (anisocytosis, poikilicytosis, polychromasia, punctate basophilia) whilst in others normal counts are present. The course of the disease is influenced by a number of factors. Some individuals reach ages of 50 and 65 years, others die younger from some intercurrent disease, but if sufferers from the condition take certain precautions to protect themselves against harmful rays, the bad effects of the condition can be controlled to a considerable extent. Up to that time no clinical case in an animal was available for examination and study.

Garrod (1892) has shown that haematoporphyrin is frequently present in minute amounts in healthy individuals and in larger amounts in urines of sufferers from a great variety of diseases.

Mackey and Garrod (1922) and (1926) describe a case of congenital porphyrinuria in a boy in whom at $4\frac{1}{2}$ years of age there was delayed ossification of the ulna and some carpal bones, but ossification was normal at $9\frac{1}{2}$ years. There is excessive vulnerability of the skin to slight injuries, for instance the rubbing of the ear with cotton-wool soaked in spirits, produces a bleeding surface. Later on the spleen and the liver become enlarged. The number of red cells remained at a level round about 4·5 millions when counted in 1921, 1925 and 1926. There are no gross changes in the differential counts, but there would seem to be an increase in the number of cells described as endothelials. There is anisocytosis, poikilocytosis, polychromasia, punctate basophilia and normoblasts. In confirmation of the views expressed by Price Jones and Robitschek, quoted by Mackey and Garrod (1926), these changes are interpreted as being indicative of an actively functioning bone marrow. This is regarded as a compensatory reaction to haemolysis which is taking place, but it is not believed that haemoglobin from broken down corpuscles is the parent substance of the porphyrins excreted. The hyperactivity of the bone marrow maintains the red cells at a fairly high level, so that no anaemia develops in spite of haemolysis, which is thought, may be due to the action of light on the sensitized blood in the peripheral circulation. The authors quote Schumm and Fischer who have shown that the staining of the bones is due to uroporphyrin and not to coproporphyrin. The authors claim that at that time their case and that of Ashby (1924), are the only ones amongst the recorded congenital porphyrinuria cases, in which there is conspicuous discolouration of the teeth including the enamel. They are inclined to believe that the explanation for this is that large amounts of porphyrins were available for staining these structures at the time they were being formed even during foetal life.

In the case of Ashby (1924) already referred to there is in addition to the pink teeth, also photosensitization and red urine. These changes were present at birth. A cream containing quinine seems to protect the skin against the harmful rays. Gray (1926) describes a case in a girl, who was apparently healthy up to 5 years of age. In addition to the presence of red urine, skin lesions, yellowish brown teeth, there is considerable hirsuties on the exposed parts of the body. She is one of a family of seven, all of whom are free from the disease, but the parents are first cousins.

Garrod (1923) points out that of the few known cases of congenital porphyrinuria in humans, in several instances more than one member of the same family suffered from the same complaint. This is apparently the only evidence suggestive of the possible hereditary nature of the condition, apart from other factors, such as its greater incidence in males, in which respect it strongly resembles other conditions such as albinism and alcaptonuria which are thought to be transmitted as recessive hereditary characters. Garrod is inclined to agree with Fischer who, at that time, favoured the view that porphyrin is an intermediate product in the conversion of haemoglobin into bilirubin.

Mason, Courville and Ziskind (1933) state that in all 27 cases of congenital porphyrinuria are recorded. They describe four cases of the acute idiopathic type of the disease. The symptoms recorded include, amongst others, abnormal urine, abdominal pain, disturbances of the nervous system, with parenchymatous degeneration of peripheral nerves, ganglion cells of dorsal root and sympathetic ganglia and central nervous system, ending sometimes in death from ascending paralysis.

Borst and Köningsdörffer (1929) give a very exhaustive description of the pathology of the case Petry. Further reference will subsequently be made to their pathological findings and to their views on the pathogenesis of the condition.

Hegler, Fraenkel and Schumm (1913) describe a case of a young woman in whom photosensitization was absent, there was no red urine at the time of the examination, but on post mortem examination the bones were found to be discoloured.

Fraenkel (1923) injected porphyrins prepared from the urine of Petry into experimental animals. The pigment was deposited in growing bone and he was further able to show that after fractures in adult animals, pigment was deposited only in the callus and not in the rest of the skeletal bones. He is of opinion that pigment once laid down in bone, remains permanently. He states that all cases of congenital porphyrinuria in man are not photosensitive and this he also finds in experimental cases.

Fischer, Hilmer, Lindner, & Pützer's (1925) work concerning the porphyrins present in Petry, is adequately reviewed by Rimington elsewhere in this journal.

Turning now to the recorded cases in domesticated animals (bovines and swine), as far as one can make out from the descriptions given, there is not a single instance in which an animal showing clinical symptoms was available for examination. In spite of the fact that Tappeiner (1885) (paper not available) quoted by Poulsen (1910) and Schmey (1913), showed that a pigment which he described as haematoporphyrin, is present in bone of a swine, which had socalled ochronosis, confusion of this condition in animals with true ochronosis of humans was maintained in veterinary literature for a number of years [see the cases of Moselman and Hebrant(1898), Rémy, Brouvier and others quoted by Schmey (1913)].

Mettam (1910) (pig) and Witte (1914) (bull and calf) describe as ochronosis what are almost certainly true cases of porphyria. Witte's cases are of special interest in that the affected bull was the sire of an affected calf, but a calf from the same bull out of a different cow was normal.

Poulsen (1910) was the first author to recognise that ochronosis of animals and man are two entirely different conditions. Whereas in man the pigment in ochronosis is melanin, in the ochronosis as described in animals the pigments are haemosiderin and haematoporphyrin. In man the bones as well as tendons, ligaments and cartilage are pigmented, whereas in animals only the bones are pigmented and tendons, cartilage and ligaments are entirely free from pigment.

Schmey (1913) briefly reviews human cases of ochronosis but gives full details of 15 cases of ochronosis described up to that time in animals. He quotes the spectroscopic examination of the pigment according to various authors: (1) Moselman and Hebrant (1898)—heifer—melanin. [Poulson (1910) states that the chemical reactions described and the spectroscopic measurements recorded by these authors, do not justify them in concluding that they were dealing with the pigment melanin].

(2) Poulson (1910)—case No. 3—cow—haematoporphyrin in bones (haemosiderin in bone marrow).

(3) Tappeiner (1885)—bones of a pig—haematoporphyrin.

(4) Ingier (1911) (2)—pig—not a blood pigment—probably melanin or a derivative of chlorophyll.

(5) His own cases (1912)—2 pigs—haemoglobin derivative, probably that of acid haematin, but not haematoporphyrin. (The two bands which he describes may equally well be that of acid uroporphyrin as that of acid haematin).

Schmey further quotes Schenk and Colberg each of whom describes discolouration of the enamel of the teeth in a cow and a three day old calf respectively.

Schmey emphasises that ochronosis in humans is an entirely different condition to what has been described as ochronosis in animals and that osteohämatochromatosis more suitably describes the condition in animals. His lead in thus designating the anomaly in animals was subsequently followed by Teutschlaender (1914); Maraev (1928) and Cohrs (1931) (Osteohämochromatose); Kitt (1921) (Haemochromatosis ossium); and by Joest (1926) (Hämochromatose).

Teutschlaender (1914) states that at that time 19 cases (including his own) of socalled pseudo-ochronosis, in animals are reported in the literature. In one of his own cases as well as one of Poulsen's referred to by him [also a case of Fikentscher (1930)], only a proportion of the bones were pigmented. In some cases the pigment is not uniformly present throughout the bones, but is laid down in alternating darker and more lightly strained rings.

Fikentscher (1930) describes changes in organs and bones from a bovine, concerning which nothing clinically abnormal was known. By the use of a fluorescence microscope the presence of porphyrins could be demonstrated. His work was confirmed by the chemical observations of Fink quoted by him.

## OWN CASES.

### History.

In view of the fact that these cases are the first to be described clinically, it is proposed to give a detailed description of the history of the whole herd. Mr. Cassie, Anniswells, Bremersdorp, Swaziland, introduced a pure bred short horn bull No. 7015, to his grade short horn herd in 1931. The owner has for some time been grading up his herd with short horn bulls. Three bulls were used. The first bull is not in any way related to bull 7015, but the second bull is out of the same herd, as the sire of bull 7015. After the introduction of bull 7015 he found that some of his young stock began to do badly, soon after weaning. Such animals loose condition and develop a rough staring coat. In some cases scabs are present in the middle of the back, where the hair parts; crusts and scabs may be present around the eyes and nose and there may be a nasal discharge. The urine is red and may remain so for months on end. These cases do not respond to treatment for redwater (piroplasmosis) by injection of trypan blue. In December 1934 one such animal was killed and apart from the red urine and a certain amount of discolouration of internal organs, Mr. Cassie did not observe anything abnormal, and the carcase was given to the natives for food. When the natives cut up the carcase they saw the pink teeth and when Mr. Cassie's attention was directed to this he noticed that not only the teeth, but all the bones were of a reddish brown colour. One of the natives who consumed a large amount of this meat had violent colic, but none of the others reported sick. During 1935, whilst on holiday in Swaziland, the Principal Veterinary Officer, Mr. W. A. Elder, M.R.C.V.S., consulted me concerning

these cases. On examining the mouths of these animals, I was amazed to see all the teeth (temporary and permanent) of a brownish pink colour. Not having seen anything like it before, I could at the time only speculate as to the nature of the condition and ventured rather boldly an opinion of a possible haemopoietic disturbance. However, arrangements were made for the collection of specimens and these were subsequently submitted by Elder, when the condition was immediately recognised to be osteochaemochromatosis as described by Kitt, Joest and Cohrs, already referred to. A piece of bone was handed to Rimington in order to determine the nature of the pigment present. After obtaining the pigment in solution, he was able to indentify it as porphyrin spectroscopically and chemically and the diagnosis of porphyrinuria was definitely established.

During 1935 Cassie had more or less 270 head of cattle. Of these 67 were cows (it is not possible to give definite figures of the numbers of breeding stock during 1931, 1932, 1933 and 1934). Since the first case was observed in December, 1934, and the beginning of 1936, 12 cases of the condition were found in Cassie's herd (porphyrins were determined chemically and spectroscopically in six of these 12 cases) and one case was found on an adjoining farm A see below). The bull 7015 was occasionally used on this farm A and this one case is his offspring. On the adjoining farm C on the South side, the bull was never used as a sire and no cases of the condition were observed in any of the animals there. Bull 7015 was the only bull used in Cassie's herd at that time.

E.

| | Adjoining farm 260 head of cattle | Cassie's farm 270 head of cattle, 67 cows, 1935 | Adjoining farm South side, bull 7015 not used. | |
|---|---|---|---|---|
| N. | A. | B. | C. | S. |
| | 1 case of porphyria sired by bull 7015 | 12 cases of porphyria all sired by bull 7015 | No cases of porphyria observed. | |

W.

Of the 13 cases, 10 are males and 3 are females. In 3 cases the abnormality was seen by Cassie immediately after birth; the teeth and bones are pink, but according to the owner, the urine is not discoloured. The last of these three cases was also seen by Mr. C. T. Nilson, B.V.Sc., Government Veterinary Officer, Bremersdorp, to whom I am indebted for a personal communication concerning it. Unfortunately this bull calf was destroyed by the owner before arrangements were made for the collection of specimens from him. The parents of this calf are blood relations. He is out of a daughter of bull 7015 and sired by the same bull. The mothers of the other 12 cases may be related to the bull, as the bull which is in all probability their sire, is out of the same herd as the sire of bull

7015. This bull, 2 normal cows (mothers of affected animals), 3 normal heifers, 7019, 7021 and 7022 (daughters of the bull) and 4 affected animals (3 steers, 7016, 7017 and 7018 and one heifer, 7023) were purchased by the Division of Veterinary Services and are now at Onderstepoort under observation and being used for breeding purposes (Figs. 1 and 2).*

Fig. 1.—Bull and four affected animals, some daughters of the bull and one cow (extreme right of picture) mother of affected bullock beside her.

Fig. 2.—Bull and four affected animals.

Since this article has gone into press two of the above heifers 7021 and 7022, both daughters of the bull and served by the bull, calved on the 8.3.37. Both calves are heifers. The calf of 7022 is normal but the calf of 7021 has pink teeth. The urine of this animal is not discoloured when it is being voided. No porphyrin bands can be recognised spectroscopically when the urine was examined within 48 hours after the birth of the animal.

*Ancestry of bull* 7015.—The bull was bred by Mr. B. of S.P.K.P. Eastern Transvaal. He is a pure bred short horn but not registered. The sire of the bull is Br. 2. This bull died in 1929. The owner does not remember seeing anything abnormal in the progeny of this bull, and those of his descendants which were available in 1935, were examined and found to be normal, by Elder, to whom I am indebted for a personal communication concerning this point. The dam, Bilsington Admiral, was never a healthy (?) cow, was in fact a bad doer and even thought to be tuberculous. The owner, however, states that she did not have tuberculosis, but it is not known on what grounds this statement is made. According to the owner animals which did not thrive were met with from time to time in his herd and he particularly remembers a bullock bred from Bilsington Admiral, he was a bad doer and was eventually destroyed. This animal may possibly have had porphyria. Bilsington Admiral died eventually on this owners property. She is supposed to be descended from Texas stock brought into South Africa after the Boer War. It is unfortunate that both the herds from which the dam and the sire of bull 7015 came, are no longer in existence, No case of porphyria could be found in a few herds to which the dispersal of the now non-existent herds could be traced. However, it is hoped that the breeding experiments referred to above, will produce conclusive evidence of the hereditary nature of the condition, already strongly suggested by the foregoing history.

## Clinical Features.

*General.*—As already stated the usual history is that affected animals do not thrive. This is particularly noticeable soon after weaning. The animals lose condition and develop rough staring coats. In one animal (7017) there is a lot of coarse, long hair on the head, behind (polar region) between and below (frontal region) the horns. This may not be analogous to hypertrichosis described in some human cases (Günther, Gray) as one sometimes finds normal bovines having this course hair about the head. No definite statement concerning temperament can be made. When the animals arrived here, they were all in rather poor condition and were usually handled with ease. However, one old cow (a normal cow herself), the mother of an affected animal, gave birth to a normal heifer calf, two days after she arrived here. She was so weak that she had to be lifted, but in spite of that she charged anybody who came near her and on account of her weak state she usually went down in the attempt. The animal with the coarse hair (7017) is bled weekly and on one occasion was so nervous and irritable that he charged the assistant and natives in his box. The cow unfortunately died from metritis within a week after arrival here. Although these animals cannot be truthfully described as friendly, these were the only occasions that they showed such a marked degree of irritability and nervousness. In the circumstances one is not justified in definitely attributing to this a pathological significance analogous to nervousness and irritability described in some human cases, especially as occasional normal animals will sometimes also behave like this.

The skin of the first animal examined, was simply one mass of lice, this animal was not regularly dipped, on account of its weak state and this was undoubtedly largely responsible for the gross parasitic infestation. In these circumstances it would not be true to say that such animals are prone to ectoparasitism. If this should be the case, it would not be unexpected on general grounds.

Details of the pulse, respirations and ruminal movements of four affected animals and one normal animal (7022) of the same breeding from the same farm will be found in the accompanying table.

| | Pulse. | | Respirations. | | Ruminal Movements during 5 Minutes. | |
|---|---|---|---|---|---|---|
| | May. | October. | May. | October. | May. | October. |
| 7016. | 70 | 74 | 36 | 39 | 8 | 10 |
| 7017. | 66 | 84 | 28 | 23 | 10 | 12 |
| 7018. | 80 | 72 | 38 | 36 | 11 | 11 |
| 7023. | 70 | 74 | 36 | 45 | 7 | 9 |
| 7022. | 72 | 90 | 44 | 36 | 8 | 10 |

No significant functional differences are present. The individual variations are probably due to excitement during handling, etc.

These animals were placed on temperatures for long periods. The temperatures recorded vary from 100° F. to 105° F. in affected as well as unaffected animals. These individual variations are probably due to a number of factors, such as handling, antimosan injections and climatic conditions.

*Photosensitization.*—Lesions are present on the skin which is not protected by hair. Scabs and crusts form around the eyes and nostrils (Fig. 3); there is a nasal discharge and sometimes ulcers

Fig. 3.—Animal from which specimens 16786 were collected. Lesions around eyes and nostrils.

on the buccal mucous membrane, particularly of the gums and the lips. In four out of the six animals, which were available for examination, scabs are present on the middle of the back, where the hair parts. In one case the scab measured 12 by 5 cm. (Fig. 4). In at least two other cases the scabs have a similar situation and look almost exactly like the one just described. In one of these cases (7017) scabs are in addition also present behind the horns and around the base of the ears. The hair seems to be efficient protection against the harmful rays of the sun, as lesions are not present even in unpigmented portions of the skin and various parts of the body, which is covered by hair.

Fig. 4.—Lesions on portions of the skin not protected by the hair. The same animal as in figure 3.

*Teeth and bones.*—In all cases the temporary and permanent teeth are conspicuously discoloured. Six out of the 13 cases were examined personally. There is no reason why the owner's word should not be accepted for the other cases. The owner's word must also be accepted for the statement that in three out of the 13 cases, pigmentation of the teeth was observed immediately after birth. When the mouth is opened, the general colour impression one gets of the teeth, is that of pink. An attempt was made to reproduce as truly as possible the colour of the incisor teeth in a portion of a formalin preserved mandible (Fig. 5). If the masticatory surface of an incisor tooth is examined with the lingual surface of the tooth towards the observer, the macroscopically clear unpigmented enamel is seen to stand out in sharp contrast to the dentine which is of a brownish pink colour. The labial surface of the tooth on the other hand has a dull brownish pink colour. It is, however, not the enamel which is discoloured, but the dentine which is seen through the somewhat translucent enamel. In the case of the molar teeth, the cement substance on the buccal and lingual surfaces is of a dark red colour. The colour of the table of the tooth varies. The outer enamel is white. The dentine immediately within the outer enamel is brownish pink. Inside this again is the infundibulum, having a white enamel ring, within which the cement substance appears

dark red, almost black in colour. Macroscopically therefore, the dentine is pigmented and the cement substance would seen to contain the pigment in even greater concentration, but the enamel is free from pigment. As far as can be determined clinically the pigment distribution in the teeth of the four living animals now at Onderstepoort is similar to that of the case just described. Schmey (1913) also found the enamel of an effected pig to be free from pigment; unfortunately the papers of Colberg and Schenk, who describe pigmentation of the enamel in the teeth of affected bovines, quoted by Schmey, are not available to me. The human cases in whom pigmentation of the enamel is claimed to be present, as described by Garrod and Mackey (1922) and (1926) and by Ashby (1924) have already been referred to.

All the bones are of a deep reddish brown colour (Fig. 6), but cartilages, articular surfaces, ligaments and tendons have a normal colour. The long bones are not uniformly discoloured. This is clearly seen in transverse sections (Fig. 7) where there are alternating darker and more lightly stained rings as described by Teutschlaender (1914) and others previously referred to. Whether this is due to an alternating increased and decreased excretion of porphyrin or to deposition of porphyrin corresponding to alternating periods of growth in summer and absence of this during the winter cannot be stated at the moment. The pathological description of the bones will not be included in this paper, but will be presented at some later date.

Fig. 8.—Bull 7015. Harness with bucket for the collection of urine.

*The Urine.*—In no case was a discolouration of the urine observed immediately or soon after birth. A slight discolouration may easily have been missed, when the urine is examined with the naked eye whilst the animal is urinating and yet such a urine may have contained significant amounts of porphyrin. The urine of five cases was available for examination. The urine is not red as occurs

PORTION OF MANDIBLE.

Fig. 5.

SCAPULA.

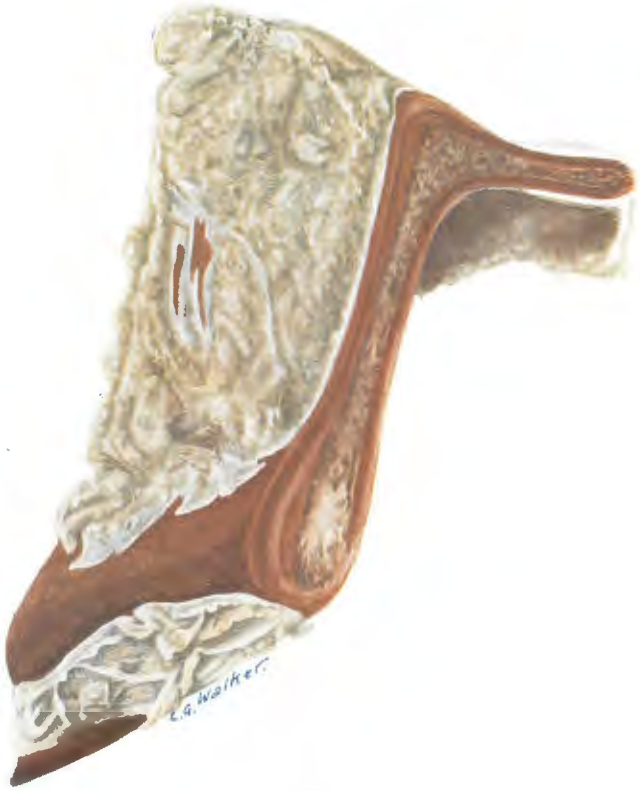

Fig. 6

METATARSUS.

Fig. 7.

in cases of haemoglobinuria. The colour of the urine as seen in the urinating animal varies from deep amber to reddish brown. When collected in a flask, even the more lightly coloured urines are seen to have a reddish tinge. For the collection of the urine in males, a special harness was designed, to which a bucket can be fastened and suspended under the abdomen of the animal. (Fig. 8.) The urine should be collected in a glass container as metal is unsatisfactory for porphyrin work. Rimington (1936) found uroporphyrin and coproporphyrin in all five cases. The one case he reports fully in this journal and the urines of the other four cases, together with that of a control unaffected animal, were determined by him as:

| | *Colour.* | *Spectrum.* | *Nature of Pigment.* |
|---|---|---|---|
| 7016 Male, 2.3.36..... | Reddish brown..... | 574·3, 537·8, 500·8... | copro- and uro-porphyrin. |
| 7017 Male, 4.3.36..... | Pinkish brown..... | 574·8, 538·7, 500·2... | copro- and uro-porphyrin and some metal complex. |
| 7018 Male, 28.2.36.... | Deep red..... | 574·4, 538·3, 504·0... | copro- and uro-porphyrin and some metal complex. |
| 7023 Female, 4.3.36... | Amber-pink tinge... | spectrum indistinct.... | copro- and uro-porphyrin. |
| 7021 Female, 4.3.36... | Daughter of bull—normal animal—urine normal appearance—no porphyrin present. | | |
| 7015 Male..... | Bull—yellow urine—turbid, undetermined protein present—but no porphyrins present. | | |

At this stage it is necessary to mention that these animals were unfortunately also infected with Schistosomes. Le Roux (1929) quotes a number of authors (Stiles, 1898), (Piller 1915), (Kaup, 1918), who describe haematuria in cattle infected with schistosomiasis. Haematuria is not present in any of these cases, neither is there any discolouration of the urine in other animals infected with bilharzia, but not affected with porphyrinuria, from the same farm. Some of the bilharzia infected animals were treated with tartar emetic, but as the use of this drug did not completely succeed in curing the animals, antimosan was used, with apparently greater success. (For details of treatment see tables 1 to 5). The excretion of porphyrins continued for some months after completion of the treatment. In the circumstances there would seem to be very little doubt that the excretion of porphyrins in the urine of these animals cannot in any way be associated with the bilharzia infection which was present. To make absolutely certain of this point, it would be necessary to examine the urine of bilharzia cases in which haematuria is present, for porphyrins. Such cases have unfortunately up to the present not been available for examination. At the moment no opinion can be given as to whether there may be a variation in the daily amount of porphyrin excreted by these affected animals.

## Haematology.

Of the four clinically affected animals, two young bullocks (7017 and 7018) seem to have the condition in a more severe form. A haematological examination of these two cases was made weekly and the other two (7016, young bullock, and 7023, heifer) were examined bi-weekly. In addition a bi-weekly examination was

made of another normal animal (7022, heifer) of the same breeding, of more or less the same age and from the same farm. This animal is being regarded as a control. Details concerning these haematological observations will be found in the accompanying graph and tables (7018—graph 1 and table 1; 7017—table 2; 7016—table 3; 7023—table 4; 7022—table 5).

These observations lose a great deal in value on account of the fact that even as late as the 20th October, 1936, bilharzia eggs were found to be present in the faeces of animal No. 7017, indicating that the antimosan treatment was not successful in completely destroying the bilharzia infection in all the animals. However, in the case of 7018 bilharzia eggs were never found in its faeces on the two or three occasions that an examination was made subsequent to treatment, the last examination being made on the 20th October, 1936. Even though this is the case, the disturbing possibility that bilharzia infection may be a contributary factor in the production of any haematological changes which may be present, cannot be ignored. In any case no marked morphological changes are present in the red cells of any of these animals, with the exception of No. 7017, in which case the anaemia is largely if not entirely due to an acute attack of anaplasmosis.

7018.—*Young ox, 2-3 years old.*—When the animal arrived here in December, 1935, this animal was undoubtedly the poorest of the lot. His red counts were 2·9 million per c.c. The red counts increased to 4·6 million per c.c. a month after the animal was treated for bilharzia and then remained at this level for a period of 3 months. (See graph 1). The animal was then placed in a dark stable (11.8.36) in order to see to what extent further improvement in its blood picture and general condition will take place if it is protected against the harmful rays of the sun. The red counts did actually increase, until they finally fluctuated around about the 5·5 million level. Whether this is due to the absence of haemolysis as a result of the protection afforded by the dark stable cannot be stated at the moment, but it is hoped to clear up this point at a later date.

The blood relations of this animal, more or less of the same age and running in a small paddock in which they can freely move about, have counts which vary from 6-8 million per c.c. of blood. Canham (1930) has shown that animals confined to pens will have red counts which are 1 to 3 million per c.c. of blood less than those of similar animals running in the veld. In an ordinary loose box, in which animal 7018 is always being kept, he cannot get any exercise at all and it is believed that his counts of 5·5 million per c.c. of blood can in the circumstances be regarded as normal for him.

No well marked morphological changes are present in the red cells of this animal. Occasional normoblasts and occasional punctuate basophiles were recognised, but not in sufficient numbers to regard them as a significant disturbance of the blood picture. It is conceivable that if the exposure of the animal to the sun had been continued, a more severe degree of anaemia may have developed, when more pronounced morphological changes in the red cells may have taken place.

GRAPH I.

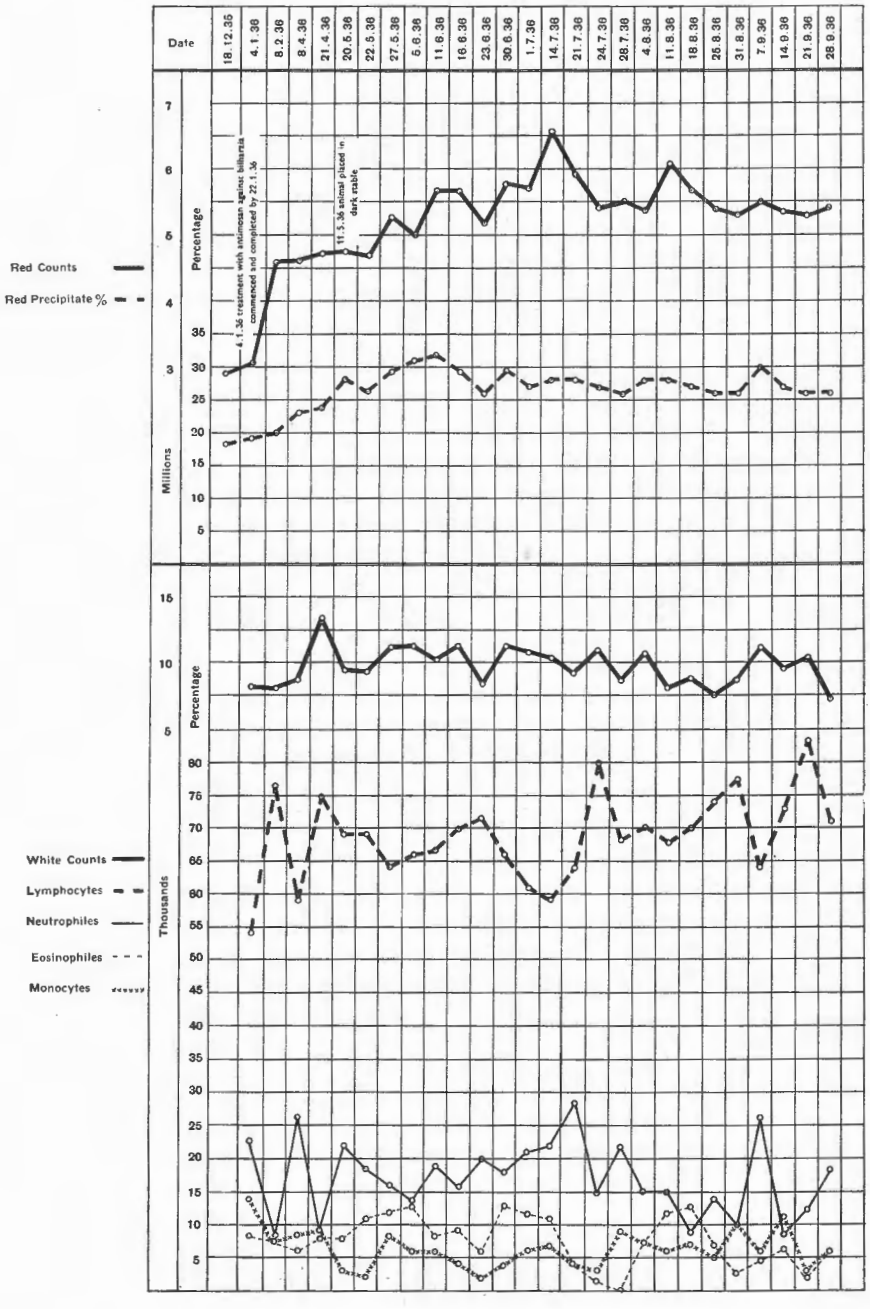
Date
18.12.35
4.1.36
8.2.36
8.4.36
21.4.36
20.5.36
22.5.36
27.5.36
5.6.36
11.6.36
16.6.36
23.6.36
30.6.36
1.7.36
14.7.36
21.7.36
24.7.36
28.7.36
4.8.36
11.8.36
18.8.36
25.8.36
31.8.36
7.9.36
14.9.36
21.9.36
28.9.36
4.1.36 treatment with antimosan against bilharzia commenced and completed by 22.1.36
11.5.36 animal placed in dark stable
Millions
Percentage
Thousands
Percentage
Red Counts
Red Precipitate %
White Counts
Lymphocytes
Neutrophiles
Eosinophiles
Monocytes

TABLE I.

*Affected Bovine* 7018—*Young Ox—2 Permanent Incisors December* 1935.

| Date. | R.C. | R.P. | W.C. | L. | M. | N. | E. | B. | Remarks. |
|---|---|---|---|---|---|---|---|---|---|
| 18/12/35..... | 2·9 | — | 5·2 | — | — | — | — | — | — |
| 4/1/36..... | 3·0 | 19 | 7·7 | 54 | 14 | 23 | 8 | 1 | Anisocytosis, jolly bodies and punctate basophilia rare. |
| 18/2/36..... | 4·6 | 20 | 7·7 | 77 | 7 | 8 | 7 | 1 | Anisocytosis, jolly bodies and punctate basophilia rare. |
| 8/4/36..... | 4·6 | 23 | 8·5 | 59 | 28 | 6 | 6 | 1 | Anisocytosis, jolly bodies and punctate basophilia rare. |
| 21/4/36..... | 4·7 | 24 | 13·3 | 75 | 9 | 9 | 7 | 0 | Cells normal. |
| 20/5/36..... | 4·6 | 28 | 9·4 | 68 | 3 | 22 | 7 | 0 | Cells normal. |
| 22/5/36..... | 4·7 | 26·5 | 9·3 | 69 | 2 | 18 | 11 | 0 | Cells normal. |
| 27/5/36..... | 5·3 | 29 | 11·2 | 64 | 8 | 16 | 12 | 0 | Cells normal. |
| 5/6/36..... | 5·0 | 31 | 11·3 | 65 | 6 | 14 | 13 | 2 | Punctate basophilia very rare, Theileria mutans rare. |
| 11/6/36..... | 5·7 | 32 | 10·1 | 67 | 6 | 19 | 8 | 0 | Cells normal. |
| 16/6/36..... | 5·7 | 29 | 11·4 | 70 | 4 | 16 | 9 | 1 | Occasional punctate basophilia. |
| 23/6/36..... | 5·2 | 26 | 8·1 | 72 | 2 | 20 | 6 | 0 | Cells normal. |
| 30/6/36..... | 5·8 | 29 | 11·5 | 66 | 4 | 17 | 13 | 0 | Occasional punctate basophilia. |
| 1/7/36..... | 5·7 | 27 | 10·8 | 61 | 6 | 21 | 12 | 0 | Occasional punctate basophilia. |
| 14/7/36..... | 6·5 | 28 | 10·2 | 59 | 7 | 22 | 11 | 1 | Occasional punctate basophilia. |
| 21/7/36..... | 5·9 | 28 | 9·5 | 64 | 4 | 28 | 4 | 0 | Occasional punctate basophilia. |
| 24/7/36..... | 5·4 | 27 | 11·1 | 80 | 3 | 15 | 2 | 0 | Occasional punctate basophilia. |
| 28/7/36..... | 5·5 | 26 | 8·7 | 68 | 9 | 22 | 0 | 1 | Occasional punctate basophilia. |
| 4/8/36..... | 5·4 | 28 | 10·4 | 70 | 7 | 15 | 7 | 1 | Occasional punctate basophilia. |
| 11/8/36..... | 6·1 | 28 | 7·9 | 67 | 6 | 15 | 12 | 0 | Cells normal. |
| 18/8/36..... | 5·7 | 27 | 8·7 | 70 | 7 | 9 | 13 | 1 | Young forms of eosinophiles and one or two eosinophile myelocytes, otherwise normal. |
| 25/8/36..... | 5·4 | 26 | 7·5 | 74 | 5 | 14 | 7 | 0 | Cells normal. |
| 31/8/36..... | 5·3 | 26 | 8·8 | 77 | 10 | 10 | 3 | 0 | Occasional Theileria mutans otherwise normal. |
| 7/9/36..... | 5·5 | 30 | 11·4 | 64 | 6 | 25 | 5 | 0 | Occasional punctate basophilia. |
| 14/9/36..... | 5·4 | 27 | 9·5 | 73 | 11 | 8 | 6 | 2 | Occasional punctate basophilia. |
| 21/9/36..... | 5·3 | 26 | 10·2 | 83 | 3 | 12 | 2 | 0 | Cells normal. |
| 28/9/36..... | 5·4 | 26 | 7·2 | 70 | 6 | 18 | 6 | 0 | Occasional normoblasts, one eosinophile myelocyte—cells normal. |

Animal was found to be infected with bilharzia—was given 25–30 cs. of 6·3 per cent. antimosan intravenously until 10 injections were given. Treatment commenced 4/1/36 and completed 22/1/36. Animal was placed in dark stable as from the 11/5/36—no parasites seen in faeces on 20/10/36.

The improvement in the blood picture was not nearly as striking as the improvement in the general appearance and condition of the animal. (See figs. 9 and 10). His weight increased from 630 lb. at the time he was placel in a dark stable (11.5.36) to 970 lb. on the 26.10.36 (5½ months in dark stable) at the rate of more or less 2 lb. per day. There are possibly a number of factors which are responsible for this remarkable general improvement in condition, etc. By eliminating the photosensitizing factor, the animal is now in a state of greater complacency and is in a position not only to relish its food, but will probably also eat more of it. Being

Fig. 9.—Animal 7017; 11.5.36; weight, 630 lb.

alone in the stable, it is no longer necessary for him to compete with its fellows, in collecting his share of the food and may possibly on this account eat more and in greater comfort. The food he is getting is otherwise practically the same as he was getting before, except that a small daily ration of green food was prescribed. in order to avoid the possibility of introducing a vitamin deficiency factor in the absence of sunlight. The improvement in his blood picture is probably also a factor, but here again, it cannot at present be stated whether the elimination of the harmful rays is directly (absence of haemolysis) or indirectly responsible for this.

The scabs around nose and eyes have disappeared and the scab on the back is completely healed. At the moment the general state of health of the animal is excellent, but the porphyrin excretion continues, and the urine remains discoloured. It is true that the urine is no longer as darkly coloured as it was some 5 month ago, before the animal was placed in the dark stable. At that time the colour of the urine was described as deep red; to-day the colour is

deep amber with a brownish tinge, but still very much darker in colour than the urine of the normal animal. It is difficult to avoid the conclusion that the changed enviroment, viz. the dark stable is responsible for this and the brown pigment in the urine is mainly due to the effects of the rays of the sun on the photosensitive porphyrin animal.

On examining the plasma of this animal it was found to be a deep yellow colour. It gave a negative direct and indirect van den Berg's reaction but on further examination the yellow colour was found to be due to the presence of lipochromes.

Fig. 10.—Same as figure 1; 26.10.36; weight, 970 lb.

The total number of leucocytes fall within normal limits, but the neutrophiles would seem to be decreased in number. At times this decrease is due to a relative increase in the lymphocytes, but on some days there would seem to be a relative increase in the monocytes and the eosinophiles. In several instances eosinophile myelocytes were recognised. The nuclei of quite a number of other eosinophiles have the appearance of young forms. The presence of these cells suggest a certain amount of immaturity. The evidence (negative faeces examination) suggests that the animal is now free form bilharzia, but in view of the fact that some of the other animals similarly treated are still definitely infected and further that even higher eosinophile counts are recorded for the control animal 7022 (table 5) which is free from porphyrinuria, but was also infected with bilharzia, one cannot at the moment attribute the presence of the eosinophiles to either a possible bilharzia infection or to the porphyrinuria, with any degree of certainty.

TABLE II.

*Affected Bovine 7017—4 Permanent Incisors December 1935.*

| Date. | R.C. | R.P. | W.C. | L. | M. | N. | E. | B. | Remarks. |
|---|---|---|---|---|---|---|---|---|---|
| 18/2/36..... | 4·0 | 26 | 14·1 | 52 | 1 | 39 | 4 | 4 | — |
| 23/4/36..... | 4·0 | 26 | 12·2 | 63 | 6 | 23 | 7 | 1 | Anisocytosis, jolly bodies, slight polychromasia, occasional normoblasts. |
| 20/5/36..... | 3·8 | 29 | 6·8 | 67 | 6 | 21 | 5 | 1 | Anisocytosis, punctate basophilia rare. |
| 27/5/36..... | 2·4 | 19 | 15·2 | 56 | 10 | 24 | 0 | 0 | Anaplasma marginale numerous, marked polychromasia and punctate basophilia. |
| 5/6/36..... | 2·6 | 25 | 24·1 | 51 | 2 | 45 | 1 | 1 | Anaplasma, punctate basophilia and polychromasia, occasional normoblasts showing basophile punctation. |
| 11/6/36..... | 3·7 | 28 | 10·1 | 64 | 5 | 30 | 0 | 1 | Anaplasma infrequent, punctate basophilia slight. |
| 16/6/36..... | 3·5 | 27 | 10·5 | 48 | 17 | 33 | 1 | 1 | Punctate basophilia rare—monocytosis. |
| 23/6/36..... | 3·4 | 27 | 13·0 | 45 | 15 | 31 | 9 | 0 | Anaplasma infrequent, Theileria mutans frequent, polychromasia and punctate basophilia monocytosis. |
| 30/6/36..... | 3·2 | 22 | 14·3 | 49 | 7 | 40 | 4 | 0 | Theileria mutans frequent, punctate basophilia slight. |
| 1/7/36..... | 3·1 | 21 | 11·2 | 37 | 12 | 44 | 7 | 0 | Theileria mutans and anaplasma—polychromasia. |
| 14/7/36..... | 3·5 | 24 | 11·9 | 48 | 20 | 31 | 2 | 0 | Theileria mutans numerous—punctate basophilia frequent. |
| 21/7/36..... | 3·3 | 23 | 14·3 | 41 | 10 | 48 | 2 | 1 | Theileria mutans numerous—punctate basophilia normoblasts frequent. |
| 24/7/36..... | 3·4 | 22 | 12·8 | 55 | 13 | 30 | 2 | 0 | Theileria mutans numerous—punctate basophilia normoblasts frequent. |
| 28/7/36..... | 2·9 | 22 | 9·3 | 67 | 9 | 15 | 10 | 0 | Theileria mutans numerous—punctate basophilia normoblasts frequent. |
| 4/8/36..... | 3·5 | 24 | 17·8 | 44 | 10 | 41 | 5 | 1 | Jolly bodies—punctate basophilia normoblasts. showing punctate basophilia. |
| 11/8/36..... | 3·7 | 22 | 10·9 | 51 | 13 | 18 | 18 | 0 | Punctate basophilia—normoblasts Theileria mutans. |
| 18/8/36..... | 3·4 | 22 | 14·0 | 45 | 12 | 31 | 12 | 0 | Theileria mutans rare, no punctate basophilia seen. |
| 25/8/36..... | 3·3 | 23 | 11·0 | 66 | 9 | 18 | 7 | 0 | Normoblasts Theileria mutans punctate basophilia rare. |
| 31/8/36..... | 3·3 | 24 | 11·9 | 59 | 11 | 25 | 5 | 0 | Occasional normoblasts punctuate basophilia rare. |
| 7/9/36..... | 3·3 | 24 | 11·0 | 69 | 7 | 12 | 12 | 0 | Occasional anaplasma punctate basophilia absent Theileria mutans. |
| 14/9/36..... | 3·2 | 23 | 9·9 | — | — | — | — | — | — |
| 20/9/36..... | 3·2 | — | 12·4 | 50 | 7 | 41 | 2 | 0 | Punctate basophilia rare normoblasts present. |
| 28/9/36..... | 3·3 | 24 | 9·4 | 65 | 6 | 28 | 2 | 0 | Normoblasts present. |

Animal was treated for bilharzia by giving five intravenous injections of antimosan commencing 18/2/36 with 20 ccs. and thereafter 30 ccs. every second day until the course of treatment completed on the 26/2/36.

On 26/5/36 animal very sick. Anaplasmosis diagnosed and injected intravenously ·75 gms. Mercurochrome and 40 gms. glucose in 200 ccs. saline. As bilharzia treatment was not successful the animal was given 10 injections of antimosan every second day commencing 23/4/36 with 20 ccs. then 25 cc. and thereafter 30 cc. until the course of treatment was completed. Even this did not completely cure the animal as bilharzia eggs were found to be present in the faeces on 20/10/36.

TABLE III.

*Affected Bovine* 7016—*Young Ox—No Permanent Incisors December* 1935.

| Date. | R.C. | R.P. | W.C. | L. | M. | N. | E. | B. | Remarks. |
|---|---|---|---|---|---|---|---|---|---|
| 18/12/35..... | 8·7 | 28 | 17·5 | — | — | — | — | — | — |
| 18/2/36..... | 6·7 | 32 | 11·8 | 69 | 8 | 17 | 6 | 0 | Several eosinophile myelocytes present otherwise cells normal. |
| 22/4/36..... | 6·0 | 32 | 13·9 | 62 | 4 | 28 | 5 | 1 | Cells are normal. |
| 3/7/36..... | 6·1 | 31 | 14·3 | 70 | 7 | 19 | 3 | 1 | Occasional Theileria mutans—cells normal. |
| 27/7/36..... | 6·9 | 35 | 15·1 | 78 | 6 | 11 | 5 | 0 | One eosinophile myelocyte counted otherwise cells normal. |
| 11/8/36..... | 7·0 | 38 | 14·2 | 79 | 8 | 9 | 4 | 0 | Cells normal. |
| 25/8/36..... | 8·0 | 39 | 13·7 | 80 | 1 | 16 | 4 | 0 | Nuclei of cells contracted, darkly staining and monocytes may have been counted as lymphocytes. |
| 7/9/36..... | 6·8 | 38 | 12·8 | 77 | 5 | 15 | 3 | 0 | Occasional Theileria mutans otherwise normal. |
| 22/9/36..... | 6·8 | 38 | 13·5 | 77 | 3 | 14 | 6 | 0 | Anisocytosis marked, some very large red cells present. |

Owing to bilharzia infection, the animal was given 5 intravenous injections of 6·3 per cent. solution of antimosan every alternate day, commencing with 20 c.s. on the 18/2/36 thereafter 25 c.s. until the course of treatment was completed on the 26/2/36. Six weeks after completion of the treatment, a badly infected animal which was similarly treated, was still found to harbour parasites and it was decided to give 10 intravenous injections of antimosan every alternate day commencing on the 23/4/36 with 25 c.s. and using thereafter 30 c.s., until the course of treatment was completed on the 13/5/36.

TABLE IV.

*Affected Bovine* 7023—*Heifer—No Permanent Incisors December*, 1935.

| Date. | R.C. | R.P. | W.C. | L. | M. | N. | E. | B. | Remarks. |
|---|---|---|---|---|---|---|---|---|---|
| 18/12/35..... | 4·6 | 25 | 8·1 | — | — | — | — | — | — |
| 18/2/36..... | 6·5 | 30 | 9·3 | 61 | 8 | 16 | 15 | 0 | Occasional Theileria mutans. Cells normal. |
| 3/7/36..... | 6·7 | 37 | 13·0 | 81 | 3 | 8 | 7 | 1 | Occasional normoblasts nuclei pycnotic and monocytes may have been counted as lymphocytes. |
| 27/7/36..... | 7·1 | 40 | 8·6 | 62 | 13 | 17 | 7 | 1 | Cells normal. |
| 18/8/36..... | 6·8 | 37 | 7·7 | 75 | 7 | 13 | 5 | 0 | Occasional Theileria mutans. Cells normal. |
| 25/8/36..... | 7·5 | 36 | 12·3 | 72 | 6 | 19 | 3 | 0 | Cells normal. One cell resembling neutrophile myelocyte seen. |
| 7/9/36..... | 6·9 | 37 | 8·2 | 72 | 3 | 18 | 7 | 0 | Cells normal. One cell resembling neutrophile myelocyte seen. |
| 22/9/36..... | 8·5 | 41 | 8·1 | 68 | 5 | 23 | 4 | 0 | — |

Treated for bilharzia by giving 5 intravenous injections of antimosan (6·3%) every second day commencing on the 18/2/36 with 20 ccs. and thereafter 25 ccs. until course of treatment completed. As treatment was not successful 10 injections were given every second day commencing on the 23/4/36 with 20 ccs. and thereafter 25 ccs. until course of treatment completed on the 13/5/36.

TABLE V.

*Bovine 7022—Heifer four Permanent Incisors December, 1935.*

*Normal animal but half sister to porphyrin animals.*

| Date. | R.C. | R.P. | W.C. | L. | M. | N. | E. | B. | Remarks. |
|---|---|---|---|---|---|---|---|---|---|
| 4/1/36..... | 6·3 | 33 | 7·3 | 56 | 4 | 21 | 19 | 0 | Occasional Theileria mutans. |
| 18/2/36..... | 6·4 | 34 | 8·7 | 57 | 6 | 19 | 16 | 2 | Occasional Theileria mutans present. |
| 22/4/36..... | 7·5 | 38 | 6·1 | 60 | 8 | 25 | 5 | 2 | Cells normal. |
| 7/5/36..... | — | — | — | 71 | 1 | 23 | 5 | 0 | Numerous pirop bigem and injected 4 ccs. acaprin intravenously—no signs of anaemia. |
| 18/5/36..... | — | — | — | 37 | 4 | 58 | 1 | 0 | Few pirop. bigem. present. |
| 27/7/36..... | 7·7 | 39 | 12·5 | 66 | 7 | 17 | 10 | 0 | Cells normal. |
| 11/8/36..... | 6·8 | 37 | 9·8 | 77 | 2 | 11 | 10 | 0 | Occasional Theileria mutans. Cells normal. |
| 25/8/36..... | 7·7 | 41 | 10·2 | 75 | 5 | 9 | 11 | 0 | Cells normal. |
| 7/9/36..... | 7·0 | 40 | 11·9 | 72 | 3 | 15 | 10 | 0 | Cells normal. |
| 22/9/36..... | 7·9 | 44 | 5·6 | 74 | 0 | 17 | 9 | 0 | Cells normal. |

Animal was treated against bilharzia with Tartar Emetic (4% solution) every second day intravenously into the ear vein. The first day 7 ccs. were given then 10 ccs. and thereafter 15 ccs. until a course of 10 injections was completed on the 8/1/36. This was not successful and a course of 10 injections of 30 ccs. of Antimosan (8% intravenously) was commenced on the 23/4/36 and completed on the 13/5/36. Animal developed acute natural Redwater on the 17/5/36 and was successfully treated with 4 ccs. of Acaprin.

On some days the monocytes are decidedly increased in number and on the whole the monocytic counts in all four porphyrinuria animals run on a rather higher level than those of the control animal 7022 (table 5). Even though this is the case, Wirth (1931) records the variations in the number of monocytes as being from 3 to 10 per cent. and although counts beyond the 10 per cent. limit are repeatedly recorded, one cannot at this stage, remembering at the same time also, the bilharzia factor, regard a monocytosis as a significant reaction in cases of porphyria. However, the possibility of this should not be lost sight of, particularly in view of the statement by Mackey and Garrod already quoted, that there would seem to be an increase of cells described by them as endothelials in the case of a boy examined by them.

7017. *Young ox. 2-3 years old.*—The effects of porphyria are well marked in this animal. It was intended to keep him exposed to the sun, as a control to 7018, when this animal was placed in a dark stable. Unfortunately animal 7017 developed an acute attack of anaplasmosis (Gallsickness). He was treated with mercurochrome on the 26.5.36 and in order to save the life of the animal the original idea, to keep him exposed to the sun, had to be abandoned and he was also continuously kept in a dark stable. In spite of getting a course of ten injections of antimosan, during April and May, 1936, bilharzia eggs were found to be present in his faeces on the 20.10.36. When this animal arrived here his red cells were also found to be decreased in number. (4 million per cm.). He then had porphyrinuria, as well as being infected with bilharzia. The five injections of antimosan given to the animal during the period 18.2.36 to the 26.2.36, made no appreciable difference to the excretion of bilharzia eggs in its faeces, and this may have been responsible for the continued low counts. Neither did the further course of ten injections of antimosan (see table 2) during the period 23.4.36 to the 13.5.36 succeed in improving the red counts, which remained more or less stationary until the 3.5.36, and were further decreased on the 27.5.36. However, this further decrease is undoubtedly due to the anaplasmosis infection, which almost certainly took place from infected ticks, which the animal must have picked up whilst he was being moved forwards and backwards to receive the antimosan injections in a crush some distance away from its quarters during the period 23.4.36 to the 13.5.36. In view of the anaplasmosis and bilharzia complications, a further discussion of the blood picture of this animal in relation to porphyrinuria will be of no value at the present time.

*Animals* 7016, 7023 *and* 7022 (*control*).—The neutrophiles seem to be on the low side in all three animals. In animal 7022 (free from porphyrinuria, but also infected with bilharzia) the eosinophiles run on a higher level, than in the case of the other two animals. In none of these animals do the monocytes call for special comment. No morphological changes are present in the red cells. Morphological changes in the red cells of the control animal 7022, may have been present as a result of an acute attack of redwater (Piroplasma bigem.) during the period 18.5.36 to 27.7.36 when an examination of its blood was not made. Theileria mutans is a parasite which is present in nearly every normal bovine in certain areas in South Africa and is not known to produce any disturbances.

## Pathology.

Specimens from two animals were available for examination. These animals were both killed in Swaziland on the owner's farm. In view of the presence of bilharzia infection in the animals which came from that farm and that bilharzia eggs were definitely found to be present in sections cut from the liver of one of these animals, it is exceedingly likely that both these animals were infected and one would consequently not be justified in regarding the various pigments present in the organs as being due to the porphyrinuria alone. It is nevertheless thought desirable to give a short description of the appearance of the pigment stained with various stains, with the hope that this may serve as a comparison for the histological description to be presented at some later date, of an animal which is free from bilharzia and which has been kept in the dark stable, to eliminate any secondary factors such as a possible haemolysis, which may take place when the porphyria animal is exposed to the sun. In view of the fact that the sections from these animals were not examined by the fluorescence microscope, any analogous reference to the pigments present, in terms of Borst and Köningsdroffer's description as $P_1$, $P_2$, $P_3$, $P_4$, $P_5$, must be regarded as speculative.

Specimen 16786, bovine, 2 years and 4 months old, killed on the farm. The biochemical details of the pigments in bone, urine, blood and other organs are described by Rimington elsewhere in this journal.

*Kidney.*—This organ is darker in colour than normal; its surface has a mottled appearance; on section marked striation is seen to be present in the cortex, dark lines alternating with greyish ones. The medulla is of a pinkish colour. The bladder contains a small amount (50 c.c.) of clear urine, having a port wine colour with a slight brownish tinge.

*Microscopic examination.*—Haem. Eosin. most of the pigment is in the form of yellowish brown granules (brown with v. Giesen) in the cells of the renal tubules of the cortex and perhaps also of the boundary zone, but very little, if any, is seen in the straight and collecting tubules of the medulla. Bigger conglomerate masses of brownish yellow (brown with v. Giesen) pigment are also present, mainly in the cortex and boundary zone but to a less extent in the medulla. Neither of these pigments are present in the structures of the glomerulus. The finely granular yellowish brown pigment described by Borst and Köningsdroffer in the endothelial cells of blood vessels, is probably also present, but was recognised with difficulty and then only with the high magnifications under oil. A structureless pink (brown with von Giesen; greenish blue, but sometimes the colour is very faint with Berliner Blue) staining substance, is present in the lumen of the renal tubules. This substance is sometimes also present in Bowman's capsule.

With Berliner Blue, there is a considerable variation in the staining reactions of the pigment, which appears as: (1) small granules of varying size in the cells of the tubules. Most of them are brown, but some are of a greenish blue colour; (2) very small

granules, which can only be seen with a 2 m.m. oil immersion lens. They are mostly of a yellowish brown colour, but occasionally they are of a greenish colour. They occur in cells, in the walls of the tubules. Practically all the cells of some tubules may contain this pigment, but the collecting tubules of the medulla contain much less of this pigment than do tubules in other parts of the kidney; (3), (*a*) big conglomorate brown pigment masses in tubular cells, (*b*) pigment masses of similar size, etc., but staining of a greenish blue colour, (*c*) what appears to be an intermediate stage between (*a*) and (*b*), where the pigment has only a slight greenish tinge.

*Sudan* III.—The pigment granules are brown and the conglomerate pigment masses are darker in colour. The granules and bigger pigment masses may occur in the same tubule and even in the same cell. The structureless material in the tubules does not stain differentially with the fat stain. It has a faded bluish colour. In Bowman's capsule a brownish staining pigment is sometimes seen to be present. In some glomeruli, pigment granules are only seen in the neck, but all other structures of the glomerulus are free from pigment. With the lower magnifiication no pigment granules can be seen in the tubules of the medulla, but with a 4 m.m. dry lens, a number of tubules in the medulla are seen to contain very fine granules. In addition to the pigmentation of the kidney, there is fairly well marked fibrosis of the organ. From the other animal kidney specimens were not available for examination.

*Diagnosis*: *marked pigmentation-fibrosis.*

*Liver: 2 animals, specimens* 16786 *and* 16719.—The stern cells are prominent and are loaded with pigment. The pigment is mostly in the form of granules, some of which stain of an almost lemon yellow colour, whilst others stain of a yellowish brown colour, with haemalum-eosin and with Sudan III. With von Giesen the granules stain brown, less frequently similarly staining conglomerate pigment masses are seen to be present, probably also in stern cells. With Berliner Blue very little iron staining pigment can be indentified under the lower magnifications; but with a 2 m.m. oil immersion lens one can easily recognise pigment which is in the form of fine blue lines or rings, especially in the sinusoids. With the same magnification one can also recognise bluish staining pigment in the liver cells themselves.

In some of the bigger vessels of the liver (specimen 16786) ovoid structures are present. They have a definite outer wall, within which cellular elements can be recognised. These structures are regarded as eggs of bilharzia parasites and in the circumstances one cannot be sure to what extent some of the pigment present may not be due to these parasites. In Giemsa stained sections, numerous eosinophiles can readily be recognised along the interstitial tissues, but some are also present in the sinusoids. Remembering the bilharzia infection one is not justified in regarding the eosinophiles as being significantly associated with the presence of porphyrins.

*Diagnosis*: *pigmentation and bilharzia infection.*

*Lymphatic glands* 16786 *and* 16719.—In freezing sections the the distribution of the pigment, mainly in the reticulum of the gland is well shewn. In embedded sections, the dominating pigment is seen to be present in the reticulum cells as fine granules, having a yellowish brown colour ($P_2$). The lymphoid follicles seem to be free of pigment, except that in the germ centres of some of them one may find structureless masses 5 to 10 times the size of the red cell, staining of a lemon-yellow colour with van Giesen. Occasionally masses of yellowish brown pigment ($P_1$) are seen in the lymphoid cords. In addition to the pigments just described one sometimes finds cells which are loaded with fine granules staining of a purplish colour with Giemsa, in the lymphoid cords.

*Berliner Blue.*—Most of the granular pigment in the reticulum cells stains of a greenish yellow colour, but a well defined iron reacting pigment as described by Borst and Köningsdroffer for Petry, could not be recognised. With a 2 m.m. oil immersion lens one does sometimes see blue rings or blue lines in or about the reticulum cells, but except for the occurrence of very occasional blue granules, this is the only evidence of stainable iron in the lymphatic glands. In places an increased number of eosinophiles would seem to be present.

*Spleen—specimens* 16786 *and* 16719. *Haemalum eosin.*—Large amounts of pigment are present. The granular ($P_2$) type of pigment is present in relatively small amounts. Most of the pigment is in the form of big masses ($P_4$) but in some cases it is also coarsely granular. Close to the pigment masses one nearly always sees a nucleus but the type of cell in which the pigment occurs, cannot be so clearly recognised as in the case of the liver. The pigment varies in colour from a light yellowish brown to a dark brown or almost black colour. In some of these pigment masses, one sometimes sees with a 2 m.m. lens small darkly staining pigment granules.

*Berliner Blue.*—In the pulp one recognises: (1) cells containing big blobs of pigment staining yellowish brown, with small darkly staining granules within the bigger pigment mass; (2) cells containing granules staining yellowish brown; (3) cells containing pigment blobs staining greenish blue; (4) cells containing granules staining greenish blue. In some cases the pigment stains between green and yellow and one has the impression that the yellowish staining pigment is being changed or converted into the stainable haemosiderin. The distribution of the pigment is well shewn with this stain. The malpighian bodies stand out prominently and do not contain any iron-staining pigment, but they do sometimes show the presence of fairly big, round, lemon yellow, pigment masses.

In the pulp one sees what can be most suitably described as *pigment rings.* On careful examination under high magnification the space enclosed by the rings which consist of bluish pigment is seen to be occupied by a red cell. It almost seems as if the blue ring represents pigment which is lying free between the red cells and which has in some way become absorbed to the periphery of the cells. In order to find out if the red cells of porphyrin animals

contain any stainable iron, smears were made from animal 7018 but no iron could be recognised after staining with Berliner Blue. In some cases it is not possible to recognise with certainty what is present in the space enclosed by the rings. The pigment is not only present in the form of rings, but occurs sometimes in the form of irregular lines, also in small amounts in the trabeculae. Although no undoubted phagocytosed red cells were encountered in the spleen, one cannot entirely ignore the possibility that some of the large pigment masses may be the remains of phagocytosed red cells. In the pulp eosinophiles are numerous.

*Lung. Only one specimen*, 16786.—There is only slight pigmentation of the lung. Pigment, in the form of fine granules ($P_3$) is present in the interalveolar walls. The granules stain yellowish brown with Haem. eosin and brown with Berliner Blue. Occasionally pigment masses are seen in cells as well as in the interalveolar walls. These stain blue with Berliner Blue, but dark brown granules can be recognised under high magnification in the substance of this bluish structureless mass.

*Myocardium.*—A small amount of non iron staining coarsely granular pigment is present in the interstitial tissues. It stains of a lemon yellow colour with Berliner Blne and of a dark yellowish brown colour with Haem. eosin.

*Muscle.*—No pigment was recognised.

A notable ommission in the pathological description is that of the bones. During 1935 Sir Arnold Theiler signified his willingness to collaborate in the further work which was contemplated and he would have been resposible for the pathological study of the bones. His unexpected death unfortunately broke this collaboration. It is a tragedy that an authority of his standing, on osteopathology, could not complete the work, for the successful execution of which he was brilliantly equipped.

## Pathogenesis.

Nothing fundamentally new and original can be contributed at this stage. Animals 7016 and 7023 seem to be affected to a mild degree only and consequently do not afford a clue as to the possible sequence of events. Animal 7017, in spite of treatment, still harbours bilharzia parasites and has in addition only recently recovered from an acute attack of anaplasmosis (Gallsickness). On account of these complications this animal is not at the moment a suitable subject for the discussion of porphyrin pathogenesis. None of these objections can be raised against the use of animal 7018 for this purpose. One should, however, remember the possibility that a negative faeces examination for bilharzia eggs, even though it is repeated 2 or 3 times, as was actually done in this case, does not necessarily mean that the animal is free from infection.

The catabolism (Abbau) theory and the anabolism (Aufbau) theory of porphyrin genesis are exhaustively discussed by Günther, Borst and Königsdörfer and others. It is consequently unnecessary to present here details of all the claims for and against these theories, but briefly stated the theories are:—

(1) *Catabolism (Abbau) theory.*—During the catabolism of the haemoglobin molecule porphyrins are formed before the final degradation to bile pigments. Chemically this is possible but it has not yet been established that this occurs in vivo, neither has it up to the present been possible to split the haemoglobin molecule to bile pigments, through a porphyrin stage in vitro.

(2) *Anabolism (Aufbau) theory.*—Here it is postulated that the synthesis of the haemoglobin molecule from pyrrol bodies passes through a porphyrin stage. Chemically this is not only possible but Fischer (1931) was actually successful in synthesising the haemoglobin molecule through a porphyrin stage, by coupling the resulting haematin with native globin. Furthermore, Borst and Königsdörffer find that porphyrins are present under normal physiological conditions in serum, liver, kidney and bones of young embryos. In the six months old human embryo, porphyrin containing cells are only present in the liver, but not in the blood and bone marrow. A proportion of the erythroblasts in the blood islands occurring in the liver of the 4-6 months old human foetus show a weak porphyrin fluorescence, and at the same time faint haemoglobin staining, with recognisable haemoglobin absorption bands. This is interpreted to mean that porphyrins form a link in the normal haemoglobin synthesis.

In their pathological investigations, they find erythro- and megalo-blasts containing porphyrin in the bone marrow, spleen and blood of humans and equines suffering from pernicious anaemia. In the bone marrow of Petry they describe erythrophagoblastosis (if one may be permitted to use this term where phagocytosis of erythroblasts occurs as against erythrophagocytosis, where the erythrocytes are phagocytosed). Porphyrins are present in phagocytosed as well as in free erythroblasts, although they also find some erythroblasts, which do not show any porphyrin fluorescence. This they regard as evidence that at least a portion of the haemoglobin synthesis occurs normally. The other portion stops short at the porphyrin stage indicating the persistence of a foetal process, in post foetal life.

The question now arises as to which of these two theories can satisfactorily explain the recorded observations on these animal cases. For reasons already stated, animal 7018 is the only one worth discussing at this stage. When this animal arrived here, its red counts were 2·9 milloin per c.c. Being infected with bilharzia, antimosan treatment was applied and within a month after treatment the red counts increased to 4·6 million per c.c. and remained at this level for some months. He was then placed in a dark stable and the red counts further increased to the 5·5 million level. This is being regarded as a normal count for this animal under conditions of inactivity in a loose box. No well marked morphological changes

are present in the red cells or the leucocytes. Without being unduly conservative the most that one can say is that the occasional normoblasts and the occasional punctate basopilic cells, as well as the eosinophile myelocytes and other young forms of eosinophiles are slightly suggestive of immaturity or youthfulness of the elements of the blood concerned. Even if the presence of immaturity is assumed, it would be necessary to establish if this is something inherent in the porphyrin animal or perhaps merely a compensatory reactive process, before definitely regarding it as evidence which favours the anabolism theory of porphyrin genesis. In a porphyrin animal exposed to the sun, secondary compensatory processes are probably present but the same compensatory processes have been eliminated in this animal by confining him to a dark stable. If the porphyrins can destroy red cells even in cases where the porphyrin animal has been adequately protected against the harmful rays of the sun, as is implied by the statement of Borst ond Königdroffer that " Die Porphyrine wirken schädigend auf die roten Blutkörperchen ", can probably only be definitely established when specimens from an animal which has been kept in a dark stable for a considerable length of time are available for pathological examination. The blood picture (5·5 milloin cells) and the marked general improvement in condition, etc., of animal 7018 in the dark stable, suggest that porphyrins do not produce markedly harmful effects on the red cells under such conditions, but previous to that, when the animal was exposed to the sun, such a destruction of the red cells possibly did take place. Except for the slight suggestion of immaturity of its blood picture, animal 7018 is in good health and daily putting on weight in the dark stable. Porphyrin excretion in the urine continues. The urine is no longer as dark in colour as it was whilst the animal was exposed to the sun, and it seems likely that a good leal of the brown discolouration of the urine may be due to the effects of photosensitization. If the porphyrins are due to the persistence of a foetal process, this would not seem to produce any disturbance of haemoglobin metabolism, in the absence of sunlight. If there is only one kind of haemoglobin, it is difficult to understand why a portion of the building stones of that kind of haemoglobin should be picked out for a maturation arrest and the remainder allowed to proceed to full maturation. If the dualism of haemoglobin postulated by Fischer were proved this would not be so difficult to accept, as the inherited deficiency may then be present only in the one haemoglobin, and the other remains unaffected. Such a maturation arrest at the porphyrin stage of haemoglobin synthesis, would bring the anomaly into line with the maturation arrest of the white cells postulated by Fitz-Hugh and Krumbhaar (1932) (Paper not available) quoted by Darling, Parker and Jackson (1936) in Agranulocytosis, as well as with the erythroblast arrest in pernicious anaemia, also quoted by Darling and co-workers.

It is of course possible that in the absence of the effects of photosensitization red cell destruction takes place, but not at the same rate as when the animal is exposed to the sun; consequently young forms of red cells as well as leucocytes may be present in the blood, as the result of compensatory hyper-activity of the haemopoietic tissues. This compensatory process in the absence of exposure to

the sun may be able to maintain the elements of the blood at a more or less normal level. No confirmatory evidence of this could be established clinically in animal No. 7018. The serum of this animal is yellow, this is due to the presence of lipochromes, but bile pigments are completely absent.

The pigments present in the two animals which were killed, seem on the whole to corespond with those described by Borst and Königsdorffer in the case of Petry. There is perhaps less iron in these animal cases. These animals were almost daily exposed to the sun and one assumes that this was not the case with Petry. In the circumstances it would seem that the effects of photosensitization are not mainly responsible for the presence of iron in the various organs of porphyrinuria cases. It is, however, almost certain that the protective mechanism of bovines against photosensitization in virtue of the hair coat and pigmented skin is much more efficient than in the case of the human subject and consequently one cannot on the above ground exclude the possibility that the effects of photosensitization may be responsible for the iron in the organs of porphyrinuria cases, even though a smaller amount of such iron is present in animal cases, in which a more intensive exposure to the harmful rays of the sun took place. In addition to prophyrinuria, Petry also suffered from other complaints. One of these two animals was definitely (and the other one probably also) infected with bilharzia. Therefore in neither instance is one dealing with an uncomplicated porphyria case and until such a case, in which the effects of photosensitization have in addition also been controlled, is available for biochemical and pathological examination, one will be at a disadvantage in interpreting some of the pathological findings.

*Nomenclature.*—There seems to be no sound reason why in the present state of our knowledge the nomenclature of this condition in animals should not be brought into line with the more correct terminology used in medical literature. Porphyria would seem to be the most suitable designation for the general state of the condition: porphyrinuria, where porphyrins are excerted in abnormal amounts in the urine, and porphyrinaemia for the abnormal presence of porphyrins in the blood plasma. Pink tooth is suggested as a popular name for animal cases, thus emphasising the clinical feature of the anomaly by means of which it can be easily differentiated from redwater (piroplasmosis).

## SUMMARY.

1. The occurrence of congenital porphyrinuria in 13 bovines, all of which are the progeny of one bull, is described.

2. 77 per cent. of the affected animals are males and 23 per cent. are females. In this respect the incidence of the condition resembles the analogous condition in humans as well as the incidence of recorded cases of Alcaptonuria and albinism, thought to be hereditarily transmitted as recessive characters.

3. The available evidence strongly suggests the hereditary transmission of the condition through a particular bull.

4. Details of the clinical symptoms of four cases are presented. These are the first animal cases of the anomaly available for clinical examination. The teeth are brownish pink, but the enamel is not pigmented. The poor condition and the scabs and crusts on parts of the skin unprotected by hair, are mainly due to the effects of photosensitization. The urine is amber, brown or reddish brown in colour and contains amongst other uro- and copro-porphyrin.

5. " Pink tooth " is suggested as the popular name for the animal cases. This emphasises the clinical feature of the anomaly by means of which it can be easily differentiated from redwater (piroplasmosis).

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