

Studies on the Photosensitisation of Animals in South Africa.

I. The Action of various Fluorescent Dye-stuffs.

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INTRODUCTION.

UNDER the title "Studies on the Photosensitisation of Animals in South Africa" it is intended to report on a series of different investigations which have been undertaken in the first place in an endeavour to elucidate the problem of "Geeldikkop" amongst small stock in South Africa. This disease has been in existence here for many years and has frequently been mentioned in the Reports of the Colonial Veterinary Surgeon of the Cape of Good Hope from 1894-1906.

In the Seventh and Eighth Reports of the Director of Veterinary Research (1918), Theiler, who carried out various experiments in different localities, describes the disease in detail. Furthermore, he definitely showed that the "dubbeltjie" (*Tribulus* species) could under certain conditions provoke geeldikkop, a belief which many stock-owners had held for years. The disease, however, occurs spasmodically and may be very difficult to reproduce as shown by Theiler's work, and also from investigations conducted subsequently by the author. According to Theiler, geeldikkop is caused exclusively by *Tribulus terrestris* in the flowering stage. It should, however, be pointed out that the term "geeldikkop" is rather misleading when used in this restricted sense, seeing that the symptom complex, viz., a generalised icterus accompanied by swelling of the head may be encountered on many different pastures in South Africa where *Tribulus* is practically unheard of. In such cases it is frequently referred to as geeldikkop, dikkop, dikoor, geelsiekte, terms which must be regarded as purely symptomatic in their description. In fact, from an examination of affected animals, the sudden onset of the condition and the post-mortem appearances, one is forced to the conclusion that the underlying factors at work are of a similar nature in each

instance. The object of these investigations was to gain a better understanding of these fundamental principles. The importance of one factor at least, is, however, realized, viz., the influence of sunlight, since the disease is definitely associated with photosensitisation. Thus affected animals always seek shade, while the subcutaneous swelling and necrosis of the skin is always localised to the exposed and unpigmented parts, viz., over the face and ears. The icterus, on the other hand, is much less clearly understood. There is, however, some damage of the liver as shown by the necrosis of hepatic cells and proliferation of the bile capillaries. Whether this is due to some specific toxic agent in the plants, or to some derangement in the metabolism of the plant or the animal body or to changes in both, it is exceedingly difficult to determine as yet. The view that some metabolic disturbance is at the root of the trouble is favoured for the following reasons:—

- (a) Animals may sicken very suddenly on a pasture which normally is considered excellent for small stock, e.g. on the Karroo where hundreds of thousands of animals are kept and where the *Tribulus* plant forms a substantial part of the diet.
- (b) A condition similar in all its aspects may at times be encountered on lucerne paddocks and where *Tribulus* can be ruled out.
- (c) On grass veld, e.g. the Transvaal highveld and in the northern Orange Free State, the condition may suddenly appear and cause heavy losses in sheep. Here again *Tribulus* can be excluded.
- (d) Farmers in certain parts of the Karroo have reported outbreaks of so-called "geeldikkop" in mid-winter, when no *Tribulus* plants are to be found (also mentioned by Theiler in his report).

It thus seems evident that *Tribulus* cannot be the only cause of geeldikkop, and that a number of widely different plants will have to be considered.

With regard to *Trifolium*, of which several species are either cultivated or found growing wild in South Africa, no complaints have been made in this respect, although Fröhner states that in Europe several species of *Trifolium* have from time to time caused photosensitisation and icterus in animals.

Buckwheat poisoning commonly noticed in unpigmented animals in Europe, is not a serious complaint in South Africa, seeing that very little buckwheat is grown. Experimentally, however, it has been shown that the typical symptoms noted in other countries can be produced in animals under South African conditions when *Polygonum fagopyrum* plants are fed. Similarly it has been demonstrated experimentally that different species of *Hypericum*, viz., *H. ethiopicum* and *H. leucoptychodes* found in South Africa, may cause photosensitisation of sheep. In these cases the symptoms noted correspond exactly with those described in European countries are due to *Hypericum perforatum* and *Hypericum crispum*, viz., marked photosensitisation of

unpigmented animals accompanied by large subcutaneous swellings of the head and ears, followed by necrosis and sloughing of the affected skin. Both in buckwheat and *Hypericum* poisoning the complete absence of icterus is an interesting point, seeing that in South Africa, outbreaks of geeldikkop occurring on the pasture are practically always characterised by an icterus of an intense nature.

On account of the great difficulty experienced in attempting to produce cases of geeldikkop experimentally, especially under laboratory conditions away from naturally occurring outbreaks, it was decided to induce photosensitisation by various other means. It was hoped that in this way a clinical picture simulating that of the true disease, could perhaps be produced. It may be mentioned that the disease appears and disappears in a most insidious manner, and at its worst only lasts for a comparatively short while during the summer months. Furthermore, poisonous *Tribulus* plants, once they are removed from their habitat seem to lose their toxicity without delay. It was for these reasons that one was forced to resort to other means of provoking the disease. Seeing that sheep and goats are practically the only animals naturally affected, most of the experiments were conducted on these animals. In a few cases white rabbits were also tried, but, as a rule, they were unsatisfactory on account of their marked sensitivity to the heat rays in strong sun-light. Many of them died after a few hours exposure from symptoms of shock probably due to "heat stroke".

In a previous paper by Quin (1931), the photosensitising influence of haematoporphyrin on sheep and goats was reported upon. In that work it was shown that injections of small amounts of haematoporphyrin (0.5 gm.) provoked an almost immediate and intense photosensitivity in unpigmented sheep and goats exposed to sunlight. The subcutaneous oedema at first, and the necrosis and sloughing of the skin afterwards, were both very marked. In all these cases, however, icterus was completely absent, as the liver appeared to maintain its normal function. Otherwise the symptoms were wholly in accordance with those of geeldikkop. Presumably haematoporphyrin only causes a simple direct photosensitisation without damaging protected organs and tissues, as no evidence of this was shown at post-mortem on animals that were killed. In geeldikkop, on the other hand, liver function seems to be definitely deranged as well, i.e. there must be some icterogenic factor operating at the same time that the animal becomes photosensitive. Seeing that haematoporphyrin did not provoke any icterus, it was decided to ascertain the effects of various fluorescent chemical substances on sheep and goats. In the earliest work in photosensitisation Raab, Jodlbauer and Busck showed that such substances as erythrosin and rose bengale when injected into rabbits produced irritability, well marked oedema, skin necrosis and loss of hair when exposed to sunlight.

The following experiments were, therefore, carried out on young Merino sheep 12-15 months old. The animals were first closely shorn, especially the head and along the back, and then kept exposed in sunlight for several hours every day.

I. EXPERIMENTS WITH DYE-STUFFS IN THE FLUORESCIN GROUP.

(a) TETRABROMFLUORESCIN OR EOSIN.

One gram eosin was dissolved in 20 c.c. saline and injected intrajugularly into a sheep. Within a few seconds all visible mucous membranes and also the exposed skin assumed an intense pink-red colour. Ten minutes after the injection the sheep became extremely restless in the sunlight. There was marked flinching of the body, shaking and scratching of the head and ears. Due to the severity of the attack of photosensitisation the animal was placed in the stable half an hour afterwards. The symptoms now soon passed off, and within 3 hours after injection the sheep was feeding quietly. The urine was of an intense red colour throughout the day due to elimination of the eosin. The next day the animal was again placed out in the sun. Photosensitisation was very slight, and the ears were slightly swollen. The blood serum now was quite clear and colourless. Subsequently two injections of 0.5 gm. eosin daily, produced only



Fig. 1. Acute photosensitisation following injection of Eosin.

slight irritation over the head when the sheep was placed in the sun. Another injection of 1 gm. eosin given on the 6th day caused extreme photosensitisation and rapid swelling of the ears (see fig. 1). The animal, after being kept in the stable for 2 days, seemed completely recovered. The head, ears and back of the same sheep was then stained thoroughly with a concentrated watery solution of eosin, and shortly afterwards 1 gm. eosin again injected intravenously. On now being placed in strong sunlight the animal showed no signs of photosensitisation, clearly indicating that the eosin applied to the skin and wool had prevented the harmful rays of the sun from affecting the body. It was then decided to ascertain the effect of oral administration of eosin. One Angora goat was dosed 10 gm. eosin in 1 litre water. The faeces rapidly became stained with eosin and a mild diarrhoea was noted for a few days. There were, however, no signs of photosensitisation.

From these experiments it was thus seen that eosin when injected intravenously produces a very marked sensitisation to sunlight, followed by oedema of the ears and face in sheep. No signs of icterus were, however, noticed. Eosin when applied to the skin and wool efficiently acts as a protection from the harmful rays when at the same time eosin is injected intravenously. Furthermore, it has been shown that the oral administration of eosin does not lead to any photosensitisation, probably due to the rapid passage through the alimentary tract and the accompanying poor absorption.

(b) TETRAIODOFLUORESCIN OR ERYTHROSIN.

One gram of erythrosin in saline injected intravenously into a sheep, caused marked sensitisation and scratching of the ears within 3 minutes after being placed out in the sun (see fig. 2). The animal was then returned to the stable. This caused a rapid disappearance of the symptoms. A subsequent injection of a further 0.5 gm. erythrosin again provoked intense sensitisation accompanied by oedematous swellings of the head, ears and also of the skin round the



Fig. 2. Acute photosensitisation following injection of Erythrosin.

anus. When the swellings had subsided, with the animal kept stabled, the head and back was thoroughly coloured with a strong solution of erythrosine. After that an intravenous injection of 1 gm. erythrosine was given and the sheep placed out in the sun. No symptoms were shown indicating that the colouring on the skin had caused protection against the sunlight. As in experiment IA it should be mentioned that no sign of icterus was ever shown, the serum remaining water clear after elimination of the dye-stuff.

(c) TETRACHLORTETRAIDO-FLUORENSCEIN OR ROSE BENGAL.

As in the previous experiments 1 gm. rose bengal was injected intravenously into a sheep. After being exposed in the sun for 8 minutes the animal showed marked irritability, scratching and flinching and frequently sitting down on the haunches, or dragging the hindquarters along the ground (see fig. 3). When placed in the stable the animal still showed symptoms 4 hours afterwards. The next morning the ears were swollen, although the sensitiveness had

passed off. Two subsequent injections of 0.5 gm. each, again provoked marked symptoms when the animal was placed in the sun. After the symptoms had subsided, the head and back was coloured with a strong solution of rose bengale and 1 gm. injected intravenously. No symptoms were shown when the animal was exposed in strong sunlight. Three weeks after the initial injection the skin

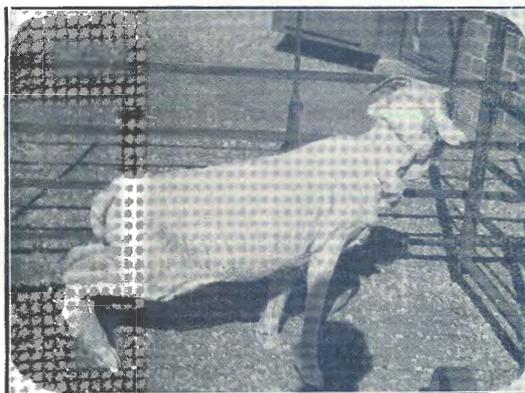


Fig. 3. Acute photosensitisation following injection of Rose Bengale.

over the back was felt to be extremely hard and causing a peculiar stiff gait. A few days later, extensive sloughing of the affected skin set in, leaving a raw bleeding surface in some parts (see fig. 4). Complete recovery took place in time, accompanied by a fresh growth of wool. No signs of icterus were ever noticed.

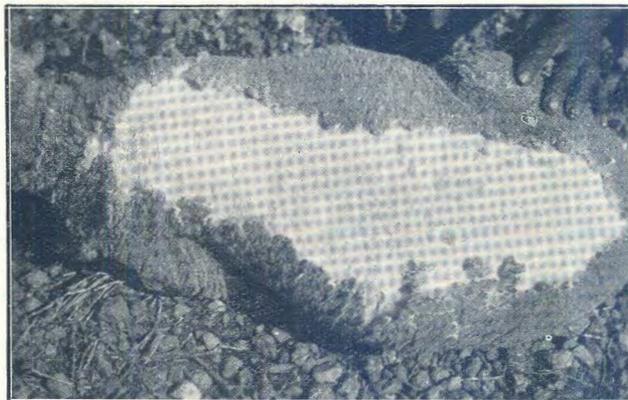


Fig. 4. Chronic skin lesions following injection of Rose Bengale.

Although the action of the three fluorescein dyes tested, was essentially the same, rose bengale produced stronger photosensitisation than the other two. From these experiments it thus became clear that although the fluorescein dyes produced striking photosensitisation, no icterus was ever shown. In this respect, the symptoms provoked seemed to be identical with those produced by haematoporphyrin.

II. EXPERIMENTS WITH DYE-STUFFS IN THE ANTHRACENE GROUP.

For this experiment dichloranthracene-disodium-sulphonate was selected. One sheep was injected intravenously 0.3 gm. the first day without showing any symptoms. On the second day 1.5 gm. was injected and on the third day 0.95 gm. The animal was kept under observation for several days but as no symptoms developed, it was discharged.

III. EXPERIMENTS WITH SUBSTANCES IN THE ACRIDIN GROUP.

In this experiment acriflavin was selected. One sheep injected 0.01 gm. acriflavin intravenously showed no symptoms when placed in sunlight. The following day 0.05 gm. was injected. Within 15 minutes the animal was markedly sensitive, running about the paddock, or repeatedly lying down and then rising. It was then placed in the stable. Two subsequent injections again caused marked symptoms. The irritation of the head was intense, as shown by the continuous rubbing against the fence. The symptoms, however, soon cleared up after the last injection. At no time were there any signs of icterus to be seen, the serum also remaining water clear.

IV. EXPERIMENTS WITH SUBSTANCES FROM THE THIAZIN GROUP.

(A) METHYLENE BLUE.

One sheep injected intravenously with 1 gm. methylene blue showed marked sensitisation in the sunlight 6 minutes after the injection. At times there was marked flinching of the body, which suddenly gave way and causing the animal to assume a crouching position or even to crawl along on the abdomen. These symptoms rapidly subsided when the animal was placed in the stable. When the sheep was again exposed in sunlight 4 hours after the injection, no symptoms were shown. This was probably due to the effective elimination of the dye from the body within a short period. Subsequent injections of methylene blue produced other toxic symptoms, e.g. fairly marked haemolysis, dullness and loss of appetite. The animal died on the 8th day after the first injection, after marked loss of condition and signs of toxæmia. Post-mortem examination revealed pulmonary oedema and cardiac dilatation. There was no icterus.

An Angora goat injected with 1 gm. methylene blue intravenously showed extreme photosensitisation which lasted only for a period of 15 minutes and then suddenly disappeared in spite of the animal being kept out in the sun. A peculiar symptom was the marked vomiting shown immediately after the injection. This, however, also soon passed off. Subsequent injections of methylene blue only produced slight photosensitisation lasting a few minutes. Haemolysis was again noticed, although this did not cause death, neither was icterus noticed at any time. The animal was discharged from experiment after two weeks.

From the above experiments it is thus clear, that methylene blue can produce very strong photosensitisation, which, however, is of short duration, probably due to rapid elimination of the dye. In addition, methylene blue also acts haemolytically on the red blood cells, and may also cause a toxæmia in other respects.

(B) THIONINE.

One Angora goat was injected 1 gm. thionine intravenously. The animal became acutely photosensitive within a few minutes (see fig. 5). There was marked flinching and scratching until the goat was placed in the stable, when the symptoms soon passed off. Subsequent injections again produced sensitisation. The elimination of the dye, however, was very rapid as noticed from the clearing of the serum, and the transitory nature of the symptoms. Slight haemolysis was noticed on one occasion, although blood was drawn and centrifuged



Fig. 5. Acute photosensitisation following injection of Thionine.

regularly every day. Another goat was dosed 10 gm. thionine in 1 litre 5 per cent. Na-bicarbonate solution and placed in the sun. Symptoms of photosensitisation were marked three hours after dosing. The animal, however, died during the night and was decomposed the following morning.

Thionine may thus be regarded as capable of producing a well-marked but transitory photosensitisation similar to that caused by methylene blue, although it seems to be less toxic than the latter dye-stuff.

(C) METHYLENE VIOLET.

One Angora goat injected intravenously with 1 gm. methylene violet showed marked photosensitisation almost immediately it was placed out in the sun. There was marked flinching and scratching until the animal was returned to the stable, when the symptoms subsided. A subsequent injection on the following day caused death from shock-like symptoms. It thus seems that, although methylene violet produces well-marked photosensitisation, it is even more toxic than methylene blue

(D) METHYL VIOLET.

To ascertain whether a non-fluorescent substance such as methyl violet could produce photosensitisation, one Angora goat was injected intravenously on three successive days with 1 gm. The animal, although it was kept out in the sunlight, never showed any signs of photosensitisation. There were, however, signs of abdominal pain and loss of appetite on the second day. As the condition did not improve the animal was killed on the 7th day. No specific changes were noted at post-mortem examination, except that there was very little food in the digestive tract.

V. EXPERIMENTS WITH QUININE SALTS.

Seeing that solutions of quinine salts fluoresce in violet and ultra-violet light, it was decided to ascertain whether photosensitisation could be induced by injections of quinine compounds. One Angora goat was injected intravenously with 0.3 gm. quinine hydrochlorate in water, without becoming sensitive when placed in the sun. A further injection of 0.5 gm. given the following day caused a well-marked haemoglobinuria without any symptoms of photosensitisation. The serum soon cleared up and the animal became apparently completely normal again 48 hours after the last injection.

Another goat was dosed with 10 gm. quinine sulphate in 1 litre water. The animal showed a transitory dullness but no signs of photosensitisation were noticeable. The serum remained water clear.

It thus appears that the quinine salts used in these experiments do not readily provoke photosensitisation, although haemolysis and haemoglobinuria may be well marked.

SUMMARY.

In an attempt to produce a clinical picture simulating that of geeldikkop in sheep, viz., generalised icterus accompanied by acute photosensitisation, various fluorescent dye-stuffs were administered to Merino sheep and Angora goats which, after being closely shorn, were kept out in strong sunlight, and carefully observed for any signs of light sensitivity.

In the fluorescein group, eosin, erythrosine and rose bengale were injected into sheep and goats. Photosensitisation in each case was observed within a few minutes, flinching and scratching becoming so marked that the most abnormal body attitudes were assumed by the animal. When the animal was protected from sunlight either by stabling or by pigmenting of the exposed skin, no untoward effects were shown by the injected dyes. In chronic cases marked sloughing of the affected skin took place, accompanied by a new growth of skin and wool underneath. In no case was there any suspicion of any derangement of the internal organs and icterus was constantly absent.

Dye-stuffs taken from the Anthracene group gave no positive results of photosensitisation.

From the Acridin group, acriflavin was found to produce marked light sensitivity which, however, soon passed off.

In the Tiazin group methylene blue was found to produce very marked sensitisation of short duration and accompanied by other toxic effects, e.g. haemolysis. Similarly thionine and methylene violet produced marked symptoms of photosensitisation, although again accompanied by other toxic effects. A non-fluorescent substance such as methyl violet, on the other hand, showed no effect of photosensitising an animal.

Experiments carried out with certain quinine salts showed that, although they were fluorescent in ultra violet light, no photosensitisation resulted when they were injected into sheep. A direct toxic effect was, however, noted in the form of haemolysis and accompanying haemoglobinuria.

From these experiments it is thus clear that, as with haematoporphyrin, marked photosensitisation can be provoked in Merino sheep and Angora goats by the injection of different fluorescent dye-stuffs, and subsequent exposure of the animals to sunlight. The condition, however, differs from true geeldikkop in that the constantly occurring generalised icterus is absent.

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