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Genetics in the Diagnosis of Bovine Congenital Porphyrinuria (Pink Tooth).

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THE animal which is the subject of this paper is a Shorthorn bull No. 7597. Fourie (1939) gave a clinical description of the animal. The bull at that time showed acute skin lesions, when the animal is exposed to the sun. (See figure 1).

When the animal is protected against the sun by stabling for instance, there is immediate improvement in the lesions. Unlike Fourie's (1936) other cases and Fourie and Rimington's (1928) Cedara case, there is no discolouration of the teeth and urine. On analysis Roets (1939) found 11·298 mgm. of total daily porphyrins excreted. This is considerably lower than is the case with undoubted bovine porphyrin sufferers. (7016—mild case—·3 gm; 7017 and 7018—severe cases—1·6 gm. and ·8 gm. respectively. This low level of total daily porphyrin excretion in bull 7597 seems nevertheless to be higher than is the case with a completely normal or apparently normal animal (3 mgm. to 8·9 mgm.) (See Fourie and Roets 1939).

In view of the fact that bull 7597 is markedly photosensitive, has a total daily porphyrin excretion on a somewhat higher level than would appear to be the case with normal bovines, and as shown by Fourie (1939) has a common ancestry, although this is admittedly very remote, with the bull Royal Regent, which Fourie (1939) has shown to be the possible transmitter of the porphyrin carrying gene to a South African Shorthorn herd, Fourie (1939) regarded this bull as a suspicious case of bovine congenital porphyrinuria which could really only be confirmed in the living animal genetically by breeding experiments. This bull was, therefore, placed in a camp with cows 7023 [a known clinical case of pink tooth (Fourie 1936, 1939)] and cow 7354 [also a known clinical case of pink tooth, the so-called Cedara case (Fourie and Rimington 1938)]. Unfortunately the bull contracted heartwater in the camp and in spite of treatment with uleron died either from heartwater or the effects of the uleron treatment. Bull 7597 was not actually seen to serve cows 7023 and 7354, but as this was the only bull running with these cows in the camp, it is reasonable to assume that this bull is the sire of the two calves which were in due course born out of cows 7023 and 7354. Both the calves are clinically normal. Fourie (1939) has shown that pink tooth in bovines is inherited as a recessive character. Consequently one would expect that if a bull which is an affected case, is mated to affected cows the progeny born from such mating must all be affected cases. In the above breeding experiment, we know that the two cows are undoubtedly cases of

congenital porphyrinuria. The bull 7597 can, therefore, not be a typical porphyrin sufferer. In other words, if the porphyrin inherited character is a simple mendelian recessive, this bull 7597 cannot be a homozygote (rr). It is of course possible that he may be a heterozygote, especially in view of his breeding. If this is the case then the outstanding clinical feature, viz. the photosensitization, which first threw suspicion on this animal as a porphyrin sufferer, must be caused by something other than the usually recognised porphyrins.

As already stated this animal died either from heartwater itself or as a result of haemolysis induced by the uleron treatment for heartwater. It was therefore possible to make a complete postmortem examination and also to examine the various organs pathologically.

Post Mortem Examination.

Lesions of photosensitization are present in places along the side of the body in the thoracic region; the skin is hairless. In other places raw surfaces are present but there is no keratinization; there is tick infestation including *Amblyomma hebraeum* (bont tick); tumour splenis; enlarged liver, hydrothorax, with petechiae on the epicardium; marked gelatinous infiltration of the pulmonary septa and marked oedema of the lungs, degenerative changes of the kidneys; haemoglobinuria, enlargement of the lymphatic glands.

There is no discoloration of the teeth or the bones, consequently the lesions of Photosensitization are the only macroscopic evidence of pink tooth. the other evidence suggestive of porphyria, especially in view of the photosensitization which is present, is the fact that the bull is related to the porphyrin carrier bull 7015 referred to by Fourie (1936, 1939).

The anatomical-pathological diagnosis is typical of heartwater, which disease the animal contracted naturally. The haemoglobinuria has however nothing to do with the heartwater infection, but was caused by the uleron, which drug is a useful curative remedy for heartwater, if used during the early stages of the disease according to Neitz (1940).

In order to obtain further evidence as to whether this is a typical case of porphyria or not, a careful histological examination of representative organs was made. Details of the examination are:—

Myocardium.—Numerous haemorrhages are present. These are irregularly distributed throughout the myocardium. Sarcosporidia are frequent. No well-defined evidence of undoubted pigmentation is present.

Bronchial Lymphatic Glands.—There is a small amount of pigment appearing almost dustlike in cells (8 mm. lens × 6 ocular), but the granular nature of this pigment is clearly seen with a 4 mm. dry lens. The pigment stains brown with haem-eosin and dark brown or almost black with v. Gieson. The pigment does not take the Sudan stain, appearing merely as refractile points with this stain. In addition to this granular pigment, there are also bigger pigment masses, staining blue with Berliner Blue. There is not much of this pigment, which is regarded as haemosiderin, due to haemolysis induced by the uleron injections.

Mediastinal Lymphatic Glands.—Pigmentation as described for the bronchial glands is present, but to a much smaller extent.

Spleen.—A good deal of blood is present in the spleen. In places pigment masses staining of a yellowish brown colour, with haem-eosin are present. With Berliner Blue, a great deal of iron staining pigment is seen to be present. (Fig. 2 and Fig. 3.)

There is undoubtedly more iron containing pigment in the spleen of this animal than is the case with the bovine cases referred to by Fourie (1936). But this iron containing pigment is not due to the same aetiological factors, described for the case Petry by Borst and Königsdorffer (1929), but rather due to the haemoglobinaemia induced by the Uleron treatment for heartwater. The finely granular pigment seen in typical cases of congenital porphyrinuria was not recognised.

Lungs.—There is marked oedema. In places the alveoli contain fibrin. Some dark, almost black pigment masses of varying size are sometimes seen. This pigment appears to be situated largely in the interstitial tissues and is regarded as consisting of carbon particles, the condition being actually anthracosis. There is in addition a good deal of iron staining pigment in the lungs.

Liver.—Some pigment is present, but most of it stains for iron with Berliner Blue and is regarded as being due to the haemoglobinaemia.

Kidneys.—Haemorrhages, small in extent, are present throughout the substance of the kidneys. In some tubules a structureless fluid staining reddish with eosin is present (haemoglobin). In some glomeruli a similar substance is present. There is also a certain amount of iron staining pigment present. These changes are due to heartwater (haemorrhages) and to the effects of the Uleron treatment (haemoglobinaemia), but undoubted porphyrin pigments were not recognised.

The macroscopic and microscopic appearances of the lesions described are typical for heartwater complicated with haemoglobinaemia. There is no undoubted evidence that porphyrin pigments are present. This is further confirmed by the work of Roets (unpublished results) who was not able to find any porphyrins in the bones of this animal. Roets, however, found as much as ·7 mg. of coproporphyrin in 100 gm. of rectal faeces (post mortem). Compare this with 3·446 mg. of coproporphyrin per 100 gm. of faeces and 6·443 mg. per litre of urine in a severe porphyrin sufferer No. 7018 and 2·813 mg. coproporphyrin per 100 gm. of faeces and 4·88 mg. coproporphyrin per litre of urine in a less severe case No. 7016. [See Fourie and Roets (1939)].

There was no uroporphyrin in the urine. It is incidentally worthy of note that there was as much as ·399 gm. of haemoglobin per 100 c.c. of urine (in bladder post mortem). These amounts of coproporphyrin in the faeces and urine are considerably higher than those recorded by Fourie and Roets (1939) when quantitative coprophyrin determinations were made from samples of faeces and urine collected from this animal when alive and not suffering from an acute disease like heartwater or complications such as haemoglobinaemia. The figures obtained by them at that time are—coproporphyrin ± ·15 mg. for 100 gm. of faeces and plus minus ·055 mg. per litre of urine.

Roets further found in bull 7597: (1) *Spleen,* chloroform soluble porphyrins 50 γ per kilo, coproporhpyrin traces.

(2) *Kidney*—no porphyrins.

(3) *Liver.*—Chloroform soluble porphyrins 184 γ per kilo and coproporphyrin 131 γ per kilo.

(4) *Bile*—Chloroform soluble porphyrin 48 γ per kilo and coproporphyrin 102 γ per kilo.

It is not known how to interpret these findings. These figures undoubtedly lose much in value in view of the fact that figures for normal animals as well as for animals suffering from various diseases are not available for comparison. This is a study which has already been undertaken by one of my colleagues at Onderstepoort but sufficient data are not yet available to allow of a comparison being made. Although it is not possible to interpret the above figures at present, it was nevertheless decided to record them for the sake of future workers. In this connection one should, however, remember that already in 1892 Garrod had shown that the substance he refers to as haematoporphyrin is frequently present in minute amounts in healthy individuals and in large amounts in urines of sufferers from a great variety of diseases.

Discussion.

This interesting case, bull 7597, has a remote common ancestry with a known porphyrin carrier bull 7015. It was further known to have had lesions of photosensitization from calfhood. Its total daily porphyrin excretion is very much lower than is the case with other known bovine porphyrin sufferers, but appears to be on a somewhat higher level, than is the case with normal animals. This evidence suggests that this animal may be a case of porphyria. However, since the teeth, bones and urine of this animal are not discoloured as is the case with typical pink tooth cases in bovines it is possible that this animal may be an atypical case of porphyria.

The evidence against porphyria is, on the other hand, undoubtedly impressive. This briefly is:—There are no porphyrins in the bones. There is no uroporphyrin in the urine. there is no discoloration of the teeth or the bones; when bred to two undoubted pink tooth females, both calves born as a result of this mating are clinically normal. This genetical evidence would therefore seem to confirm the evidence furnished by (i) chemical analysis of urine, faeces and organs and (ii) macroscopic and microscopic examination of representative organs, that this animal, in spite of being in a continuous state of photosensitization, is not a porphyrin sufferer, or if it is a case of porphyria, it must be an atypical and hitherto unrecognised type of porphyrin sufferer.

Summary.

A Shorthorn bull 7597 remotely related to a porphyrin carrier (Dr) bull (7015) and showing skin lesions of photosensitization from calfhood, was bred to two cows suffering from congenital porphyrinuria (pink tooth). Both calves born out of this mating are clinically normal. The bull died from heartwater or haemoglobinuria induced by Uleron treatment for heartwater. Histologically no undoubted evidence of porphyria was recognised. Chemically no porphyrins were found in the bones and no uroporphyrin in the urine. The teeth are not discoloured. This chemical and histological evidence tends to confirm the genetical evidence that this animal is not a porphyrin sufferer, or, if it is, it must be a very unusual and atypical case of porphyria.

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Fig. 1.—Bull 7597, showing acute lesions (photosensitization).

Fig. 2.—Spleen 20×, showing iron staining pigment. Berliner Blue.

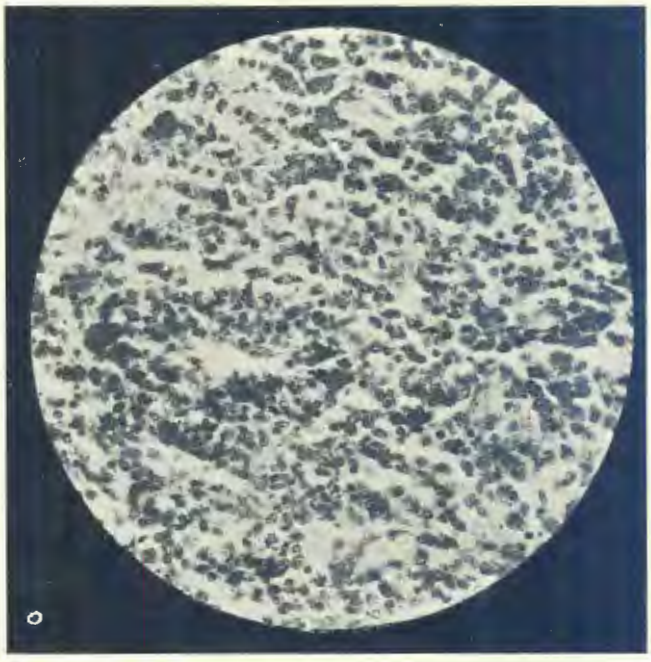

Fig. 3.—As fig. 2. ×330.