Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 17, Numbers 1 and 2, July and October, 1941.

Printed in the Union of South Africa by the Government Printer, Pretoria.

Studies on the Photosensitisation of Animals in South Africa. XI.—The Reaction of the Sensitised Merino Skin to Radiation in Different Regions of the Spectrum.

By GERTRUD RIEMERSCHMID, Department of Public Health, and JOHN I. QUIN, Section Physiology, Onderstepoort.

Introduction.

During the period 1931 to 1937 Quin, Rimington and Roets published a series of articles on investigations concerning the problem of geeldikkop in South Africa. In these publications it was demonstrated that a part of the symptom complex of this disease in sheep was associated with extreme photosensitivity. This in turn was provoked by the presence, in the peripheral circulation, of minimal amounts of phylloerythrin, a photo-active plant porphyrin derived from the degradation of chlorophyll in the digestive tract. The only parts of the skin which were clinically affected by light were the unpigmented and exposed areas of the head and ears and at times the lower parts of the limbs. Quin (1931, 1933) showed that haematoporphyrin and such fluorescent dye-stuffs as eosin, erythrosin and rose bengale all produced photosensitisation in sheep, the clinical symptoms of which were identical with those seen in geeldikkop caused by phylloerythrin. Likewise the effects of hypericin obtained from the plant *Hypericum ethiopicum* var. *glaucescens* Sond. and *Hypericum leucoptychoides* (syn. *H. lanceolatum* Lam.) on sheep were found to be identical with those of phylloerythrin. At the same time it was found that a variety of pigments applied to the exposed parts of the head and ears protected the underlying skin of sheep rendered photosensitive, i.e. they afforded protection somewhat similar to that of true skin melanin against the penetration of those light rays which ordinarily cause tissue damage.

As a result of this finding stock owners were advised to apply black marking fluid to the head and ears of sheep as a preventive against the acute symptoms of photosensitisation in geeldikkop. This measure proved beneficial especially in areas where natural shade against sunlight was scarce or even completely absent as, for instance, on many Karroo farms. In the above-mentioned experiments a variety of pigments were tested on the skin of sheep for their efficacy in warding off the harmful influence of sunlight. On the whole it was found that black and dark brown afforded more protection than the other colours. Sunlight filtered through ordinary window glass still caused oedema of the head and ears of animals sensitised with haematoporhyrin, although this was not as intense as that caused by unfiltered sunlight.

Exposure of haematoporphyrin sensitised sheep to a quartz mercury vapour lamp (Quin, 1931) (Hanovia artificial Alpine Sun) produced none of the symptoms of photosensitisation, although these were elicited in the same animals by ten minutes exposure to sunlight. On the other hand, light from a strong carbon arc lamp did produce some photosensitisation with subsequent oedema formation although again not as severe as that seen in sunlight.

None of these experiments gave definite information as to which of the rays in the solar spectrum were responsible for the typical symptoms so readily produced by a variety of photodynamic substances.

Physical Aspects.

In order to gain more precise information, artificially photosensitised sheep were exposed to sunlight transmitted through glass filters. The sheep were photosensitised with two different agents, namely, group 1 with eosin and group 2 with phylloerythrin, which is responsible for causing geeldikkop.

The experiments were undertaken in accordance with the following physical considerations:—

The Grotthus-Draper Law states that "Radiation must be absorbed in order to bring about the reactions which its effect produces." Accordingly in any study on the effect of light, two factors have to be taken into account:—

1. The light source and its quality, i.e. the spectral distribution of light emitted from the source;
2. the ability of the medium to absorb the incoming light. This ability varies for the different parts of the spectrum. Therefore the spectral distribution of absorption in the medium sets a limit to the possible effect of the light. When these theoretical considerations are applied to the case of geeldikkop, the position is as follows:—

   The source of the light which causes the damage is the sun. The amount of energy emitted in the different parts of the solar spectrum is known. This energy is reduced on its way through the atmosphere before it arrives at the surface of the earth. It influences the exposed skin of the sheep, but, according to the above law reactions would be expected only in those parts of the spectrum in which rays are readily absorbed by the photosensitising substance in the peripheral circulation of the animal.

The aim of the following experiments was to determine which portions of the solar spectrum produce a reaction in artificially photosensitised sheep, and to find out whether these portions coincide with absorption bands of the dye-stuffs used for photosensitisation.

A. *Spectral Distribution of the Intensity of the Solar Radiation at Onderstepoort.*

The reduction of the light emitted by the sun on its way to the surface of the earth is due to absorption and scattering by gas molecules and small particles. The reduction depends on the thickness of the atmospheric layer which has to be traversed by the rays before arriving at the earth's surface. The length of the path through the air depends on the altitude of the sun. The lower the sun, the thicker is the reducing layer of air. Thus the path is much longer in the early morning and late afternoon than at midday. The spectral

distribution of the solar rays accordingly varies during the course of the day. Consequently it is essential to take into account a mean altitude of the sun prevailing during the experiments. As this mean altitude and hence the average thickness of the air layer was known during the periods of exposure, it was possible to compute the mean spectral energy distribution curve of the solar radiation at Onderstepoort during the experimental periods. For this calculation the experiments were divided into two groups with a mean sun altitude of 65° and 35° respectively. The calculations were based on values for normal solar energy spectral distribution as determined by Abbot and his collaborators. Their fundamental data on the transmission coefficient for various wavelengths and on the solar intensity outside the atmosphere were used in our calculations.

For the group 1 experiments (sun height 65°) the mean air mass at Onderstepoort was 1·22 (Bemporad tables) corrected for 665 mm. Hg. mean barometric pressure, which gives a corrected air mass of 1·05. Abbot's values of the atmospheric transmission coefficient for air mass 1·0, were considered to be close enough to the conditions prevailing during the experiments at Onderstepoort to be used in computing the spectral distribution of solar energy. The short-wave end of the spectral distribution curve was taken from Richard's (1939) determination of the shortest wavelengths observed at Johannesburg. These were 299·6 mμ for 65°, and 303·3 mμ for 35° sun altitude. The same method was used for the curve of the group 2 experiments with a mean sun height of 35° (mean air mass 1·74, 665 mm. Hg. mean barometric pressure; corrected air mass, 1·5).

B. *Spectral Distribution of Absorption in Eosin and Phylloerythrin.*

The second factor which had to be considered was the possible absorption of the light by the absorbing medium. Dealing with this subject we will refrain from considering the question of how the rays, which arrive at the skin of the exposed sheep, are reduced and changed on their way into and through the skin, since, as will be shown, the absorption of solar radiation does not cause any severe damage to the skin of unphotosensitised sheep. If, therefore, the photosensitising dye-stuff is responsible for the damage, we may assume that reactions are to be expected only with radiation from that part of the spectrum in which the photo-active agent shows absorption.

The ability of a medium to absorb rays of certain wavelengths is represented by its absorption spectrum. This shows a varying intensity for the different wavelengths thus indicating that not all wavelengths are absorbed to the same extent. The amount of absorption is partly dependent on the thickness of the absorbing layer. Part of the light is absorbed in the outer layers of the medium, a reduced amount penetrating to the deeper layers. This results in a change in the quality and quantity of the radiation which is available for absorption in the various layers. To determine the distribution of absorption over the whole spectrum, however, it is necessary to refer to a unit of absorption irrespective of the thickness of the absorbing medium. This unit is the molecular absorption coefficient which indicates how much of the light energy of various wavelengths can be absorbed by a solution of known concentration and thickness. The absorption coefficient was calculated for eosin (Fig. 1) and phylloerythrin (Fig. 3). Where both the absorption spectrum of the dye-stuff and the spectral distribution of the incoming light are known, a combination of these two curves shows how much energy is absorbed by eosin and phylloerythrin respectively in the different parts of the spectrum. Absorption curves corrected for the light intensity are given in Fig. 2 (eosin) and Fig. 4 (phylloerythrin).

(a) Eosin Absorption.

The eosin calculations are based on the absorption spectrum given by Weigert (1916). This graph, unfortunately, only refers to the visible part of the spectrum and had to be completed for the ultraviolet part from readings given by Krüss (1905). As his readings are not given in absolute units, the height of the absorption maxima in this region could not be deduced with certainty. The position of the bands, however, is clearly indicated.*

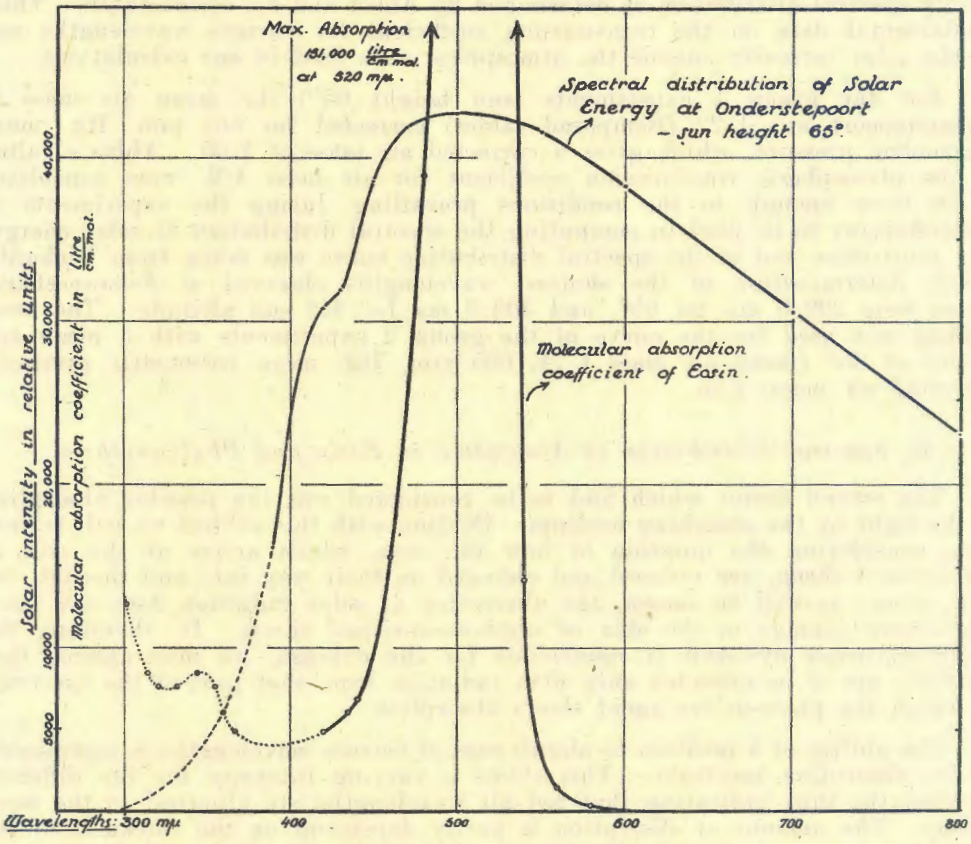


Fig. 1.—Absorption coefficient of Eosin and spectral distribution of solar energy.

The information gained from Fig. 1 may shortly be summarised as follows:—

The graph shows that in the eosin experiments we are concerned only with a comparatively narrow range of the spectrum, namely from 600-300 mμ. Wavelengths longer than 600 mμ are not absorbed by eosin, while wavelengths shorter than 300 mμ are absorbed by ozone on their way through the atmosphere. The maximum absorption between 470 and 540 mμ coincides with the maximum

*Footnote.—The molecular absorption coefficient was calculated according to the following formula:—

$I_z = I_o e^{-kcz}$ where I_z = the intensity at a depth z cm, I_o = the original intensity, c = concentration in gr. mol/litre, e = base of natural logarithms and k = molecular absorption-coefficient.

intensity of the solar radiation. A second region of increased absorption, between 370 and 300 mμ corresponds to a rapidly decreasing solar intensity. The amount actually absorbed in eosin is practically confined to one band, namely between 470 and 540 mμ, the amount of solar energy absorbed on either side of this band being negligible. This can be seen clearly in the graph in Fig. 2 which was obtained by multiplying the absorption coefficients of eosin by the solar intensities at corresponding wavelengths.

This graph indicates that reactions on the sheep's skin can be expected only under filters which transmit part or all of this absorption band.

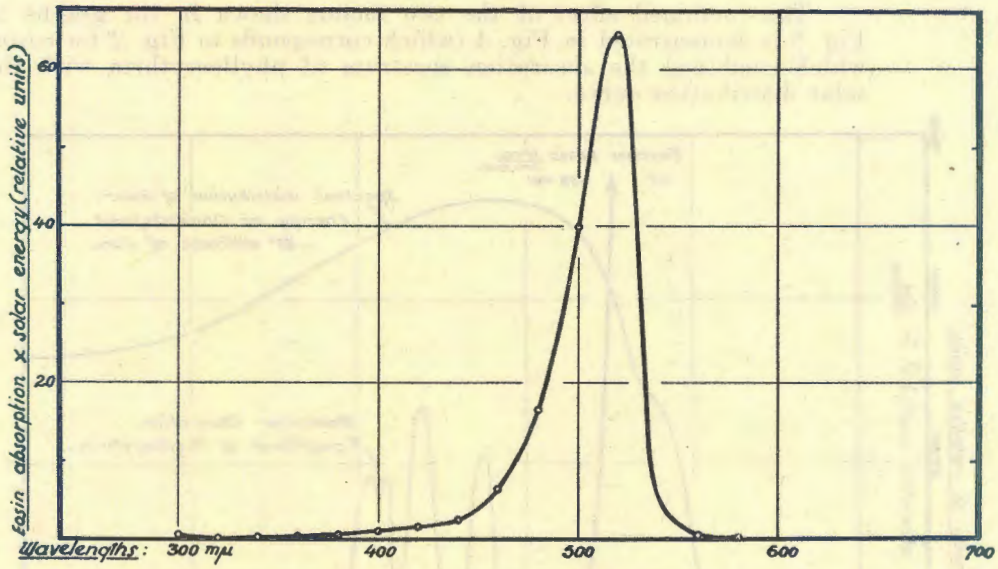


Fig. 2.—Eosin absorption corrected for solar energy at Onderstepoort, 65° altitude of sun.

(b) Phylloerythrin Absorption.

The phylloerythrin absorption coefficient was calculated from spectral transmission data provided by Messrs. C. Zeiss (Jena) for various wavelengths. The molecular absorption coefficients are given in Fig. 3, the circles representing actual spectrophotometric determinations. As these determinations were not made at sufficient points to show the structure of the absorption bands in great detail, the approximate shape of the absorption curve in this zone was inferred from the wedge spectrogram provided by the above firm. With wavelengths shorter than 438 mμ, the absorption was so great that the transmitted light was too weak to be measured by the method applied. Consequently no accurate value of the absorption coefficient in this region was determined, but the absorption must have been greater than at 438 mμ, where the transmitted intensity was just great enough to be measurable. The absorption coefficient at this wavelength was therefore used in calculations at shorter wavelength to indicate the lower limit of the possible absorption of energy.

The significant points of the information gained in Fig. 3 are:—

(*a*) Infra-red part of the spectrum. In the region of wavelengths 800-700 mμ the relative amount of light obtained from the sun is fairly great, but there is practically no absorption by phylloerythrin.

(*b*) Visible part of the spectrum. Below 700 mμ the solar intensity steadily increases until it reaches a maximum at 520 mμ. The absorption in phylloerythrin in this part of the spectrum also shows an increase below 700 mμ with a small maximum at 638 mμ; following a minimum there is a rapid increase with three maxima between 600 and 520 mμ. In this region the absorption maxima coincide with maximum intensity of solar radiation. A minimum absorption at wavelength 488 mμ is followed by a strong absorption in the blue, violet and ultraviolet part of the spectrum, where the solar intensity decreases rapidly.

The combined effect of the two factors shown in the graphs in Fig. 3 is demonsrated in Fig. 4 (which corresponds to Fig. 2 for eosin) which combined the absorption spectrum of phylloerythrin with the solar distribution curve.

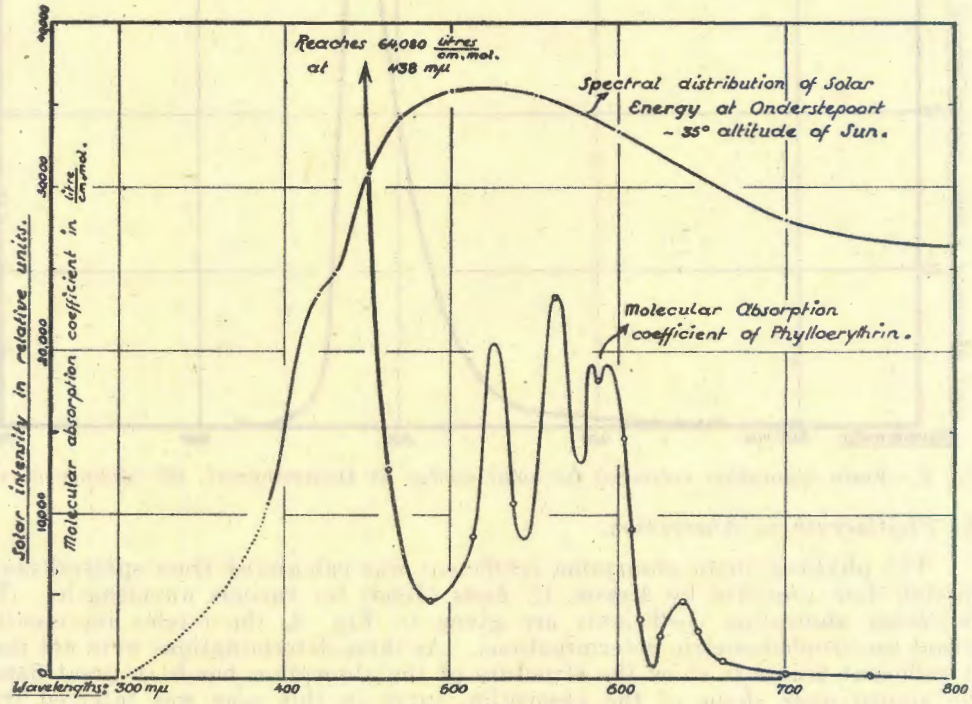


Fig. 3.—Absorption coefficient of phylloerythrin and spectral distribution of solar energy.

2. Experimental.

A. *Procedure.*

Test exposures were conducted on the skin of 33 artificially photosensitised adult merino sheep. An area of skin large enough to take a cardboard frame (10 by 30 cm.) containing the filters was shorn slightly to the side of the vertebral spine extending forwards from the tuber coxae. Various methods of obtaining a complete depilation of the skin after shearing were tried out but the exceptional tenderness of the merino skin offered considerable difficulty. In many cases the skin reacted to shaving or barium sulphide depilation and showed

excessive hyperaemia followed by varying degrees of cyanosis and oedema. Sometimes petechiae and subsequent scab formation were noted. These reactions made the skin unsuitable for testing the light effect. The method eventually adopted was the close clipping of the wool with a pair of fine scissors. Although depilation was not quite complete, vascular disturbances of the skin were thereby avoided.

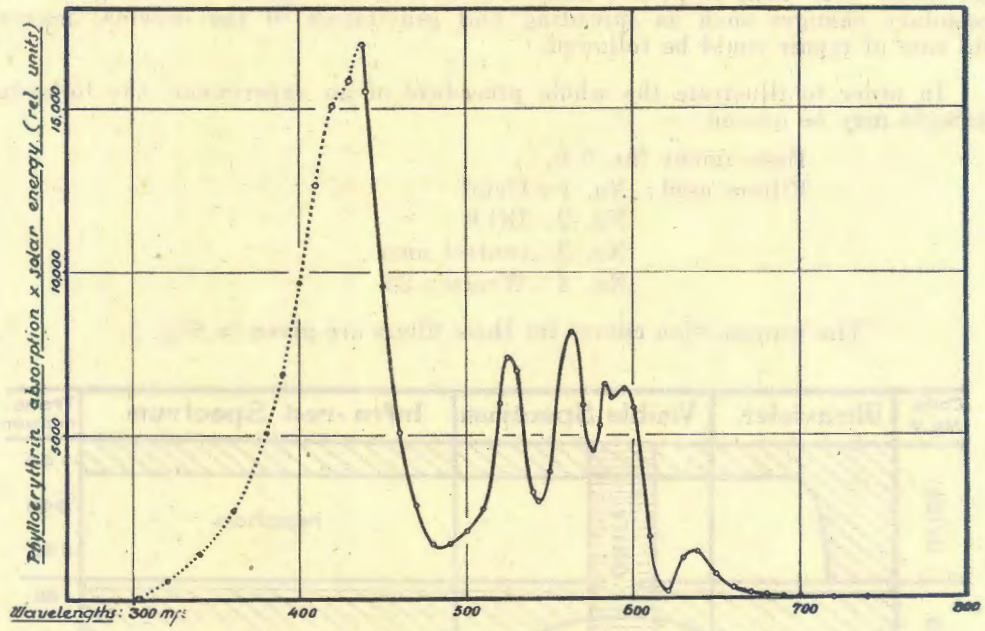


Fig. 4.—Phylloerythrin absorption corrected for solar energy at Ondestepoort, 35° altitude of sun.

Following the clipping of the skin, the animal was sensitised by intravenous injection in the eosin experiments, or by dosing with poisonous plant material such as *Lantana camara* in the phylloerythrin experiments.

After being removed from the stable, the animal was placed in a specially constructed and movable crush pen. In this it could stand normally but not move about. The cardboard frame containing the filters was then fixed in position. At the same time the colour of the skin was noted. In order to protect the head and other parts of the body from sunlight a hessian screen, with a window large enough to allow sunlight to fall on the test areas of the back, was fixed to the sunny side of the crush pen.

In the actual reading of the skin tests some difficulty was experienced at times as a result of the rapidity and irregularity of the colour changes. This was perhaps only to be expected since the type and intensity of colour change depends primarily on the vasomotor response of the skin capillaries, a factor which, in itself, is capable of very wide variations in a brief space of time. Moreover, the area of skin along the back used in these tests is normally covered by varying lengths of wool throughout the life of the animal. Consequently the capillaries in this locality are more fully protected against the influence of direct solar radiation than the capillaries in the skin over the face and ears,

which are more exposed to sunlight. However, in spite of these complications, the type and severity of the skin reactions permitted the comparison of the findings in the different squares. The procedure usually followed was to expose the test area continuously for a period up to six hours. During this time observations were made at short intervals. After completion of the exposure the animal was returned to a darkened stable and the test area covered with cotton wool. The test areas were then inspected daily but not again exposed to sunlight. The secondary changes such as spreading and gravitation of the oedema, necrosis and rate of repair could be followed.

In order to illustrate the whole procedure of an experiment, the following example may be quoted:—

Experiment No. 3 b,
Filters used: No. 1 = Uviol
No. 2 = BG 9
No. 3 = control area
No. 4 = Wratten 23

The transmission curves for these filters are given in Fig. 5.

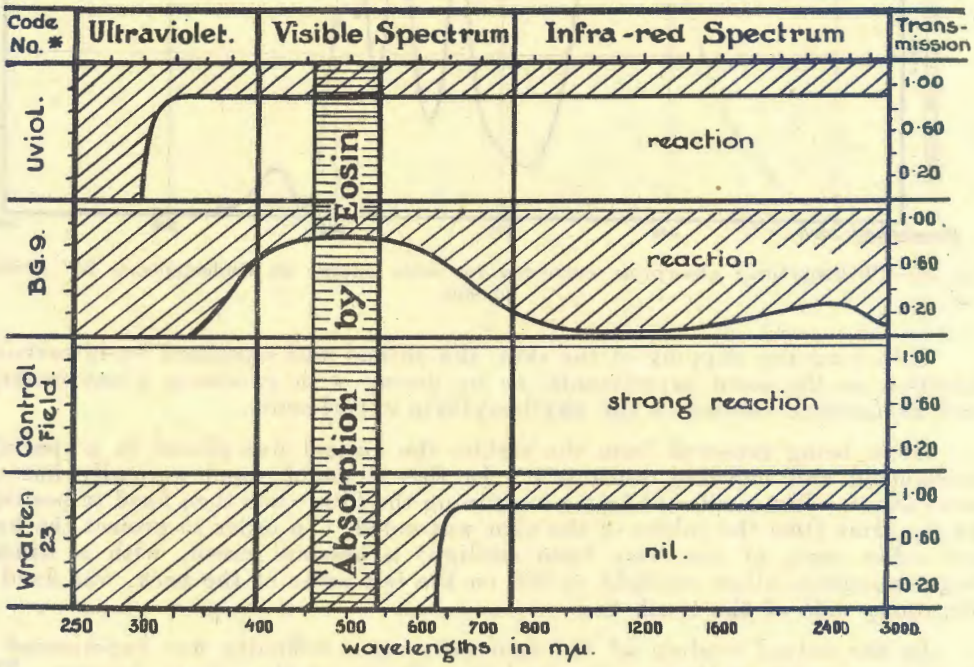


Fig. 5.—Filters used in eosin experiment No. 3 (*b*).

Procedure:

10.35 a.m.—Injected 1 gram eosin in 20 c.c. saline.

10.43 a.m.—Exposed to sunlight; skin pinkish within 10 minutes of the injection of eosin.

11.10 a.m.—Animal flinching as a result of the sunlight striking the back along the test areas, i.e. the first definite symptoms of photosensitisation were noticeable within 35 minutes of eosin injection.

Observations:

Filter No. 1. (*Uviol*).

11.10 a.m.—Skin bright red, swollen.
11.30 a.m.—Skin deeper red, sharply defined within square.
12.00 a.m.—Skin purplish red (cyanotic), slightly swollen.
12.30 p.m.—Purplish red, definite oedema present.
2.30 p.m.—As at 12.20 p.m.
3.30 p.m.—Condition unchanged.

Filter No. 2 (*B.G.* 9).

11.10 a.m.—Skin red, somewhat swollen.
11.30 a.m.—Skin darker red, sharply demarcated within square.
12.00 a.m.—Purplish red, swelling indefinite.
12.30 p.m.—Slight purplish red as in filter No. 1, although slightly more oedema.
2.00 p.m.—Light purplish red, slightly swollen.
3.30 p.m.—Condition unchanged.

No. 3 *Control* (*No Filter*).

11.10 a.m.—Skin red, swollen.
11.30 a.m.—Red discolouration demarcated by edge of square.
12.00 a.m.—Slightly less red than No. 2, swelling indefinite.
12.30 p.m.—Light purplish red.
2.00 p.m.—Deep purple red and markedly swollen, more than No. 1 and No. 2.
3.30 p.m.—Still dark red and markedly swollen.

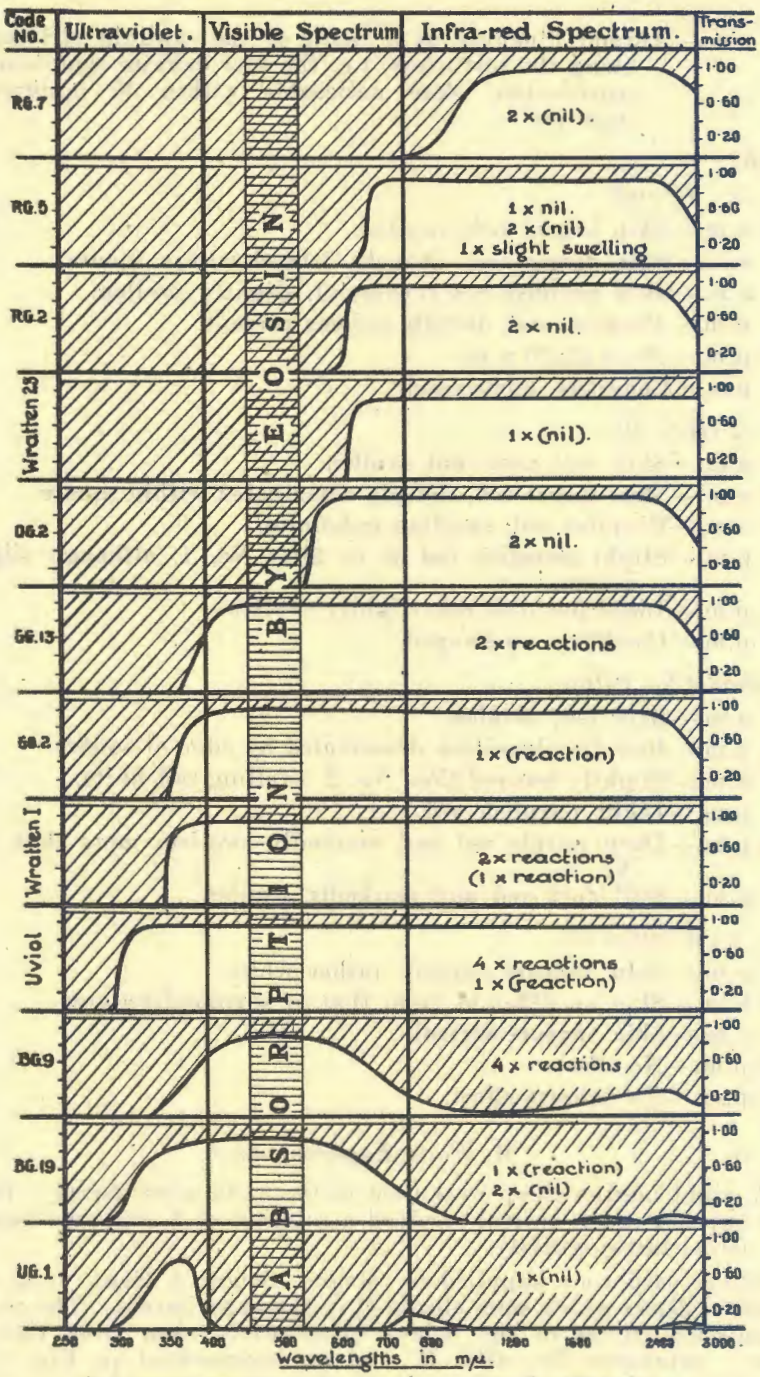
Filter No. 4 (*Wratten* 23).

11.10 a.m.—Skin appears normal, rather white.
11.30 a.m.—Skin no different from that of surrounding area.
12.00 a.m.—Skin appears normal.
2.00 p.m.—No effect.
3.30 p.m.—No definite effect.

B. *Eosin Experiments.*

In all, eight merino sheep were used in the eosin experiments. Immediately before the exposure each animal received a solution of 1 gm. pure eosin (yellow) in 20 c.c. saline intrajugularly.

The filters used were supplied by Messrs. Schott & Gen., Jena, except for a few Wratten filters which were also used in the experiments. The code numbers and transmission curves of the Schott filters were taken from their "optical glass filter" catalogue No. 4892 E, and are represented in Fig. 6. In this graph the eosin absorption band is also shown, superimposed on the filter transmission curves. The limit of the eosin band was taken at the wavelengths where the absorption was $^1/_{10}$th of the maximum absorption.



The reactions given in brackets indicate that the simultaneous reaction in the control area was very slight.

Fig. 6.—Filters used in eosin experiments.

Discussion.

The results of the eosin experiments are given in Table 1. They showed clearly that the Draper-Grotthus law holds in the case of sheep photosensitised with eosin. Reactions on the skin were (except once) only observed in those regions of the spectrum where light is transmitted through the filters and where eosin absorbs strongly. In one case [experiment No. 9 (*a*), filter RG 5] a slight swelling occurred under a filter which transmitted light not absorbed in eosin. Otherwise reactions were observed only within the region of eosin absorption. The intensity of these reactions was dependent on the degree of photosensitisation as can be seen from a comparison of the readings of the control area with simultaneous filter readings (see Table 1).

C. *Phylloerythrin Experiments.*

Lantana camara is a widely distributed indigenous verbenaceous shrub. It is closely related to *Lippia rehmanni Pears*, which was shown to cause icterus and photosensitisation in sheep (Quin, 1933; Rimington and Quin, 1934, 1937). The leaves of *Lippia rehmanni* contain an icterogenic principle (icterogenin) that causes a paralysis of bile flow, with symptoms of regurgitative jaundice, and photosensitisation. The photosensitisation is due to phylloerythrin in the peripheral circulation following the liver damage. Dried leaves of *Lantana camara* also contain an icterogenic principle termed *Lantanin* which provokes symptoms in sheep similar to those caused by dry *Lippia* material. As a supply of dried leaves of *Lantana camara* was available, a series of experiments was conducted on sheep photosensitised with it and also with lantanin, derived from it.

(*a*) *Effect of Sunlight on Normal Skin under Different Glass Filters.*

Before ascertaining the influence of sunlight on the skin of sheep sensitised with *Lantana*, all animals, during the preperiod of lucerne feeding, were clipped on one side along the back and a series of filters, selected for the subsequent experiments, tested out beforehand on the normal skin. As usual, one of the squares was kept as a control i.e. continuously exposed to direct sunlight without a filter. In these tests all the filters used completely inhibited any visible skin reaction, even after five hours insolation. The control area, however, frequently revealed a typical circumscribed bright red erythema within one hour or more, depending on solar intensity. The erythema was, however, always of a light grade and subsided rapidly without oedema formation.

(*b*) *Dosing of the Sheep in the Phylloerythrin Experiments.*

In all 12 adult merino sheep were used for the phylloerythrin experiments. As mentioned before the formation of phylloerythrin was stimulated by feeding lucerne for a fortnight preceding the poisoning of the sheep. 250 gm. of dry powered *Lantana camara* leaves were used as a dose. When the supply of this plant material was exhausted, lantanin crystals were used to produce geeldikkop conditions.

The reactions, even when using the same amount of poisonous material, were, however, not always of equal severity. The control areas (without glass filters), which could be taken as an indication of the degree of damage caused by the poison, showed varying degrees of reaction.

This can be readily explained as follows:—

1. Idiosyncrasy of the animals where the phylloerythrin concentration and solar irradiation remained constant.

2. Variation of the phylloerythrin concentration in the peripheral blood circulation. This depends on the amount of chlorophyll ingested, the rate of phylloerythrin formation and its absorption from the intestines, the severity of the disturbance in liver function and finally on the rate and efficiency with which phylloerythrin is eliminated by the kidneys in the urine.
3. Variation in the amount of solar irradiation to which the animals were exposed.

(*c*) *Effect of Varying the Duration of Exposure of Sheep Poisoned with* Lantana camara.

The following experiment indicates the type and severity of the reaction when test squares of skin on a sheep poisoned with *Lantana camara* were exposed to direct sunlight for varying lengths of time. A sheep was placed on a diet of green lucerne on the 10th of June, 1940. The object of this was to increase the total intake of chlorophyll, so that more phylloerythrin would be available for absorption and sensitisation afer the liver had been damaged by dosing with *Lantana camara*. Repeated bleeding during the fourteen days on this diet revealed a water-clear blood serum free from bile pigments. It should be mentioned here that this is the normal condition for healthy adult merino sheep in which a physiological bilirubinaemia is usually completely absent. A fortnight later the animal was dosed by stomach tube with 125 grams of dry powered *Lantana camara* leaves in 1·5 litres water. The same dose was repeated the following day. Two days later the blood serum was a deep yellow and gave a positive direct v.d. Bergh reaction. On the same day at 10 o'clock a.m., six different squares on a previously clipped test area of skin were exposed to direct sunlight for varying lengths of time, the last square being covered at 3 p.m. Readings were taken on the squares at 3 p.m. on the same day and at 9 a.m. the following day. The following results were noted:—

Duration of Exposure.	Results at 3 p.m. same Day.	Results at 9 p.m. next Day.
15 Minutes	No discolouration, no swelling	Faint orange tinge.
30 Minutes	No discolouration, no swelling	Very slight orange tinge.
1 Hour	Faint orange, very slight swelling	Slight orange.
2 Hours	Slightly deeper orange, slight swelling	Definite purple.
3 Hours	Orange brown, marked swelling	Marked purple.
5 Hours	Light greyish, very marked diffused swelling	Very marked purple.

It will be noted that no swelling was recorded in the observations taken at 9 a.m. the following morning. It had disappeared or gravitated to a level below that of the original test area. These results indicated the progressive nature of the reactions when sensitised skin was exposed to sunlight for varying periods.

The following macroscopic changes were noted in the irradiated skin when the animal was killed 17 days later for the collection of skin material for histological examination

(*a*) 15 min. exposure—no change at all.
(*b*) 30 min. exposure—slight hyperaemia, slight oedema.
(*c*) 1 hour exposure—more definite hyperaemia and more oedematous swelling.
(*d*) 2 hours, (*e*) 3 hours, (*f*) 5 hours } exposure—more distinct oedematous swelling of the skin and subcutaneous tissues and also deeper purplish discolouration.

The orange tinge noted above was due to the presence of bile pigments in the peripheral circulation even before clinical jaundice could be definitely established on the skin, and represented a blending of the yellow colour of the biliary pigments with the bright red of the arterial blood during the stage of acute erythema.

The same sequence of colour changes noted above occurred in each test in which a reaction was observed (see Fig. 7 *a* and 7 *b*). The first colour change varied from a circumscribed greyish white, due to vasoconstriction, to a bright red erythema, following vasodilation. These reactions were soon followed by purple discolouration accompanied by swelling and later by oedema. Skin sections showed marked stasis of red cells in the damaged blood vessels. Eighteen hours after exposure the purple discolouration was more intense and more diffuse and the oedema showed a tendency to gravitate. The centre of the test areas remained swollen and became darker, almost purple black, in colour. Later the skin lost its elasticity and acquired a tough, dry, leathery texture. The breakdown of haemoglobin in the affected areas resulted in the formation of bile pigments. These pigments together with those accompanying the generalised icterus caused an intense, localised deep yellow-green discolouration which usually persisted until the final sloughing of the necrosed skin. Wool growth in such areas was suppressed for a considerable period.

In this connection it should be explained that the skin reaction which is noted during the acute stage of naturally occurring cases of geeldikkop may be slightly different from that described above. Thus the oedematous swelling of the head and ears in true geeldikkop is not preceded by the same degree of erythema and cyanosis as revealed on the back of the artificially photosensitised sheep. This may be due to either a difference in the reactivity of the skin capillaries in the two areas or to slightly greater protection of the face and ears by its covering of hair and dust as compared to the closely shorn, clean skin of the test areas. However, the extent and severity of the intra- and subcutaneous oedema as well as the chronic lesions of skin necrosis are fully comparable in the natural and in the experimentally produced conditions.

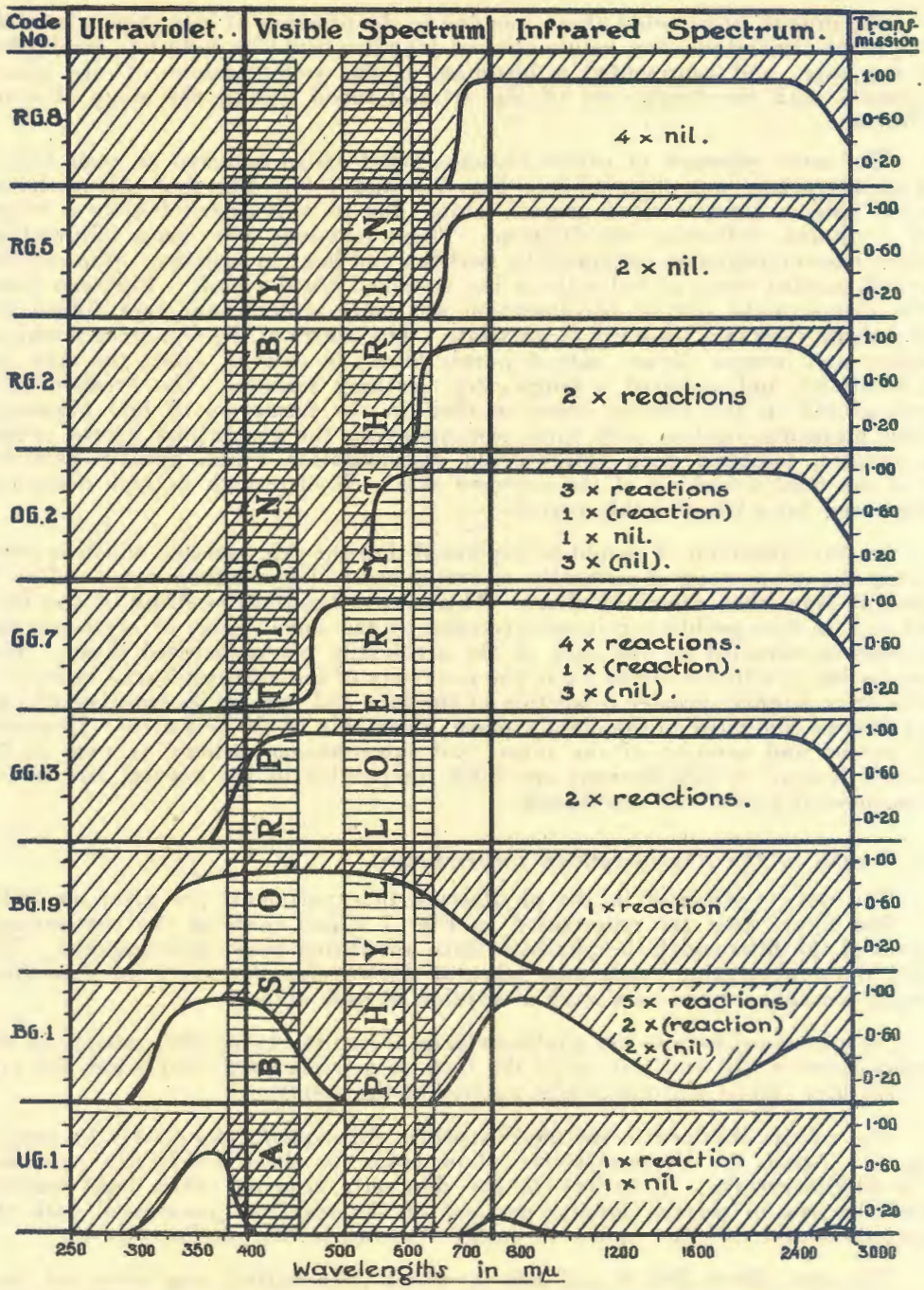
(*d*) *Results of the Phylloerythrin Experiments.*

The results obtained in the phylloerythrin experiments are given in Table 2. The filters used are represented in Fig. 7 which contains the transmission curves of the filters with the phylloerythrin absorption bands superimposed. The limit of the absorption bands were taken at the wavelengths where the absorption was 40 per cent. of the maximum absorption in each area.

As mentioned before, the phylloerythrin absorption takes place mainly in the region between 610 and 510 mμ in the form of a triple band, and below 460 mμ, i.e. the blue, violet and ultraviolet portion of the spectrum.

The results obtained in the phylloerythrin experiments can clearly be seen in Fig. 7. Again the Draper-Grotthus Law holds for sheep artificially sensitised with phylloerythrin. Reactions in the skin only occurred when light emitted from the sun in certain definite portions of the spectrum penetrated both the atmosphere and the filter and were finally absorbed by the phylloerythrin.

The two filters RG 8 and RG 5, which transmitted long-wave red rays outside the absorption bands prevented reactions. As soon as any of the phylloerythrin absorption bands were transmitted, reactions occurred, as shown by all the remaining filters.



(The reactions given in brackets indicate that the simultaneous reaction in the control area was very slight.)

Fig. 7.—Filters used in phylloerythrin experiments.

The next point of interest was, whether all three areas of absorption (see Fig. 4) in phylloerythrin were responsible for reactions. Readings taken under filter RG 2 and BG 1 proved that this was the case. Filter RG 2 only includes the small absorption band with a maximum at 638 mμ but showed definite reactions which were proportional to the severity of simultaneous reactions in the control field. Filter BG 1, which excludes both this narrow band and the triple band between 610 and 510 mμ, proved that the intensive absorption of phylloerythrin in the blue, violet and ultraviolet part of the spectrum gave definite reactions on the sheep skin. Filter UG 1, which only transmits a small part of this absorption area of phylloerythrin gave one positive and one negative reaction.

Summary.

1. The effect of sunlight on sheep artificially photosensitised with eosin and phylloerythrin was investigated. Glass filters were used to ascertain whether any of those rays readily absorbed by these dye-stuffs produced skin reactions. Small square test areas on the back of photosensitised sheep were exposed under these filters to solar radiation for various lengths of time, up to six hours.

2. A spectral distribution curve was calculated for the solar radiation at Onderstepoort for an altitude of the sun of 65° and 35° respectively. When this spectral distribution curve was combined with the absorption spectrum of eosin and phylloerythrin the resulting curve delimitated those regions of the spectrum which might be expected to yield skin reactions in the photosensitised sheep.

The results may be summarised as follows:—

(*a*) The skin of closely shorn, non-photosensitised, adult merino sheep, exposed to solar radiation for several hours showed only a light erythema which rapidly subsided without oedema formation.

(*b*) Sheep photosensitised with phylloerythrin (dried leaves of the *Lantana camara* plant) and subsequently exposed to sunlight, showed reactions which increased in severity depending on the duration of exposure.

(*c*) The reactions of the skin of sheep photosensitised with eosin, were strictly confined to those parts of the spectrum where eosin strongly absorbs radiation, namely from wavelength 540-460 mμ, i.e. in the green and blue part of the spectrum.

(*d*) The phylloerythrin absorption is spread over a much wider area than that of eosin. The phylloerythrin shows three regions of absorption namely a single band from 654 to 626 mμ (orange), a triple band between 610 and 510 mμ (yellow and green) and high absorption below 460 mμ (blue, violet and ultraviolet). The filter experiments showed that reactions in the skin of sheep photosensitised with phylloerythrin were restricted to parts of the spectrum where radiation is absorbed by phylloerythrin, namely the region between 650 and 380 mμ, which includes practically all visible light.

REFERENCES.

ANNALS of the Astrophysical Observatory of the Smithsonion Institution, Vol. III.

BLUM, H. F., AND PACE, N. (1937). Studies on photosensitisation by Porphyrins, *Br. Journ. Derm. and Syph.*, Vol. 49, pp. 465-485.

KRUSS, P. (1905). Über die Absorption organischer Farbstoffe im Ultraviolett, *Zeitschr. f. Phys. Chemie*, Vol. 51, pp. 257-296.

QUIN, J. I. (1931). The photosensitising influence of Haematoporhyrin on sheep and goats (1931), 17*th Report of the Director of Vet. Serv. and Anim. Industry.*

QUIN, J. I., RIMINGTON, C., AND ROETS, C. G. S. (1) Studies on the photosensitisation of animals in South Africa, Nos. 1-11, *Onderstepoort J.*, Vol. 1, No. 2, 1933; Vol. 3, No. 1 (1934); Vol. 4, No. 2 (1935); Vol. 7, No. 1 (1936).

RICHARDS, S. J. (1939). A survey of the ultraviolet solar radiation at Johannesburg, *S. Afr. J. Sci.*, Vol. 36, pp. 132-149.

WEIGERT, F. (1916). Über Absorptionspektren und über eine einfache Methode zu ihrer quantitativen Bestimmung, *Berichte der deutschen Chemischen Gesellschaft*, Vol. 49, No. 1, pp. 1496-1532.