Photosensitising Agent in 'Geel-dikkop' Phylloerythrin

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AS reported recently¹, we are engaged upon the study of a disease 'geel-dikkop' or 'yellow-thick-head' in sheep, characterised by intense photosensitivity and a well-marked, generalised icterus. Our view, previously put forward, was that a special set of physiological conditions, both of the plant and of the animal, were necessary for the appearance of the

FIG. 1. Bile porphyrin from pyridine-methyl alcohol.

disease. We have now pursued the study further upon chemical lines.

Since photosensitisation is a characteristic symptom, the first step, naturally, was to look for the photosensitising pigment. No porphyrins or pigments of the 'hypericin' type could be discovered in *Tribulus*, so it became necessary to turn to the

FIG. 2. Symptoms of photosensitisation 30 minutes after intravenous injection of 0.1 gm. bile porphyrin.

animals' blood for the isolation and identification of the factor responsible.

In this we were hampered by the uncertain, even erratic, occurrence of the natural disease; an observation of fundamental importance was, however, made by the discovery² that simple ligation of the

common bile duct in sheep gave rise to symptoms almost identical with those encountered in the natural disease, that is to say, generalised icterus and a marked photosensitivity followed by oedema of the unpigmented parts, especially the face, lips, etc., and later, necrosis and sloughing of the hardened skin. The serum of ligation icterus cases was investigated and a fluorescent substance with porphyrin-like properties and absorption spectra in ethereal and acid solution was found to be present. Normal sheep's bile (laboratory flock) was found to contain traces of a similar, if not identical, pigment. In order to procure larger quantities of bile for isolation purposes, we introduced bile fistulae instead of ligating the duct in a number of sheep and in every case it was found that within 48 hours after the operation, the concentration of porphyrin-like pigment in the bile had risen to about ten times that found in normal bile, probably owing to the ingestion of more green feed.

Conditions were such that bacterial growth in the

FIG. 3. Same animal on the following morning. Edematous swellings and pendulous, swollen ears.

fistula fluid and infection of the gall bladder were carefully avoided. The hypothesis that normal excretion might be largely in the form of a leuco-compound has been entertained but cannot, so far, be substantiated by direct experiment.

Tying off the fistula tube so that a condition equivalent to simple ligation of the duct ensued, again led to marked photosensitivity.

We have now had an opportunity of comparing these findings with samples of bile, serum, etc., taken from actual geel-dikkop cases in two different outbreaks. From every serum examined, the same porphyrin-like pigment was isolated and this in no way differed from that found in the experimental obstruction cases. Further, the biles of the sick animals in each instance contained large amounts of the same pigment, ranging from four to fifteen times the normal bile concentration. The identity of the porphyrin-like pigment in the experimental and geel-

dikkop cases has been established with certainty by means of comparing absorption spectra, crystalline forms from different solvents and its conversion into the same methyl-ester by esterification (Fig. 1).

With larger quantities of pigment derived from the working up of dried fistula bile we have now been able to recognise the pigment as the chlorophyll derivative, phylloerythrin, a true porphyrin as shown by Fischer *et al.*³, and first discovered in bile by Löbisch and Fischler⁴.

The identification of the pigment was based upon elementary analysis, absorption spectra in acid and in ethereal solutions and elementary analysis and melting point of its methyl ester.

Marchlewski⁵ showed that the excretion of phylloerythrin was related to the amount of chlorophyll in the diet. We are at present investigating the factors responsible for its formation.

Phylloerythrin has not, so far, been recognised as a photosensitising substance. That it is photoactive we have proved by injecting 0.1 gm. intravenously into an unpigmented merino sheep. Within about thirty minutes, the animal became intensely sensitive, crouching in an endeavour to avoid the sun's rays, flinching, shaking the head and ears and finally lying down in a peculiarly contorted position (Fig. 2). Swellings of the lips and ears were soon noticeable and the next day a large pouch of oedematous fluid distended the loose skin in the intermandibular space (Fig. 3). Smaller quantities injected into white mice, subcutaneously, killed these animals within half an hour when exposed to the sun's light. An identical result was obtained using a specimen of phylloerythrin

prepared from chlorophyll by chemical means. The final proof is therefore obtained that the photosensitivity which characterises the disease 'tribulosis' or 'geel-dikkop' is due to the passage into the bloodstream of the plant porphyrin, phylloerythrin, derived ultimately from ingested chlorophyll and, for some reason or other, present in the bile of these cases in abnormally large quantities.

Of course, the reason for the icterus in geel-dikkop is still being sought. It would appear that affected animals suffer from an insufficiency of the liver, whereby biliary secretion fails and icterus consequently develops; there is no demonstrable obstruction of the bile ducts. A further possibility, which we have not lost sight of, is the hypothetical production of bilirubinoid substances from pigments of plant origin.

The finding that a chlorophyll derivative is the cause of photosensitivity in geel-dikkop explains why not only *Tribulus* but also other plants can cause the disease. No light is yet thrown, however, upon the peculiarly erratic nature of its appearances, and we still incline to the view that the physiological factors are here of paramount importance. The production of porphyrins by the action of micro-organisms upon chlorophyll is being investigated. These results together with full details of the above findings will be published shortly.

¹ Quin and Rimington, *NATURE*, **130**, 926, Dec. 17, 1932.

² Quin, forthcoming publication. Nineteenth Report of the Director of Veterinary Services, Union of South Africa.

³ Fischer, Moldenhauer and Süss, *Liebigs Ann.*, **485**, 1; 1931.

⁴ Löbisch and Fischler, *Monatsh. Chem.*, **24**, 335; 1904.

⁵ Marchlewski, *Z. Physiol. Chem.*, **48**, 208; 1904-5.

Electrical Interference and Broadcasting

THE modern widespread use of broadcasting receivers of continually increasing sensitivity has made most listeners familiar with the interference which arises from the operation of various types of machinery and apparatus from the electric supply mains. It may not be generally known how much the listening public is indebted to the experts of the Post Office for doing whatever lies in their power to prevent or cure interference of this kind.

At last year's Radio Exhibition at Olympia, a very interesting demonstration was given of the manner in which the interfering noises on a wireless receiver, which arise from the operation of electric fans, vacuum cleaners, refrigerators and flashing signs, may be eliminated by the connexion of a suitable choke-condenser by-pass arrangement to the machine or apparatus causing the disturbance. More recently the Institution of Post Office Electrical Engineers has published a paper by Col. A. S. Angwin containing an account of what has been accomplished in combating this 'man-made' interference.

This paper states that the Post Office organisation is dealing with cases of electrical interference at the rate of some ten thousand a year and the information resulting therefrom is centralised in the Radio Section of the Engineer-in-Chief's office in London. All complaints reach that office after investigations have been made, and there has been in consequence a gradual accumulation of knowledge of the various types of interference and the means found most suitable for minimising it.

The interference associated with supply mains and with electrical machinery and apparatus can be classified into two main groups, comprising radio

and audio-frequency disturbances respectively. Col. Angwin's paper states that most of the interference belongs to the first group, and is attributable to sudden changes in current in the electrical circuits, such as may be caused by switch gear, commutators, faulty contacts, etc. By connecting suitable by-pass arrangements in the manner referred to above, the high frequency transients resulting from these effects are restricted to small areas of the mains as remote as possible from the receiver affected.

There would appear to be some reason for adopting the suggestion put forward recently in the *Wireless World*, that the responsibility for fitting these interference-preventing devices should rest with the manufacturers of the electrical machinery and appliances. Medical high-frequency apparatus is particularly difficult to deal with; it appears that operation inside screened enclosures is the only satisfactory solution. In the case of electric tramways, and particularly trolley buses with an overhead wire supply, a remedy was found in the fitting of special chokes in the trolley arms. These chokes are constructed to have a high impedance at the frequency of the broadcasting transmitter serving the area concerned, and in many cases this remedy has proved very successful indeed.

With the increased use of short and ultra-short waves for broadcasting and television purposes, a further type of interference is experienced. This arises from the ignition systems of internal combustion engines, which are capable of setting up direct radiation on very short wave-lengths. This trouble has already been encountered in acute form in aeroplanes equipped with wireless, and it appears that